37th Meeting of the American Society for Photobiology San Diego, California

June 14–19, 2014
Conference Chair: Tayyaba Hasan

PROGRAM AND ABSTRACTS

AMERICAN SOCIETY FOR PHOTOBIOLOGY
LUX ET VITA
MCMLXXII

PROGRAM AND ABSTRACTS

37th Meeting of the

American Society for Photobiology

1972-2014

Hard Rock Hotel

San Diego, California

June 14-19, 2014

Scientific Program Chair:

Tayyaba Hasan

A Heartfelt Welcome to the 37th Meeting of the American Society for Photobiology

Welcome to the 37th meeting of the American Society for Photobiology (ASP) being held at the Hard Rock Hotel in San Diego, California from June 14–19, 2014. I hope you will enjoy your stay at the conference, with excellent science, stimulating conversation and a lot of fun to be had in beautiful San Diego.

With the help of the organizing committee, we have put together an exciting program, including cutting edge symposia on a wide range of topics. Along with exciting new developments in optical and medical technology, sessions will address recent advances in environmental photobiology, DNA damage and repair, UV carcinogenesis, photodynamic therapy, photovaccines, sunscreens, and new applications of light in dermatology - to name just a few. You will hear about enabling photobiology in the dark and spiders, silk and light. We also have Dr. Roger Tsien, speaking in a plenary session on “Cells in Health and Disease, Seen Mostly in Pretty Colors.” Additional exciting symposia include sessions on optogenetics, imaging and PDT, a joint ASP-ESP symposium on the photoinactivation of pathogens, pigment cell photobiology, death mechanisms, photoimmunology, and new this year is the introduction of a hands-on workshop on photobiology. I thank the chairs and the organizing committee for their help with developing the conference program.

I anticipate that the conference will be a constructive forum for those already in the field, as well as for those contemplating a career in photobiology or photomedicine. This is a particularly good opportunity for laboratory scientists, clinicians, and practitioners of related disciplines, who would otherwise not have the opportunity to meet, exchange ideas, and build collaborations for future endeavors.

As at the last meeting, with ASP’s commitment to developing scientists of the future, there will be networking and career development events, including grant-writing and mentoring workshops, poster sessions, and a variety of awards recognizing achievements in photobiology. In addition to our traditional awards, appearing for a second time are the ASP Editor’s Award for Outstanding Student Research, the Light Path Award, and the PhotoCite Awards recognizing individuals’ contributions to literature and their impact on the research environment. This year, a record number of students and postdocs were awarded travel assistance through the Frederick Urbach Memorial Student Travel Award, and we welcome them all to the meeting.

The meeting will take place at the Hard Rock Hotel in sunny San Diego’s historic Gaslamp Quarter, and the banquet will be held at the Harbor House, offering great views of the San Diego harbor. In addition to providing a vibrant venue for scientific exchange, San Diego is a great place for fun, history and cultural exploration. Located in Southern California, San Diego is an important center of international trade, technology development, biotechnology, and military/defense research. The city is well connected with local public transport including both buses and 102.6 miles of light rail, allowing you to get around the city with ease. San Diego is also home to numerous attractions, including Balboa Park, the San Diego Zoo, and SeaWorld.

I hope you will enjoy this unique opportunity for scientific exchange and social interaction with your fellow photobiologists, and I look forward to seeing you in San Diego!

Tayyaba Hasan

THE AMERICAN SOCIETY FOR PHOTOBIOLOGY THANKS THE 2014 MEETING ORGANIZING COMMITTEE

Chair:

Tayyaba Hasan

Organizing Committee:

Theresa Busch

Jean Cadet

Alexander Greer

Rüdiger Greinert

Yu-Ying He

David Kessel

Henry Lim

Jonathan Lovell

Edward Maytin

David Mitchell

Patrycja Nowak-Sliwinska

Imran Rizvi

Pål Selbo

John Streicher

Theo Theodossiou

THE AMERICAN SOCIETY FOR PHOTOBIOLOGY

THANKS THE FOLLOWING SPONSORS

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2014 AMERICAN SOCIETY FOR PHOTOBIOLOGY AWARDS

LIFETIME ACHIEVEMENT AWARD

2014 –

PHOTON AWARD

2014 –

NEW INVESTIGATOR AWARD

2014 – Angel Marti & Kaushal Rege

RESEARCH AWARD

2014 – Juan Scaiano

PHOTOCITE-A AWARD

2014 – Majid Montazer & Esfandiar Pakdel

PHOTOCITE-B AWARD

2014 – Wolfgang Gärtner & Aba Losi

EDITOR'S STUDENT RESEARCH AWARD

2014 – Glaucia Fragoso & Mai Thao

LIGHT PATH AWARD

2014 – David Mitchell

2014 AMERICAN SOCIETY FOR PHOTOBIOLOGY AWARDS

FREDERICK URBACH MEMORIAL STUDENT TRAVEL AWARD

2014 –

Neha Aggarwal

Saroj Kumar Amar

Emma Briars

Gwendolyn Cramer

Emilia Della Pietra

Marie-Catherine Drigeard Desgarnier

Carl Fisher

Glaucia Fragoso

Shannon Gallagher-Colombo

Ashwini Ghogare

Rebecca Gilson

Shruti Goyal

Huang-Chiao Huang

Justin Mallet

Srivalleesha Mallidi

Cristina Mari

Syed Fiaz Mujtaba

Bibi Petersen Marvin

Pollum Ramya

Raghunathan Imran Rizvi

Kishore Rollakanti

Bryan Spring

Amir Taslimi Jennifer

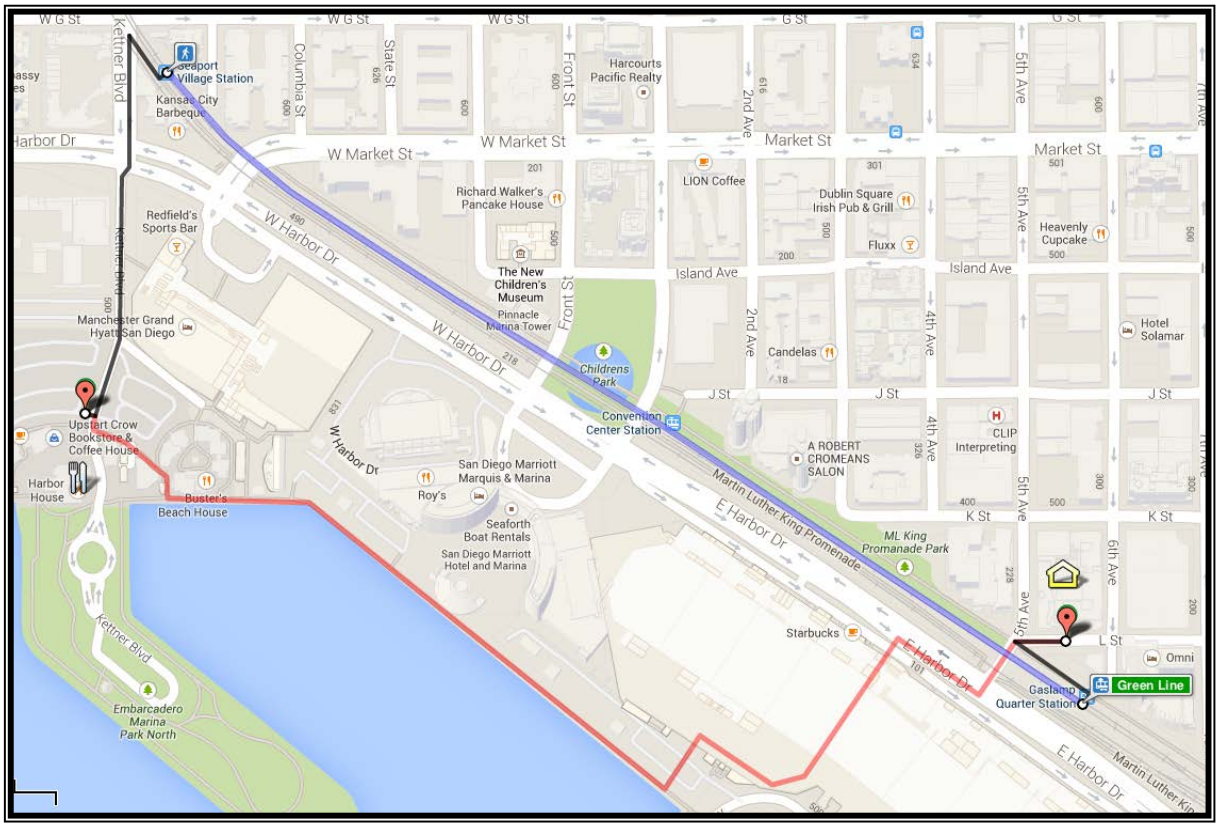
Tournear Xue Yang

ASP BANQUET & AWARDS CEREMONY

Harbor House

Tuesday June 17, 2014, 7:15—10:00 p.m.

Banquet ticket required for entrance. Drink tickets will be handed out at the door.



A ASP CONFERENCE

Hard Rock Hotel- San Diego
207 Fifth Avenue
San Diego, CA 92101
(619) 702 3000

B BANQUET & AWARDS

Harbor House
831 West Harbor Drive
San Diego, CA 92101
(619) 232 1141

MAP KEY

Red line: Walking directions
Blue line: Trolley route
Black line: Walking to and from trolley stops

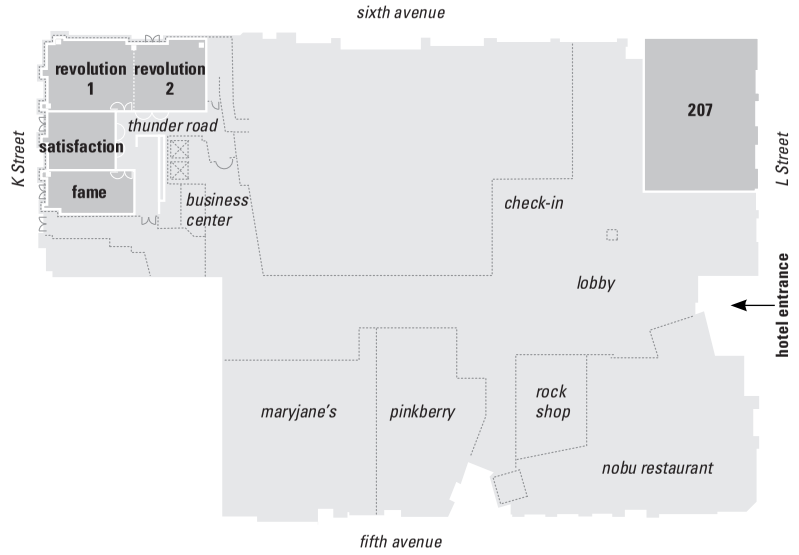
Parking at Harbor House

Parking will be validated with a minimum $10 purchase from any Seaport Village establishment. Validated parking is $3 for the first 2 hours and $2 for every 30 minutes thereafter. Non-validated parking is $8 per hour.

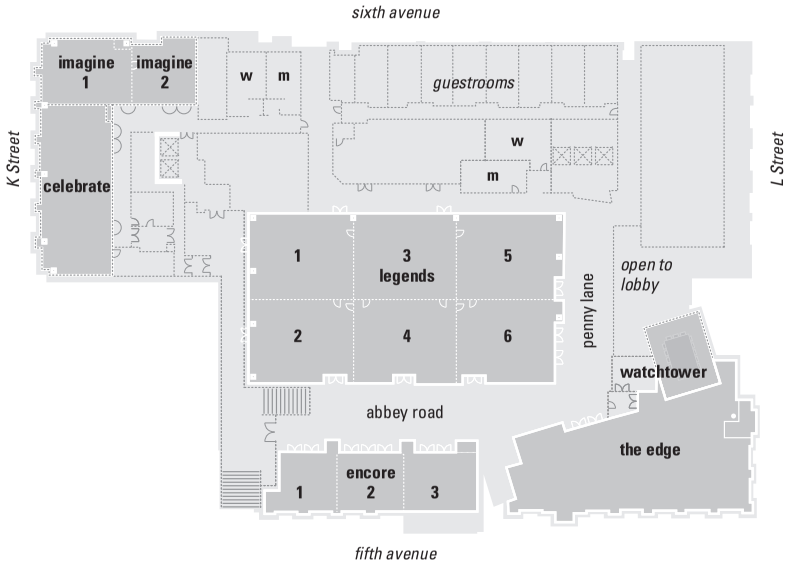
San Diego Trolley Information

Take the Gaslamp Quarter trolley in front of the Hard Rock Hotel (Stop number 75098) to Seaport Village (Stop number 75095) in front of Harbor House Restaurant. Fares are $2.50 each per person one-way. Exact fare is required on buses. The trolleys arrive every 15 minutes from 4 a.m. to 11:30 p.m. Monday-Sunday. Travel time is approximately 11 minutes.

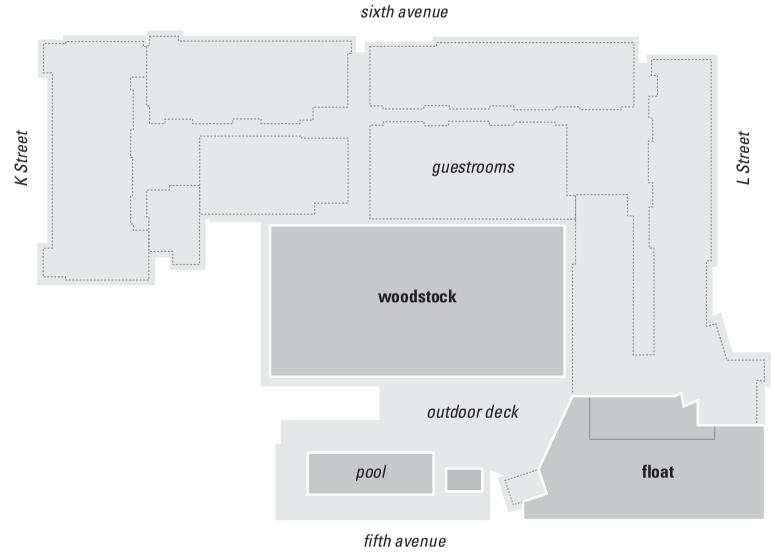
FIRST FLOOR



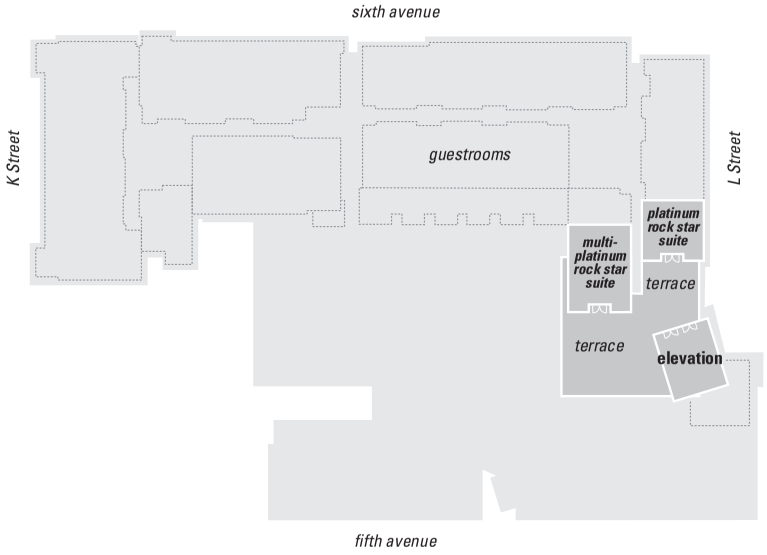
SECOND FLOOR



FOURTH FLOOR



ELEVENTH FLOOR



DAY 1 (SATURDAY JUNE 14)

All meeting rooms are located on the 2nd floor unless otherwise noted.

TIME	EVENT	CHAIR(S)	ROOM
10:00–8:00	REGISTRATION OPEN		Penny Lane
11:30–4:00	Council Meeting (New officers to arrive at 11:00)	Gaillard	Revolution 1st Floor
1:00-5:00	Exhibitors and Poster Set-up		Legends
6:00–8:00	WELCOME RECEPTION		Encore

DAY 2 (SUNDAY JUNE 15)

All meeting rooms are located on the 2nd floor unless otherwise noted.

TIME	EVENT	CHAIRS)	ROOM
8:00–5:30	REGISTRATION OPEN		Penny Lane
8:00-10:30	Exhibitor Set-up		Legends
9:00–10:30	**Kendric C Smith Symposium**: David Kaplan - "Spiders, Silk, and Light" Andy Yun - "Bio-optics: Enabling Photobiology in the Dark" Duncan Graham - "Biosensing of Molecules, Cells, and Tissue Using Metallic Nanoparticles and SERS"	Hasan	The Edge
10:30–11:00	BREAK WITH EXHIBITORS		Legends
11:00–12:30	**New Frontiers in the Development of Theranostic Agents**	Zheng/Lovell	Encore
11:00–12:30	**DNA Repair of Chromatin**	Johnson	Celebrate
11:00–12:35	**Joint ASP-ESP: Photodestruction of Parasites and Fungi**	Haidaris/Dai	The Edge
12:30–2:00	LUNCH		
2:00–3:40	**Joint ASP-ESP: Light-based Inactivation of Bacteria**	Faustino/Maisch	The Edge
2:00–3:30	**Low Level Light Therapy**	Hamblin	Celebrate
2:00–3:30	**Photochemical Properties of Metal-Organic and Nanoparticle Systems and Supramolecular Containers**	Greer	Encore
3:30–4:00	BREAK WITH EXHIBITORS		Legends
4:00–5:40	**New Probes and Approaches in Biological Optical Imaging**	Vinogradov/Lin	The Edge
4:00–5:30	**Nanotechnology for Photobiology**	Rege/Huang	Celebrate
4:00–6:00	**Structure-activity Relationships in DNA Photoproduct Formation and Repair**	Taylor	Encore
6:00–9:00	PRESIDENT'S PIZZA PARTY FOR ASSOCIATE MEMBERS	Gaillard	
7:00–10:00	EDITOR'S DINNER (by invitation)		

DAY 3 (MONDAY JUNE 16)

All meeting rooms are located on the 2nd floor unless otherwise noted.

TIME	EVENT	CHAIR(S)	ROOM
8:00–9:00	REGISTRATION OPEN		Penny Lane
8:00-1:30	Poster Set-up *(judging will begin after 1:30)*		Legends
9:00–10:40	**UVA/UVB Signaling in Skin Carcinogenesis**	He/Wu	The Edge
9:00–10:40	**Photoimmunology and PDT-induced Immunology**	Ullrich/Gollnick	Celebrate
9:00–10:40	**Optogenetics and Biotechnological Applications of Biological Photoreceptors**	Gärtner	Encore
10:30–11:00	BREAK WITH EXHIBITORS		Legends
11:00–12:30	**Channelrhodopsins & Molecular Responses**	Gärtner	Encore
11:00–12:40	**PDT Planning and Tumor Microenvironment**	Busch/Robinson	Celebrate
11:00–12:30	**UV-epigenetics—From DNA Damage Induction to Photocarcinogenesis**	Greinert/Rapp	The Edge
12:30–2:00	PAST PRESIDENTS LUNCH (by invitation)		Maryjane's 1st floor
12:30–2:00	LUNCH		
2:00–3:40	**Focusing Light on Stem Cells: Challenges for Imaging and PDT**	Selbo/Morgan	The Edge
2:00–3:30	**Small Molecule Modulators In Photsensitization pt. 1**	Maytin/Korbelik	Celebrate
2:00–3:30	**Interactions of UV & Other Stressors in the Survival of Extremophiles**	Connelly/Mitchell	Encore
3:30–4:00	BREAK WITH EXHIBITORS		Legends
4:00–5:40	**Death Pathways in Photodynamic Therapy**	Kessel/Oleinick	Encore
4:00–5:30	**Small Molecule Modulators In Photsensitization pt. 2**	Maytin/Korbelik	Celebrate
4:00–5:30	**Platform Session - Cellular Photobiology**	Black/Shirmanova	The Edge
6:00–9:00	POSTER SESSION (with refreshments)		Legends

DAY 4 (TUESDAY JUNE 17)

All meeting rooms are located on the 2nd floor unless otherwise noted.

TIME	EVENT	CHAIR(S)	ROOM
7:30–2:30	REGISTRATION OPEN		Penny Lane
8:30–9:30	**New Investigator Award Lecture**: Angel Marti & Kaushal Rege	Noonan	The Edge
9:30–10:30	**Research Award Lecture**: Juan "Tito" Scaiano	Noonan	The Edge
10:30–11:00	BREAK WITH EXHIBITORS		Legends
10:30–12:00	BUSINESS MEETING (with refreshments)		The Edge
12:00–2:00	GRANT-WRITING WORKSHOP WITH LUNCH (pre-registration required)	Busch/Turner	Encore
12:00–1:00	LUNCH		
1:00–2:30	**Theoretical and Clinical Validation of the Utility of Dosimetry: Pretreatment Planning and Online Treatment Monitoring**	Lilge/Kanick	Celebrate
1:00–2:30	**Platform Session - Targeted Photosensitization**	Samkoe	The Edge
1:00–2:30	**Platform Session - Photosensitizers and Contrast Agents**	Mari/Ohayon	Imagine
2:30–7:15	NETWORKING TIME		
7:15–10:00	BANQUET AND AWARDS CEREMONY		Harbor House

DAY 5 (WEDNESDAY JUNE 18)

All meeting rooms are located on the 2nd floor unless otherwise noted.

TIME	EVENT	CHAIR(S)	ROOM
8:00–5:30	REGISTRATION OPEN		Penny Lane
9:00–9:45	**Kendric C Smith Innovations Lecture**: David Boas: Optical Spectroscopy and Tomography of Oxygen Delivery—From Macro to Micro and Back	Hasan	The Edge
9:45–10:45	**Plenary Lecture:** Nobel laureate, Roger Tsien -"Cells in health and disease, seen mostly in pretty colors"	Lovell/Hasan	The Edge
10:45–11:00	BREAK WITH EXHIBITORS		Legends
11:00–12:00	**ASP President's Lecture**: Beth Gaillard	Forbes	The Edge
12:00–1:30	MENTOR LUNCH (pre-registration required)	Busch/Turner	Encore
12:00–2:00	LUNCH		
2:00–3:35	**Interrogating Disease with Light: Preclinical and Clinical Progress**	Lange/Mallidi	The Edge
2:00–3:35	**Vascular Effects of PDT & Interaction with Molecular-targeted Agents**	Chen/Nowak-Sliwinska	Imagine
2:00–3:30	**Sunscreens**	Lim/Noonan	Celebrate
3:30–4:00	BREAK WITH EXHIBITORS		Legends
4:00-5:00	Exhibitors and Posters Tear Down		Legends
4:00–5:30	**Light, Biology and Mechanics**	Scarcelli/Celli	The Edge
4:00–5:35	**UV & Melanoma / Pigment Cell Photobiology**	Noonan/De Fabo	Celebrate
4:00–5:40	**Distribution of UV Irradiance in the Environment: Models, Measurements, and Applications**	Streicher/Turner	Imagine

DAY 6 (THURSDAY JUNE 14)

All meeting rooms are located on the 2nd floor unless otherwise noted.

TIME	EVENT	CHAIR(S)	ROOM
8:00–11:00	REGISTRATION OPEN		Penny Lane
8:30–10:05	**Nitric Oxide as a PDT Modulator**	Girotti	Imagine
8:30–10:05	**Bidirectionally Informed Photobiology: Developing Relevant Preclinical Tools**	Cengel/Rizvi	The Edge
8:30–10:15	**DNA Structure, Photoproducts and Mutagenesis**	Drouin	Celebrate
10:00–10:30	BREAK		Abbey Road
10:30–12:10	**Photocleavable Materials in Health Sciences**	Almutairi	Imagine
10:30–12:00	**Dosimetry and Treatment Monitoring in Photobiology**	Cengel/Rizvi	The Edge
10:30–12:10	**Impact of Climate, Environment and Personal Factors on UVR Exposure and its Health Consequences**	Young	Celebrate
12:30–4:00	ASP Council Meeting with Lunch	Cengel	The Edge

General Information

Registration Desk Hours

Location: Penny Lane *(2nd Floor)*

Day	Time
Saturday June 14	10:00 a.m. – 8:00 p.m.
Sunday June 15	8:00 a.m. – 5:30 p.m.
Monday June 16	8:00 a.m. – 9:00 p.m.
Tuesday June 17	7:30 a.m. – 2:30 p.m.
Wednesday June 18	8:00 a.m. – 5:30 p.m.
Thursday June 19	8:00 a.m. – 11:00 a.m.

Morning Refreshments

Coffee, hot tea, and breakfast bars will be available near the registration desk each morning during the hour before presentations begin.

Sunday, Monday, and Wednesday from 8:00-9:00 a.m.
Tuesday and Thursday from 7:30-8:30 a.m.

Drink Ticket Key

There are two blue raffle tickets and two red raffle tickets tucked in each name badge. Now you know when to use them.

Blue tickets = Welcome Reception in Encore, Sunday June 15th 6-8 p.m.
Red tickets = Poster Session Reception in Legends, Monday June 16th 6-9 p.m.

*Drink tickets for the **Banquet & Awards Ceremony** at the Harbor House on Tuesday June 17th will be handed out at the door.*

Explore San Diego!

The San Diego Convention & Visitors Bureau will be available during Saturday evening's Welcome Reception to answer all of your questions about our host city. Whether you're looking for the best pizza in town or fresh seafood, this is where you'll find the answers. They can also recommend museums, local tours, outdoor adventures, and local shopping areas.

If you aren't able to connect with the San Diego CVB on Saturday night, no worries! City maps and visitor's guides will be available at the registration check-in desk.

Overall Conference hashtag: #ASP14

You can use this hashtag for all tweets/posts. However, for a specific session you can also use the following additional hashtags.

#KCSS	Session 1:	Kendric C. Smith Symposium
#BMCT	Duncan Graham	Biosensing of Molecules, Cells, and Tissue Using Metallic Nanoparticles and SERS
#BEPD	Andy Yun	Bio-optics: Enabling Photobilogy in the Dark
#SSL	David Kaplan	Spiders, Silk, and Light
#NFDTA	Session 2:	New Frontiers in the Development of Theranostic Agents
#DNARC	Session 3:	DNA Repair of Chromatin
#PPF	Session 4:	Joint ASP-ESP: Photodestruction of Parasites and Fungi
#LBIB	Session 5:	Joint ASP-ESP: Light-based Inactivation of Bacteria
#LLLT	Session 6:	Low Level Light Therapy
#PPMONSSC	Session 7:	Photochemical Properties of Metal-Organic and Nanoparticle Systems and Supramolecular Containers
#NPABOI	Session 8:	New Probes and Approaches in Biological Optical Imaging
#NFP	Session 9:	Nanotechnology for Photobiology
#SRDPFR	Session 10:	Structure-activity Relationships in DNA Photoproduct Formation and Repair
#UUSS	Session 11:	UVA/UVB Signaling in Skin Carcinogenesis
#PPDTI	Session 12:	Photoimmunology and PDT-induced Immunology
#OBAB	Session 13:	Optogenetics and Biotechnological Applications of Biological Photoreceptors
#CMR	Session 14:	Channelrhodopsins & Molecular Responses
#PDTPTM	Session 15:	PDT Planning and Tumor Microenvironment
#UVEPI	Session 16:	UV-epigenetics—From DNA Damage Induction to Photocarcinogenesis
#FLSC	Session 17:	Focusing Light on Stem Cells: Challenges for Imaging and PDT
#SMMP1	Session 18:	Small Molecule Modulators In Photsensitization pt. 1
#IUVOSSE	Session 19:	Interactions of UV & Other Stressors in the Survival of Extremophiles
#DPP	Session 20:	Death Pathways in Photodynamic Therapy
#SMMP2	Session 21:	Small Molecule Modulators In Photsensitization pt. 2
#PLAT1	Session 22:	Platform Session 1 - Cellular Photobiology
#TCVUD	Session 23:	Theoretical and Clinical Validation of the Utility of Dosimetry: Pretreatment Planning and Online Treatment Monitoring:
#PLAT2	Session 24:	Platform Session 2 - Targeted Photosensitization
#PLAT3	Session 25:	Platform Session 3 - Photosensitizers and Contrast Agents

Overall Conference hashtag: #ASP14

You can use this hashtag for all tweets/posts. However, for a specific session you can also use the following additional hashtags.

#IDL	Session 26:	Interrogating Disease with Light: Preclinical and Clinical Progress
#VEPDTIMA	Session 27:	Vascular Effects of PDT & Interaction with Molecular-targeted Agents
#SUNSCR	Session 28:	Sunscreens
#LBM	Session 29:	Light, Biology and Mechanics: Optical Methods in Tissue Mechanics and Mechanobiology
#UVMPCP	Session 30:	UV & Melanoma / Pigment Cell Photobiology
#DUVIE	Session 31:	Distribution of UV Irradiance in the Environment: Models, Measurements, and Applications
#NOPDTM	Session 32:	Nitric Oxide as a PDT Modulator
#BIP	Session 33:	Bidirectionally Informed Photobiology: Developing Relevant Preclinical Tools
#DNASPM	Session 34:	DNA Structure, Photoproducts and Mutagenesis
#PMHS	Session 35:	Photocleavable Materials in Health Sciences
#DTMP	Session 36:	Dosimetry and Treatment Monitoring in Photobiology: Hands on Demonstrations
#ICEPF	Session 37:	Impact of Climate, Environment and Personal Factors on UVR Exposure and its Health Consequences

#WELREC	Welcome Reception
#BQAC	Banquet & Awards Ceremony
#PPL	Past President's Lunch
#EDD	Editor's Dinner
#NIAL	New Investigator Award Lecture
#RAL	Research Award Lecture
#KCSIL	Kendric C. Smith Innovations Lecture
#PLEN	Plenary Lecture
#ASPPRES	ASP President's Lecture
#POSTSES	Poster Session
#GWW	Grant Writing Workshop
#BUSMTG	Business Meeting
#MTRL	Mentor Lunch

Saturday, June 14, 2014

Registration Open
June 14, 10:00am - 8:00pm **Penny Lane**

ASP Council Meeting & Luncheon
June 14, 11:30am - 4:00pm **Revolution** **Chair: Beth Gaillard**

New Council members to arrive at 11:00am

Welcome Reception
June 14, 6:00pm - 8:00pm **Encore**

Sunday, June 15, 2014

Registration
June 15, 8:00am - 5:30pm **Penny Lane**

Kendric C. Smith Symposium
June 15, 9:00am - 10:30am **The Edge** **Chair: Tayyaba Hasan**

SUN1
9:00am-9:30am
Biosensing of Molecules, Cells, and Tissue Using Metallic Nanoparticles and SERS
Duncan Graham
University of Strathclyde, Glasgow, UK

SUN2
9:30am-10:00am
Bio-Optics: Enabling Photobiology in the Dark
S. H. Andy Yun
Harvard Medical School, Cambridge, USA

SUN3
10:00am-10:30am
Spiders, Silk and Light
David Kaplan
Tufts University, Medford, MA, USA

Break with Exhibitors
June 15, 10:30am - 11:00am **Legends**

New Frontiers in the Development of Theranostic Agents
June 15, 11:00am-12:30pm **Encore** **Chair: Gang Zheng & Jonathan Lovell**

SUN4
11:00am-11:20am
Dark Materials for Molecular Imaging
Zhen Cheng
Stanford University, Stanford, USA

SUN5
11:20am-11:35am
Fluorescent and Singlet Oxygen-Activatable Conjugate of Photosensitizer and Anticancer Drug, Overcoming the Problems of Heterogeneity of PDT by Bystander Effect
Youngjae You, Moses Bio, Pallavi Rajaputra, Gregory Nkepang
OUHSC College of Pharmacy, Oklahoma City, OK, USA

SUN6
11:35am-11:55am
Image-Guided Cancer NanoTheranostics with Hollow Gold Nanospheres and CuS Nanoparticles
Chun Li
UT MD Anderson Cancer Center, Houston, TX, USA

SUN7
11:55am-12:10pm
Tumor-targeted, Activatable Photoimmunotherapy for Selective Destruction of Cancer Micrometastases
Bryan Spring[1], Adnan Abu-Yousif[1], Akilan Palanisami[1], Imran Rizvi[1], Xiang Zheng[1], Zhiming Mai[1], Sriram Anbil[1], R. Bryan Sears[1], Lawrence Mensah[1], Ruth Goldschmidt[1], S. Sibel Erdem[1], Esther Oliva[2], Tayyaba Hasan[1]
[1]Harvard Medical School, Boston, MA, USA, [2]Massachusetts General Hospital, Boston, MA, USA

SUN8
12:10pm-12:30pm
In Vivo Rapid Cancer Detection and Therapy Based on Rationally Designed Activatable Fluorescence and Photosensitizing Probes
Yasuteru Urano
The University of Tokyo, Tokyo, Japan

DNA Repair of Chromatin

June 15, 11:00am - 12:30pm Celebrate **Chair: David Johnson**

SUN9
11:00am-11:20am
Novel Cellular Activities Targeting UV Damage Recognition in Chromatin
Ling Zhang, Abigail Lubin, Hua Chen, Leah Nemzow, Feng Gong
University of Miami, Miami, FL, USA

SUN10
11:20am-11:35am
Absence of UV-induced Cancer in the Human Cornea; A Comparative Study of UV-induced Pyrimidine Dimers Repair and Cell Death in Human Corneal Epithelium and Epidermis
Justin D. Mallet, Marie-Catherine Drigeard Desgarnier, Sébastien P. Gendron, Patrick J. Rochette
Axe Médecine Régénératrice, Centre de Recherche FRQS du CHU de Québec, Hôpital du Saint-Sacrement and Centre LOEX de l'Université Laval, Québec, Qc, Canada

SUN11
11:35am-11:55am
E2F1 and RB Direct Histone Acetylation at Sites of DNA Damage
David Johnson, Renier Velez-Cruz, Swarnalatha Manickavinayaham, Anup Biswas, David Mitchell
University of Texas MD Anderson Cancer Center, Science Park, Smithville, Texas, USA

SUN12
11:55am-12:10pm
Impact of Iirradiating Skin Diploid Fibroblasts with Chronic Low Dose of UVB on Nucleotide Excision Repair
Marie-Catherine Drigeard Desgarnier[1], Régen Drouin[2], Patrick Rochette[1]
[1]Centre de recherche du CHU de Québec, Axe Médecine Régénératrice, Québec, Qc, Canada, [2]Laboratory of Transdisciplinary Research in Genetics, Medicines and Social Sciences,Division of Genetics, Department of Pediatrics, Faculty of Medicine and Health Sciences, Sherbrooke, Qc, Canada

SUN13
12:10pm-12:30pm
Retinoblastoma Protein Influences Susceptibility to DNA Damage via Chromatin Regulation
Ashby Morrison
Stanford University, Stanford, CA, USA

Joint ASP-ESP: Photodestruction of Parasites and Fungi

June 15, 11:00am - 12:35pm The Edge **Chair: Constantine Haidaris & Tianhong Dai**

SUN14
11:00am-11:25am
Antimicrobial Blue Light Against Skin and Soft Tissue Infections
Tianhong Dai, Yunsong Zhang, Rehab Amin, Michael Hamblin
Massachusetts General Hospital, Boston, MA, USA

SUN15
11:25am-11:40am
Photodynamic Control of Malaria Vector and Other Parasites in Infested African Swamps
Mahmoud Abdel Kader[1]
[1]German University in Cairo, 5th Settelment, New Cairo, Cairo, Egypt, [2]Cairo University, Cairo, Egypt

SUN16
11:40am-11:55am
Photodynamic Inactivation of Plant-pathogenic Fungi - So What is Stopping Us?
Henrique D. de Menezes[1], Gabriela B. Rodrigues[1], Simone de Pádua Teixeira[1], Nelson S. Massola Jr[2], Luciano Bachmann[1], Mark Wainwright[3], Gilberto U. L. Braga[1]
[1]Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil, [2]Universidade de São Paulo, Piracicaba, São Paulo, Brazil, [3]Liverpool John Moores University, Liverpool, UK

SUN17
11:55am-12:20pm
Carbon Flux Modulates the Sensitivity of the Pathogenic Fungus Candida albicans to PDT.
Constantine Haidaris
University of Rochester Medical Center, Rochester, NY, USA

SUN18
12:20pm-12:35pm
UVB Radiation Induces Both Beneficial and Deleterious Effects in a Localized Skin Infection with Mycobacterium Ulcerans in the Hairless Guinea Pigs
Amminikutty Jeevan[1], Vijaya Dirisala[1], Pam Small[2], Charlie Hoxmeier[3], Karen Dobos-Elder[3], Veronica Sanchez[1]
[1]Texas A&M Health Science Center, College Station, TX, USA, [2]University of Tennessee, Knoxville, USA, [3]Colorado State University, Fort Collins, USA

Lunch

June 15, 12:30pm - 2:00pm

Joint ASP-ESP: Light-based Inactivation of Bacteria

June 15, 2:00pm - 3:40pm **The Edge** **Chair: Maria Faustino & Tim Maisch**

SUN19
2:00pm-2:25pm

Bad Bugs – New Photosensitizers – No "ESKAPE" Against Antimicrobial PDT
Anja Eichner[1], Andreas Spaeth[2], Anita Gollmer[1], Fabian Cieplik[3], Tim Maisch[1]
[1]University Hospital, Department of Dermatology, Antimicrobial Photodynamic & Cold Plasma Research Unit, Regensburg, Germany, [2]Institute of Organic Chemistry, University of Regensburg, Regensburg, Germany, [3]Department of Operative Dentistry and Periodontology, University Hospital, Regensburg, Germany

SUN20
2:25pm-2:50pm

A Lipidomic Approach to Identify Minute Differences Among Staphylococcus aureus Strains. Possible Role in Antimicrobial Photoinactivation.
Joanna Nakonieczna[1], Weronika Hewelt-Belka[2], Michalina Filipiak[1]
[1]Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Gdansk, Poland, [2]Faculty of Chemistry, Gdansk University of Technology, Gdansk, Poland

SUN21
2:50pm-3:15pm

Polysaccharides and Photosensitizers: New Materials and Surfaces for Antimicrobial Photodynamic Therapy
Vincent SOL
University of Limoges, Laboratoire de Chimie des Substances Naturelles, Limoges, France

SUN22
3:15pm-3:40pm

Porphyrins In the Photodynamic Inactivation of Microorganisms Beyond the Medical Scope
Eliana Alves[2], Maria G P M S Neves[1], Angela Cunha[2], Adelaide Almeida[2], Maria A F Faustino[1]
[1]Department of Chemistry and QOPNA of University of Aveiro, Aveiro, Portugal, [2]Department of Biology and CESAM of University of Aveiro, Aveiro, Portugal

Low Level Light Therapy

June 15, 2:00pm - 3:30pm **Celebrate** **Chair: Michael Hamblin**

SUN23
2:00pm-2:20pm

Unblinded by the Light: Photobiomodulation in Retinal Injury and Disease
Janis Eells[1], Mahsa Ranji[1], Joseph Carroll[2], Sandeep Gopalakrishnan[1]
[1]University of Wisconsin-Milwaukee, Milwaukee, Wisconsin, USA, [2]Medical College of Wisconsin, Milwaukee, Wisconsin, USA

SUN24
2:20pm-2:30pm

Effects of Blue LED Light on Hemodynamic Parameters of Human Skin In Vitro and In Vivo
Christine M. Volkmar[1], Kim Kotte[1], Christian Opländer[1], Matthias Born[2], Jörg Liebmann[2], Joachim Windolf[1], Christoph V. Suschek[1]
[1]Department of Trauma and Hand Surgery, Medical Faculty of the Heinrich-Heine-University, Düsseldorf, Germany, [2]Innovative Technologies, Philips Technology GmbH, Aachen, Germany

SUN25
2:30pm-2:50pm

Can Near-infrared Light Induce the Brain to Heal Itself?
Michael Hamblin[1], Weijun Xuan[2], Liyi Huang[1], Fatma Vatansever[1]
[1]Massachusetts General Hospital, Boston, MA, USA, [2]Harvard Medical School, Boston, MA, USA, [3]Harvard-MIT Division of Health Science Techology, Cambridge, MA, USA

SUN26
2:50pm-3:10pm

Near Infrared Light-induced Protection of Heart During Reperfusion
Agnes Keszler, Christopher Hwe, Shelley Baumgardt, Martin Bienengraeber
Medical College of Wisconsin, Milwaukee, WI, USA

SUN27
3:10pm-3:30pm

Induction of Regulatory T cells by 670nm Light in a Model of Autoimmunity
Jeri-Anne Lyons
University of Wisconsin-Milwaukee, Milwaukee, WI, USA

Photochemical Properties of Metal-Organic and Nanoparticle Systems and Supramolecular Containers

June 15, 2:00pm - 3:30pm **Encore** **Chair: Alexander Greer**

SUN28
2:00pm-2:20pm
Learning from Nature - Supramolecular Photocatalysis Mediated By Cucurbiturils
Sivaguru Jayaraman, Barry Pemberton
North Dakota State University, Fargo, USA

SUN29
2:20pm-2:40pm
The Use of Metallic Nanoparticles to Enhance the Production of Singlet Oxygen
Belinda Heyne, Sara Mooi, Nicolas Macia
University of Calgary, Calgary, Alberta, Canada

SUN30
2:40pm-2:50pm
Photosensitization in Drug - Cucurbit[n]uril - protein Ternary Complexes
Denis Fuentealba, Karina Scholtbach, Ítalo Venegas
Pontificia Universidad Católica de Chile, Santiago, Chile

SUN31
2:50pm-3:10pm
Moving Metal-Based Photosensitizers for Photodynamic Therapy from Concept to Reality
Sherri McFarland, Susan Monro, Huimin Yin, Ge Shi, Jordan Gibson, Mat Stephenson, Tariq Sainuddin
Acadia University, Wolfville, NS, Canada

SUN32
3:10pm-3:30pm
Reactions of Singlet Oxygen with Metal Thiolates
Dong Zhang, Lorillee Tallorin, Blanca Hernandez, Matthias Selke
California State University, Los Angeles, Los Angeles, CA, USA

Break with Exhibitors

June 15, 3:30pm - 4:00pm **Legends**

New Probes and Approaches in Biological Optical Imaging

June 15, 4:00pm - 5:40pm **The Edge** **Chair: Sergei Vinogradov & Charles Lin**

SUN33
4:00pm-4:20pm
Tools for High Resolution Optical Imaging of Neuronal, Glial, Vascular, and Metabolic Activity for Neuroscience Studies In Vivo
Anna Devor , UCSD, La Jolla, USA

SUN34
4:20pm-4:40pm
Bright Porphyrin Phosphors and Click-Assembled Dendrimers: A Modular Platform for Tissue Oxygen Tension Imaging
Conor Evans[1], Emmanouil Rousakis[1], Alexander Nichols[1], Benjamin Sun[2], Oliver Klein[1]
[1]Wellman Center for Photomedicine / Harvard Medical School, Boston, MA, USA, [2]Harvard University, Cambridge, MA, USA

SUN35
4:40pm-5:00pm
Engineering of Bacterial Phytochromes for In Vivo Imaging.
Vladislav Verkhusha
Albert Einstein College of Medicine, Bronx, NY 10461, USA

SUN36
5:00pm-5:20pm
Two-photon Microscopy with Continuous Wave Laser Sources and Upconverting Nanoprobes
Sergei Vinogradov
University of Pennsylvania, Philadelphia, PA, USA

SUN37
5:20pm-5:40pm
Direct Measurement of Local Oxygen Concentration in the Bone Marrow of Live Animals by Two-photon Phosphorescence Lifetime Microscopy
Charles Lin
Massachusetts General Hospital, Boston, MA, USA

Nanotechnology for Photobiology

June 15, 4:00pm - 5:30pm **Celebrate** **Chair: Kaushal Rege & Huang Chiao Huang**

SUN38
4:00pm-4:20pm
Near Infrared Laser-tissue Welding Using Plasmonic Nanocomposite as a Photothermal Converter
James Ramos[1], Huang Chiao Huang[2], Kaushal Rege[1]
[1]Arizona State University, Tempe, AZ, USA, [2]Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

SUN39
4:20pm-4:40pm
Nuclear Delivery of Photo Immunoconjugates
Sijia Wang[1], Shifalika Tangutoori[2], Gereon Hüttmann[1], Tayyaba Hasan[2], Ramtin Rahmanzadeh[1]
[1]University of Lübeck, Lübeck, Germany, [2]Massachusetts General Hospital, Boston, USA

SUN40
4:40pm-5:00pm
Photoimmunotherapy; Basis, Applications and Beyond
Hisataka Kobayashi
NCI/NIH, Bethesda, USA

SUN41
5:00pm-5:10pm
Nanobody-photosensitizer Conjugates for Targeted Photodynamic Therapy
Raimond Heukers, Paul van Bergen en Henegouwen, Sabrina Oliveira
Utrecht University, Utrecht, The Netherlands

SUN42
5:10pm-5:30pm
In Vivo Evaluation of Nanoliposomal Photochemotherapy for Pancreatic Cancer
Huang Chiao Huang, Srivalleesha Mallidi, Imran Rizvi, Zhiming Mai, Chun Te Chiang, Joyce Liu, Dmitriy Timerman, Tayyaba Hasan
Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Structure-activity Relationships in DNA Photoproduct Formation and Repair

June 15, 4:00pm - 6:00pm **Encore** **Chair: John Taylor**

SUN43
4:00pm-4:30pm
Ultrafast Spectroscopy of DNA: Connecting Excited States and Photoproducts
Bern Kohler, Yuyuan Zhang, Ashley Beckstead, Jordan Dood
Montana State University, Bozeman, MT, USA

SUN44
4:30pm-5:00pm
Repair of the (6-4) Photoproduct by its DNA Photolyase: Experimental Evidence for a Two-photon Mechanism
Klaus Brettel
UMR 8221 (CEA-iBiTecS, CNRS, Univ Paris-Sud), Gif-sur-Yvette, France

SUN45
5:00pm-5:30pm
Photochemistry of G-quadruplex Forming Sequences in Telomeric and Promoter DNA
Jillian Smith, Chen Lu, John-Stephen Taylor, Washington University, St. Louis, USA

SUN46
5:30pm-5:45pm
Unraveling the Potential of Sulfur-Substituted DNA and RNA Bases as UVA Photosensitizers
Marvin Pollum, Carlos E. Crespo-Hernández
Case Western Reserve University, Cleveland, OH, USA

SUN47
5:45pm-6:00pm
Impact of the Methylation Site of Cytosine on the Formation of Bipyrimidine Photoproducts
Thierry DOUKI[1], Jarah MEADOR[2], Aude WACK[1], Izabel BERARD[1]
[1]CEA / UJF-Grenoble 1, INAC/SCIB UMR E3, Grenoble, France, [2]Center for Radiological Research, Columbia University, New York, NY, USA

President's Pizza Party for Associate Members

June 15, 6:00pm-9:00pm **BASIC Pizza & Bar** **Chair: Elizabeth Gaillard**

Editor's Dinner (By Invitation)

June 15, 7:00pm - 10:00pm

Monday, June 16, 2014

Registration

June 16, 8:00am - 9:00pm **Penny Lane**

UVA/UVB Signaling in Skin Carcinogenesis

June 16, 9:00am - 10:40am **The Edge** **Chair: Yu-Ying He & Shiyong Wu**

MON1
9:00am-9:25am

Mechanism Of Action Of Prohibitin In Regulation Of UVB-induced Apoptosis
Shiyong Wu, Qiong Wu
Ohio University, Athens, Ohio, USA

MON2
9:25am-9:50am

Effects of the Pharmacological Inhibition of Macrophage Inhibitory Factor on Ultraviolet Light Induced Inflammation and Tumor Development.
Priyadharsini Nagarajan, Kathleen Tober, Abhay Satoskar, Tatiana Oberyszyn
The Ohio State University, Columbus, OH, USA

MON3
9:50am-10:15am

Dual Role of SIRT1 in UVB-induced Skin Tumorigenesis
Mei Ming[1], Keyoumars Soltani[1], Christopher Shea[1], Xiaoling Li[2], Yu-Ying He[1]
[1]University of Chicago, Chicago, IL, USA, [2]NIH/NIEHS, Research Triangle Park, NC, USA

MON4
10:15am-10:40am

Roles of C/EBP Family Transcription Factors in UV-Induced Carcinogenesis
Sanjay Anand[1], Kishore Rollakanti[1], Nikoleta Brankov[1], Edward Maytin[2]
[1]Department of Biomedical Engineering, Cleveland Clinic, Cleveland, OH, USA, [2]Department of Dermatology, Cleveland Clinic, Cleveland, OH, USA

Photoimmunology and PDT-induced Immunology

June 16, 9:00am - 10:40am **Celebrate** **Chair: Stephen Ullrich & Sandra Gollnick**

MON5
9:00am-9:20am

UV-induced Platelet Activating Factor Activates Systemic Immune Suppression
Stephen Ullrich
UT MD Anderson Cancer Center, Houston, Texas, USA

MON6
9:20am-9:40am

UV Radiation-induced DNA Hypermethylation Promotes Immunosuppression in UV Exposed Mice
Santosh Katiyar[1], Ram Prasad[1]
[1]University of Alabama at Birmingham, Birmingham, AL, USA, [2]Birmingham VA Medical Center, Birmingham, AL, USA

MON7
9:40am-10:00am

Photodynamic Therapy Can Induce a Non-specific Protective Immune Response Against a Bacterial Infection
Michael Hamblin[1], Masamitsu Tanaka[4], Pawel Mroz[2]
[1]Massachusetts General Hospital, Boston, MA, USA, [2]Harvard Medical School, Boston, MA, USA, [3]Harvard-MIT Division of Health Science Techology, Cambridge, MA, USA, [4]Department of Integrated Physiology and Bio-Nano Medicine, National Defense Medical College, Tokorozawa,, Saitama, Japan

MON8
10:00am-10:20am

Photodynamic therapy induced immune response towards tumor antigens.
Pawel Mroz[1], Michael Hamblin[0]
[1]Department of Pathology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA, [2]Wellman Center for Photomedicine, Mass. General Hospital, Harvard Medical School, Boston, MA, USA

MON9
10:20am-10:40am

Intraoperative Photodynamic Therapy for Malignant Pleural Mesothelioma – Evidence Suggesting a Positive Immunologic Effect
Joseph Friedberg
University of Pennsylvania, Philadelphia, PA, USA

Optogenetics and Biotechnological Applications of Biological Photoreceptors

June 16, 9:00am - 10:40am **Encore** **Chair: Wolfgang Gärtner**

MON10
9:00am-9:25am
Biological Photoreceptors As Tools In Superresolution Microscopy and Optogenetics Applications
Wolfgang Gärtner
Max-Planck-Institute Chem. Energy Conversion, Mülheim, Germany

MON11
9:25am-9:50am
Increasing the Light-sensitivity of Lov2 Domain-based Optogenetic Tools
Svetlana Usherenko, Hilke Stibbe, Lars-Oliver Essen, Ekaterina Kostina, Christof Taxis
Philipps-Universität Marburg, Marburg, Germany

MON12
9:50am-10:15am
Molecular Properties of Channelrhodopsin and Their Impact on Optogenetics
Christian Bamann[1], Thomas Sattig[1], Christian Rickert[2], Heinz-Jürgen Steinhoff[2], Ernst Bamberg[1]
[1]Max Planck Institute of Biophysics, Frankfurt, Germany, [2]University of Osnabrück, Osnabrück, Germany

MON13
10:15am-10:40am
Channelrhodopsin et al.: Photoreceptors for Optogenetic Applications
Georg Nagel
University Wuerzburg, Wuerzburg, Bavaria, Germany

Break with Exhibitors

June 16, 10:30am - 11:00am **Legends**

Channelrhodopsins & Molecular Responses

June 16, 11:00am - 12:30pm **Encore** **Chair: Wolfgang Gärtner**

MON14
11:00am-11:25am
A New Cryptochrome-based Optogenetic Tool for Probing Protein Interaction and Function
Amir Taslimi, Justin Vrana, Daniel Chen, Matthew Kennedy, Chandra Tucker
University of Colorado Denver, Colorado, USA

MON15
11:25am-11:50am
Engineered Microbial Rhodopsins for All-optical Electrophysiology
Daniel Hochbaum[1], Yongxin Zhao[2], Sami Farhi[1], Nathan Klapoetke[3], Ed Boyden[3], Robert Campbell[2], Adam Cohen[1]
[1]Harvard, Cambridge, MA, USA, [2]University of Alberta, Alberta, Canada, [3]MIT, Cambridge, MA, USA

MON16
11:50am-12:05pm
Use of Hypothermia During PDT Treatment of Malignant Glioma
Carl Fisher[1], Carolyn Nui[2], Lothar Lilge[2]
[1]University of Toronto, Toronto, Ontario, Canada, [2]University Health Network, Toronto, Ontario, Canada

MON17
12:05pm-12:30pm
Cell Type-specific Optogenetic Vision Restoration Strategies
Volker Busskamp
Harvard Medical School, Boston, MA, USA

PDT Planning and Tumor Microenvironment

June 16, 11:00am - 12:40pm **Celebrate** **Chair: Theresa Busch & Dominic Robinson**

MON18
11:00am-11:20am
Combination Therapy Incorporating PDT
Charles Gomer[1], Angela Ferrario[2], Marian Luna[2], Natalie Rucker[2]
[1]University of Southern California, Los Angeles, CA, USA, [2]Children's Hospital Los Angeles, Los Angeles, CA, USA

MON19
11:20am-11:40am

Novel Ways of Targeting the Tumor Vasculature
Arjan W. Griffioen[1], Judy R. van Beijnum[1], Patrycja Nowak-Sliwinska[2]
[1]Angiogenesis Laboratory, Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands, [2]Institute of Chemical Sciences and Engineering, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland

MON20
11:40am-12:00pm

Studying the Effects of Photodynamic Therapy on Tumor Oxygenation and Blood Flow Toward Better Treatment
Theresa Busch
University of Pennsylvania, Philadelphia, PA, USA

MON21
12:00pm-12:20pm

Evaluating the Roles of Stromal Rheology and Heterotypic Cross-talk in the Pancreatic Tumor Microenvironment to Inform PDT Treatment Strategies
Dustin Jones[1], William Hanna[1], Gwendolyn Cramer[1], Ljubica Petrovic[1], Hamid El-Hamidi[1], Imran Rizvi[2], Tayyaba Hasan[2], Jonathan Celli[1]
[1]University of Massachusetts, Boston, MA, USA, [2]Massachusetts General Hospital, Boston, MA, USA

MON22
12:20pm-12:40pm

Treatment Planning and Microenvironment in PDT of Head and Neck Cancer
Dominic Robinson[1], Floor van Leeuwen – van Zaane[1], Pieter van Driel[2], Thomas Snoeks[2], Henriette de Bruijn[1], Angelique van der Ploeg – van den Heuvel[1], Arjen Amelink[1], Clemens Lowik[2]
[1]Erasmus University Medical Center, Rotterdam, The Netherlands, [2]Leiden University Medical Centre, Leiden, The Netherlands

UV-epigenetics—From DNA Damage Induction to Photocarcinogenesis

June 16, 11:00am - 12:30pm **The Edge** **Chair: Ruediger Greinert & Alexander Rapp**

MON23
11:00am-11:30am

Genome-wide Repair Kinetics of UVC Induced CPDs and Correlation to Epi-genetic Chromatin States
Alexander Rapp, Wei Yu, M. Cristina Cardoso
TU Darmstadt, Department of Biology, Cellbiology and Epigenetics, Darmstadt, Germany

MON24
11:30am-12:00pm

Acute Exposure to Solar UV drives the Cutaneous Formation of Photodamage-associated Protein Epitopes That Are Prevalent in Melanoma and Nonmelanoma Skin Cancer
Joshua Williams[3], Yira Bermudez[2], Amit Patel[2], Georg Wondrak[4]
[1]The University of Arizona, Tucson, AZ, USA, [2]The University of Arizona Cancer Center, Tucson, AZ, USA, [3]Department of Biomedical Engineering, Tucson, AZ, USA, [4]College of Pharmacy, Tucson, AZ, USA

MON25
12:00pm-12:30pm

UV-induced Epigenetic Alterations in Human Keratinocytes - From DNA Damage Induction to Skin Cancer
Ruediger Greinert, Beate Volkmer, I Peng Chen, Faust Alexandra, Henning Stefan
Elbekliniken Stade/Buxtehude, Research Center, Dept. Mol. Cell Biology, Buxtehude, Germany

Past President's Lunch (By Invitation)

June 16, 12:30pm - 2:00pm **Satisfaction**

Lunch

June 16, 12:30pm - 2:00pm

Focusing Light on Stem Cells: Challenges for Imaging and PDT

June 16, 2:00pm - 3:40pm **The Edge** **Chair: Pål Selbo & Janet Morgan**

MON26
2:00pm-2:25pm

Enhancing Photodynamic Therapy by Regulating ABCG2 Expression and Activity in Cancer Cell Side Populations.
Janet Morgan, University of Buffalo, Buffalo, USA

MON27
2:25pm-2:50pm

In Vivo Imaging of Normal Stem Cells
Charles Lin
Massachusetts General Hospital, Boston, MA, USA

MON28
2:50pm-3:15pm

Specific and Efficient Targeting of Cancer Stem Cells by Photochemical Internalization
Pål Selbo[1], Monica Bostad[1], Marius Eng[1], Anders Høgset[2], Kristian Berg[1]
[1]*Norwegian Radium Hospital, Oslo, Norway,* [2]*PCI Biotech, Lysaker, Norway*

MON29
3:15pm-3:40pm

Stem Cells, T Cells, and Selective Targeting with PDT—The Role of the Amide-thioamide 'Switch' in Rhodamine Photosensitizers in P-gp Expressing Cells
Michael Detty[1], Kellie Davies[1], Michelle Linder[1], Jackie Hill[1], Mark Kryman[1], Gregory Schamerhorn[1], Tymish Ohulchanskyy[1], Janet Morgan[2], Zachary McIver[3]
[1]*University at Buffalo, Buffalo, NY, USA,* [2]*Roswell Park Cancer Institute, Buffalo, NY, USA,* [3]*Wake Forest University, Winston-Salem, NC, USA*

Small Molecule Modulators In Photsensitization Part 1

June 16, 2:00pm - 3:30pm **Celebrate** **Chair: Edward Maytin & Mladen Korbelik**

MON30
2:00pm-2:25pm

LCL521, Sphingolipid Metabolism Modulator, is a Potent Enhancer of Antitumor Effect of Photodynamic Therapy
Mladen Korbelik[1], Judit Banath[1], Zdzislaw Szulc[2], Alicja Bielawska[2], Duska Separovic[3]
[1]*British Columbia Cancer Agency, Vancouver BC, Canada,* [2]*Medical University of South Carolina, Charleston SC, USA,* [3]*Wayne State University, Detroit MI, USA*

MON31
2:25pm-2:45pm

Ceramide-generating Drugs Enhance Cancer Cell Killing After PDT
Duska Separovic[1], Nithin Boppana[1], Mladen Korbelik[2]
[1]*Wayne State University, Detroit, MI, USA,* [2]*British Columbia Cancer Agency, Vancouver, BC, Canada*

MON32
2:45pm-3:00pm

Histone Acetyltransferase p300 Involves in Autophagy induced by Photodynamic Therapy and Is a Target to Improve PDT Response
chintin chen[1], Yi-Chen tsai[1], tsuimin tsai[2], hsiung-fei chien[1]
[1]*National Taiwan University, Taipei, Taiwan,* [2]*Taipei Medical University, Taipei, Taiwan*

MON33
3:00pm-3:15pm

Combination of Oral Vitamin D3 with Photodynamic Therapy Enhances Tumor Cell Death in a Murine Model of Cutaneous Squamous Cell Carcinoma
Sanjay Anand[1], Kishore Rollakanti[1], Tayyaba Hasan[3], Edward Maytin[2]
[1]*Department of Biomedical Engineering, Cleveland Clinic, Cleveland, OH, USA,* [2]*Department of Dermatology, Cleveland Clinic, Cleveland, OH, USA,* [3]*Wellman Center for Photomedicine, Harvard Medical School, Boston, MA, USA*

MON34
3:15pm-3:30pm

The Tryptophan Photoproduct and Endogenous AhR-ligand 6-formylindolo[3,2-b]carbazole (FICZ) is a Nanomolar UVA- and Visible Light-activated Photosensitizer in Epidermal Keratinocytes and Reconstructed Human Skin
Sophia L. Park, Justiniano Rebecca, Christopher M. Cabello, Joshua D. Williams, Shuxi Qiao, Georg T. Wondrak
Department of Pharmacology and Toxicology, College of Pharmacy, College of Engineering, & Arizona Cancer Center, University of Arizona, Tucson, AZ, USA

Interactions of UV & Other Stressors in the Survival of Extremophiles

June 16, 2:00pm - 3:30pm **Encore** **Chair: Sandra Connelly & David Mitchell**

MON35
2:00pm-2:30pm

Life and UV in Yellowstone: As if Boiling Acid and Arsenic Were Not Enough
Tim McDermott[1], David Mitchell[2], Ted Weatherwax[1], Jill Wilconson[1], John Schroeder[1]
[1]*Institute on Ecosystems, Montana State University, Bozeman, MT, USA,* [2]*Department of Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Smithville, TX, USA*

MON36
2:30pm-2:45pm

Enhanced Cold Resistance of Zoysiagrass Cultures Through Overexpression of Wild Type and Ser599Ala-mutant Phytochrome A Genes
Markkandan Ganesan[1], Mayank Anand Gururani[3], Jeong Il Kim[2], Hyo Yeon Lee[3], Pill Soon Song[3]
[1]Department of Biological Sciences, Presidency University, Kolkata, West Bengal, India, [2]Department of Biotechnology and Kumho Life Science Laboratory, Chonnam National University, Gwangju, Republic of Korea, [3]Subtropical Horticulture Research Institute and Faculty of Biotechnology, Jeju National University, Jeju, Republic of Korea

MON37
2:45pm-3:00pm

Extreme Resistance of Geodermatophilus Obscurus and Hymenobacter Gelipurpurascens to UV-C Irradiation
Ivan Glaucio Paulino Lima, Lynn Rothschild
NASA Ames Research Center, Moffett Field, USA

MON38
3:00pm-3:30pm

The Fine Structure of DNA Damage in Marine Microbial Communities; Geographical and Temporal Distribution Along a Latitudinal Transect in the Pacific Ocean
Jarah Meador[1], Amy Baldwin[2], Joseph Pakulski[2], Wade Jeffrey[2], David Mitchell[3], Thierry Douki[4]
[1]Columbia University, New York, New York, USA, [2]University of West Florida, Pensacola, Florida, USA, [3]University of Texas MD Anderson Cancer Center, Smithville, Texas, USA, [4]Universite Joseph Fourier, Grenoble, France

Break with Exhibitors
June 16, 3:30pm - 4:00pm **Legends**

Death Pathways in Photodynamic Therapy
June 16, 4:00pm - 5:40pm **Encore** **Chair: David Kessel & Nancy Oleinick**

Introduction - Nancy Oleinick 4:00pm-4:05pm

MON39
4:05pm-4:30pm

The Role of Autophagy-related Proteins [ATGs] in the Efficacy of Photodynamic Therapy
David Kessel
Wayne State University, Detroit, Michigan, USA

MON40
4:30pm-4:45pm

Increased PDT Efficacy When Associated With Nitroglycerin. A study on Retinoblastoma Xenografted on Mice.
Carole D. Thomas[2], Florent Poyer[1], Philippe Maillard[3], Mihaela Lupu[1]
[1]Institut Curie, Orsay, France, [2]Inserm U759, Orsay, France, [3]Cnrs UMR176, Orsay, France

MON41
4:45pm-5:00pm

Enhanced Efficacy of Photodynamic Therapy (PDT) via an Iron-Lysosome-Mitochondria Connection: Studies with Pc 4 and Dual Responsive Nanoparticles
Hsin-I Hung[1], Justin Schwartz[1], Huacheng He[2], Peisheng Xu[2], John Lemasters[1], Anna-Liisa Nieminen[1]
[1]Medical University of South Carolina, Charleston, USA, [2]University of South Carolina, Columbia, USA

MON42
5:00pm-5:15pm

Photo-activated Psoralen Binds the ErbB2 Catalytic Kinase Domain, Blocking ErbB2 Signaling and Triggering Tumor Cell Apoptosis
Wenle Xia[1], David Gooden[3], Leihua Liu[2], Sumin Zhao[2], Erik Soderblom[6], Eric Toone[3], Wayne Beyer[5], Harold Walder[4], Neil Spector[1]
[1]Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA, [2]Duke Cancer Institute, Duke University Medical Center, Durham, NC 27710, USA, [3]Department of Chemistry, Duke University, Durham, NC 27710, USA, [4]Immunolight ,LLC, Detroit, MI 48226, USA, [5]QNS Group, LLC, Bahama, NC 27503, USA, [6]Proteomic Core Facility Duke University, Durham, NC 27710, USA

MON43
5:15pm-5:40pm

Integrin-Targeted, PEG-Enhanced Photosensitizer Constructs for Lysosome-Mediated Cell Death
Oliver Klein[1], Hushan Yuan[2], Lee Josephson[3], Conor Evans[1]
[1]Wellman Center for Photomedicine, MGH, Boston, MA, USA, [2]Center for Translational Nuclear Medicine and Molecular Imaging, MGH, Boston, MA, USA, [3]Martinos Center for Biomedical Imaging, MGH, Boston, MA, USA

Small Molecule Modulators In Photsensitization Part 2

June 16, 4:00pm - 5:30pm **Celebrate** **Chair: Edward Maytin & Mladen Korbelik**

MON44
4:00pm-4:25pm

Improving Tumor Responses to Photodynamic Therapy by Pretreatment with Small Molecule Enhancers of Cellular Differentiation
Edward Maytin
Cleveland Clinic, Cleveland, OH, USA

MON45
4:25pm-4:40pm

Using Coordination Chemistry to Develop Light-activated Anticancer Agents
Edith (Phoebe) Glazer, David Heidary, Brock Howerton, Erin Wachter, Yang Sun
University of Kentucky, Lexington, KY, USA

MON46
4:40pm-5:00pm

UV and Vitamin D: What Are We Aiming for and What Are We Achieving?
Mark Farrar, Ann Webb, Richard Kift, Jacqueline Berry, Lesley Rhodes
University of Manchester, Manchester, UK

MON47
5:00pm-5:15pm

Vitamin D Pretreatment Enhances the Therapeutic Efficacy of Aminolevulinate Based Photodynamic Therapy in Basal Cell Carcinoma Model
Kishore Reddy Rollakanti[1], Sanjay Anand[2], Edward Maytin[2]
[1]Cleveland State University, Cleveland, Ohio, USA, [2]Cleveland Clinic, Cleveland, Ohio, USA

MON48
5:15pm-5:30pm

ALA-mediated PDT Induces Vascular Response and Photobleaching in Superficial Oral Cavity Lesions
Jarod Finlay[1], Shannon Gallagher-Colombo[1], Harry Quon[2], Peter Ahn[1], Kelly Malloy[3], Theresa Busch[1]
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]Johns Hopkins University, Baltimore, MD, USA, [3]University of Michigan, Ann Arbor, MI, USA

Platform Session - Cellular Photobiology

June 16, 4:00pm - 5:30pm **The Edge** **Chair: Homer Black & Marina Shirmanova**

MON49
4:00pm-4:30pm

Role of Nutritional Lipids and Antioxidants in UV-carcinogenesis
Homer Black
Baylor College of Medicine, Houston, Texas, USA

MON50
4:30pm-4:45pm

Photoreactivity of Human Retinal Lipid Extracts From Different Age Groups.
Anna Maria Pawlak[1], Agnieszka Broniec[1], Andrzej Zadlo[1], Mariusz Duda[1], Olivier Berdeaux[2], Stephane Gregoire[2], Lionel Bretillon[2], Tadeusz Sarna[1]
[1]Dept. of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland, [2]INRA, Centre des Sciences du Gout et de l'Alimentation, Universite de Bourgogne, Dijon, France

MON51
4:45pm-5:00pm

Blue-light (420–453 nm) Induced Non-enzymatic Nitric Oxide Generation From Photolabile Nitric Oxide Derivates in Human Skin In Vitro and In Vivo
Joerg Liebmann[1], Christian Oplaender[2], Matthias Born[1], Christine Volkmar[2], Christoph V. Suschek[2]
[1]Philips Technologie GmbH, Innovative Technologies, Aachen, Germany, [2]Department of Trauma and Hand Surgery, Medical Faculty, University Hospital, Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany

MON52
5:00pm-5:15pm

Measurement of Intracellular pH in Cancer Cells In Vivo Using New Genetically Encoded Indicator
Marina Shirmanova[1], Irina Druzhkova[1], Maria Lukina[1], Vsevolod Belousov[2], Natalia Prodanetz[1], Ludmila Snopova[1], Sergey Lukyanov[2], Elena Zagaynova[1]
[1]Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russia, [2]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia

MON53
5:15pm-5:30pm

Environmental UV-mediated Photomodification and DNA Damage Induced Apoptosis by Benz(a)anthracene via Mitochondrial Mediated Pathway
Syed Faiz Mujtaba, Ashish Dwivedi, Neera Yadav, Ajeet K. Srivastav, Ratan S. Ray
Photobiology Division, CSIR-Indian Institute of Toxicology Research, Lucknow, U.P, India

Poster Session with Refreshments

June 16, 6:00pm - 9:00pm **Legends**

POS1
6:00pm-9:00pm

Towards Prevention of Infectious Diseases: Microbial Control of Wastewater by Photoactivated ZnO Nanoparticles
Kristina Aponiene[1], Tomas Serevicius[2], Zivile Luksiene[1]
[1]Vilnius University Institute of Applied Research, Vilnius, Lithuania, [2]Vilnius University Faculty of Physics, Vilnius, Lithuania

POS2
6:00pm-9:00pm

Dendrimeric-like Hexadecahydroxylated Zinc Phthalocyanine. Synthesis And in Vitro Evaluation of Photodynamic Efficiency.
Serkan Alpugan[1], Guillaume Garcia[2], Florent Poyer[2], Mahmut Durmus[1], Philippe Maillard[2], Vefa Ahsen[1], Fabienne Dumoulin[1], Guillaume Garcia[3], Guillaume Garcia[4], Guillaume Garcia[5], Florent Poyer[4], Florent Poyer[5], Florent Poyer[6], Philippe Maillard[3], Philippe Maillard[4], Philippe Maillard[5]
[1]Gebze Institute of Technology, Department of Chemistry, P.O. Box 141, Gebze, 41400, Kocaeli, Gebze, Turkey, [2]Institut Curie, Section de Recherche, Bât 110-112, Centre Universitaire, F-91405, Orsay, France, [3]UMR 176 CNRS, Bât 110, Centre Universitaire, F-91405, Orsay, France, [4]Université Paris-Sud, Centre Universitaire, F-91405, Orsay, France, [5]CNRS GDR 3049 PHOTOMED, UMR 5623 Université Paul Sabatier, F-31062, Toulouse, France, [6]U759 INSERM, Bât 112, Centre Universitaire, F-91405, Orsay, France

POS3
6:00pm-9:00pm

UVB Radiation Increases MCPIP-1 Expression in HaCaT Cells.
Beata Bugara[1], Marta Smejda[2], Leopold Eckhart[3], Elzbieta Boratyn[4], Piotr Konieczny[1], Jolanta Jura[1], Agnieszka Wolnicka-Glubisz[2]
[1]Jagiellonian University, Department of General Biochemistry, Kraków, Poland, [2]Jagiellonian University, Department of Biophysics, Kraków, Austria, [3]Medical University of Vienna, Department of Dermatology, Vienna, Austria, [4]Jagiellonian University, Laboratory of Molecular Genetics and Virology, Kraków, Poland

POS4
6:00pm-9:00pm

UV-Stressed *Daphnia pulex* and Freshwater Algal Species Increase Fitness Through Uptake of Vitamin D
Sandra Connelly[1], Kelly Walling[1], Steven Wilbert[1], Diane Catlin[1], Cailin Monaghan[1], Sofiya Hlynchuk[1], Pamela Meehl[1], Lauren Resch[1], J. Valerie Carrera[1], Stephanie Bowles[1], Michael Clark[1], Zachary Kopp[1], Rob Keith[1], Loraine Tan[2], Jeremy Cody[1]
[1]Rochester Institute of Technology, Rochester, NY, USA, [2]Ramapo College of New Jersey, Mahwah, NJ, USA

POS5
6:00pm-9:00pm

Hair Dye Induced DNA Damage and Differential Protein Expression in Human Keratinocyte Under Environmental UV Radiation
SHRUTI GOYAL[1], SAROJ KUMAR AMAR[1], SYED FAIZ MUJTABA[1], R.S. RAY[1]
[1]CSIR-IITR, LUCKNOW, India, [2]AcSIR, DELHI, India

POS6
6:00pm-9:00pm

Oxidative Stress Mediated Apoptosis and Identification of Marker Proteins by Benzophenone Under Environmental UV Radiation
Saroj Kumar Amar[1], Shruti Goyal[1], Faiz Mujtaba[1], Divya Dubey[1]
[1]CSIR Indian institute of toxicology research, Lucknow, India, [2]AcSIR Delhi, delhi, India

POS7
6:00pm-9:00pm

DRPDT2: A New Compound to Improve Photodynamic Therapy
Emilia Della Pietra[1], Greta Varchi[2], Benjamin Bonavida[3], Luigi E Xodo[1], Valentina Rapozzi[1]
[1]Department of Medical and Biological Science, University of Udine, Udine, Italy, [2]National Research Council Institute for Organic Syntheses and Photoreactivity ISOF, Bologna, Italy, [3]Dep. of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, Jonsson Comprehensive Cancer Center, University of California Los Angeles, Los Angeles, USA

POS8
6:00pm-9:00pm

Differences in Expression of Genes Controlling Metabolic Equipment of Co-cultured Human Melanocytes and Keratinocytes. Modulation by Solar UV or H2O2 Exposure.
Laurence Denat, Maureen Dutordoir, Christophe Jones, Yohann Phalente, Laurent Marrot
L'OREAL R&I, Aulnay sous Bois, France

POS9
6:00pm-9:00pm

Comparative Ttudy of In-vitro Photodynamic Effect of Free and Liposome-encapsulated Chlorophyll Derivative in De-pigmented Melanoma
Aya Sebak, Iman Gomaa, Samar Mansour, Mahmoud Abdel Kader
German University in Cairo, Cairo, Egypt

POS10
6:00pm-9:00pm

Evaluation of Growth, Biomarker Expression and Matrix Remodeling in 3D Cultures of Drug-resistant Pancreatic Cancer Cells Reveals Elevated Invasiveness and Increased Sensitivity to PDT
Gwendolyn Cramer[1], Dustin Jones[1], William Hanna[1], Imran Rizvi[2], Joshua Hempstead[1], Sai Gourishetti[1], Sathish Kasina[1], Jill Macoska[1], Tayyaba Hassan[2], Jonathan Celli[1]
[1]University of Massachusetts Boston, Boston, MA, USA, [2]Massachusetts General Hospital, Boston, MA, USA

POS11
6:00pm-9:00pm

Sequential [4+2] Diels Alder Reaction of 3,4',5 Trimethoxy-Trans-Stilbene with Singlet Oxygen
Abegail Tadle, Matthias Selke
Cal State University Los Angeles, Los Angeles, USA

POS12
6:00pm-9:00pm

Degradation of Bio-based Oligomer/Polymers From Sustainable Materials
Saravanakumar Rajendran, Ramya Raghunathan, Dean Webster, Mukund Sibi*, Sivaguru Jayaraman**
North Dakota State University, Fargo, North Dakota, USA

POS13
6:00pm-9:00pm

Anticancer Effect of Blebbistatin Under Blue Light
Aliaksandr Mikulich[1], Simona Kavaliauskiene[2], Petras Juzenas[2]
[1]B.I. Stepanov Institute of Physics of the National Academy of Sciences of Belarus, Minsk, Belarus, [2]Institute for Cancer Research, Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway

POS14
6:00pm-9:00pm

Longitudinal Monitoring of Cancer Micrometastases Using Activatable Immunoconjugates and Fluorescence Microendoscopy
Bryan Spring[1], Adnan Abu-Yousif[1], Akilan Palanisami[1], Imran Rizvi[1], Xiang Zheng[1], Zhiming Mai[1], Sriram Anbil[1], R. Bryan Sears[1], Lawrence Mensah[1], Ruth Goldschmidt[1], S. Sibel Erdem[1], Esther Oliva[2], Tayyaba Hasan[1]
[1]Harvard Medical School, Boston, MA, USA, [2]Massachusetts General Hospital, Boston, MA, USA

POS15
6:00pm-9:00pm

Long Term Stability of Isotropic Detectors Calibration Using an LED-coupled Integrating Sphere
Andreea Dimofte, Jarod Finlay, Timothy Zhu
University of Pennsylvania, Philadelphia, PA, USA

POS16
6:00pm-9:00pm

Repair-dependent Cell Radiation Survival and Transformation: an Integrated Theory
John Sutherland
East Carolina University, Greenville, North Carolina, USA

POS17
6:00pm-9:00pm

In Vitro Photodynamic Inactivation of Candida Species with Chloroaluminium Phthalocyanine Nanoemulsion

Gabriela B. Rodrigues[1], Mariana S.L. Rambaldi[1], Fernando L. Primo[2], Antonio C. Tedesco[2], Gilberto U. L. Braga[1]

[1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto/USP, Ribeirão Preto/São Paulo, Brazil, [2]Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto/USP, Ribeirão Preto/São Paulo, Brazil

POS18
6:00pm-9:00pm

Evaluating the Efficacy of Photodynamic Therapy With Glioblastoma Neurospheres Enriched in Cancer Stem-like Cells

Kohei Watanabe[1], Bryan Spring[2], Srivalleesha Mallidi[2], Tayyaba Hasan[2]

[1]Healthcare Optics Research Laboratory, Innovation Center, Canon U.S.A. Inc., Cambridge, Massachusetts, USA, [2]Wellman Center for Photomedicine, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts, USA

POS19
6:00pm-9:00pm

Autocatalytic-Assisted Photorelease of a Sensitizer Drug Bound to a Silica Support

Dorota Bartusik, Mihaela Minnis, Goutam Ghosh, Alexander Greer

City University of New York, Brooklyn College, Brooklyn, New York, USA

POS20
6:00pm-9:00pm

Synergism Between Airborne Singlet Oxygen and a Trisubstituted Olefin Sulfonate for the Inactivation of Bacteria

Rajib Choudhury, Alexander Greer

City University of New York, Brooklyn College, Brooklyn, New York, USA

POS21
6:00pm-9:00pm

Incorporation of an 18O-Label in the Photooxidation of Aromatic Nitrosoamines with Singlet Oxygen (18[1O2])

Marilene Silva Oliveira[1], Ashwini Ghogare[2], Inna Abramova[2], Fernanda Manso Prado[1], Paolo Di Mascio[1], Alexander Greer[2]

[1]University of São Paulo, São Paulo, Brazil, [2]City University of New York, Brooklyn College, Brooklyn, New York, USA

POS22
6:00pm-9:00pm

Singlet Oxygen Generation on Porous Superhydrophobic Surfaces: Effect of Gas Flow and Sensitizer Wetting on Trapping Efficiency

Yuanyuan Zhao[1], Yang Liu[1], Qianfeng Xu[1], Mark Barahman[1], Dorota Bartusik[2], Alexander Greer[2], Alan Lyons[1]

[1]City University of New York, College of Staten Island, Staten Island, New York, USA, [2]City University of New York, Brooklyn College, Brooklyn, New York, USA

POS23
6:00pm-9:00pm

Superhydrophobic Photosensitizers. Mechanistic Studies of 1O2 Generation in the Plastron and Solid/Liquid Droplet Interface

David Aebisher[1], Dorota Bartusik[2], Yang Liu[3], Yuanyuan Zhao[3], Mark Barahman[3], Qianfeng Xu[3], Alan Lyons[3], Alexander Greer[3]

[1]Shorter University, Rome, Georgia, USA, [2]City University of New York, Brooklyn College, Brooklyn, New York, USA, [3]City University of New York, College of Staten Island, Staten Island, New York, USA

POS24
6:00pm-9:00pm

Bacterial Inactivation by a Singlet Oxygen Bubbler: Identifying Factors Controlling the Toxicity of 1O2 Bubbles

Dorota Bartusik[1], David Aebisher[1], Alan Lyons[2], Alexander Greer[1]

[1]City University of New York, Brooklyn College, Brooklyn, New York, USA, [2]City University of New York, College of Staten Island, Staten Island, New York, USA

POS25
6:00pm-9:00pm

Treatment of Systemic Sclerosis with Extracropreal Photopheresis is not Associated with an Increase in Lung Cancer

Sabrie Topuzoglu[1], Robert Knobler[1], Ventzislav Petkov[2], Ulrike Just[1], Christian Jantschitsch[1]

[1]Department of Dermatology, Medical University of Vienna, Vienna, Austria, [2]Department of Internal Medicine II, Medical University of Vienna, Vienna, Austria

POS26
6:00pm-9:00pm

Phosphorescence of Bilirubin and Efficiency of Bilirubin-sensitized Generation of Singlet Oxygen
V. Yu. Plavskii, V.N. Knukshto, A.S. Stasheuski, A.I. Tretyakova, A.V. Mikulich, L.G. Plavskaya, I.A. Leusenko, B.M. Dzhagarov
B.I. Stepanov Institute of Physics of the National Academy of Sciences of Belarus, Minsk, Belarus

POS27
6:00pm-9:00pm

Effect of Laser Radiation of Red and Near Infrared Spectral Regions on the Zooplankton Artemia Salina L.
V.Yu. Plavskii[1], N.V. Barulin[2], A.S. Grabtchikov[1], I.A. Khodasevich[1], A.V. Mikulich[1], L.G. Plavskaya[1], A.I. Tretyakova[1], V.A. Orlovich[1]
[1]B.I. Stepanov Institute of Physics of the National Academy of Sciences of Belarus, Minsk, Belarus, [2]Belarusian State Agricultural Academy, Gorki, Belarus

POS28
6:00pm-9:00pm

Growth Under Visible Light Increases Mucilage and Conidia Production and Tolerance to UV-B Radiation in the Plant-pathogenic Fungus Colletotrichum Acutatum
Henrique D. de Menezes[1], Gabriela B. Rodrigues[1], Drauzio E. N. Rangel[2], Luciano Bachmann[1], Gilberto U. L. Braga[1]
[1]Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil, [2]Universidade do Vale do Paraiba, São José dos Campos, São Paulo, Brazil

POS29
6:00pm-9:00pm

Electron Transfer Processes in Cytochrome-cytocrome Oxidase System Studied by Laser Induced Optoacoustic Spectroscopy.
Pedro David Gara[1], Gabriel Bilmes[1], Silvia Braslavsky[2]
[1]Centro de Investigaciones Opticas and UNLP, La Plata, Bs.As., Argentina, [2]Max-Planck-Institut für Chemische Energiekonversion, Mülheim an der Ruhr, Germany

POS30
6:00pm-9:00pm

Modeling Heterotypic Communication in Tumor Growth and Treatment Response: The Role of Tumor Endothelial Cells and Stromal Fibroblasts
Imran Rizvi[1], Emma Briars[1], Arnav Chandra[1], Sriram Anbil[1], Jonathan Celli[2], Heather Gudejko[1], Shazia Khan[1], William Hanna[2], Dustin Jones[2], Tayyaba Hasan[1]
[1]Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA, [2]Department of Physics, University of Massachusetts, Boston, MA, USA

POS31
6:00pm-9:00pm

Cytometric Approach for a Rapid Evaluation of Candida Albicans Susceptibility to Photodynamic Antimicrobial Chemotherapy with Phenotiazinium Photosensitizers
Gabriela B. Rodrigues, Mariana S. L. Rambaldi, Emerson de S. Santos, Sérgio A. Uyemura
Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil

POS32
6:00pm-9:00pm

"Pointsource" Delivery of a Photosensitizer Drug and Singlet Oxygen: Eradication of Glioma Cells in Vitro
Ashwini Ghogare[1], Imran Rizvi[2], Tayyaba Hasan[2], Alexander Greer[1]
[1]City University of New York - Brooklyn College, Brooklyn, New York, USA, [2]Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA

POS33
6:00pm-9:00pm

Rapid Optical Determination of Beta-Lactamase based Antibiotic Susceptibility
Shazia Khan[1], Ulysses W Sallum[1], Xiang Zheng[1], Gerard J Nau[2], Tayyaba Hasan[1]
[1]Wellman Centre for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA, [2]University of Pittsburgh School of Medicine,, Pittsburgh, PA, USA

POS34
6:00pm-9:00pm

Silicon Phthalocyanine (Pc) 4 Phototoxicity in c. albicans Biofilm
Matthew Dimaano, Minh Lam
Case Western Reserve University, Cleveland, OH, USA

POS35
6:00pm-9:00pm

50th Anniversary of the Foote/Wexler Discovery: A Milestone for Singlet Oxygen Research
Alexander Greer
City University of New York, Brooklyn College, Brooklyn, New York, USA

POS36
6:00pm-9:00pm

The Effects of Modified Fibronectin on ARPE-19 Cells as Model Systems for Ageing and Inflammation in Human Bruch's Membrane
Mai Thao, James Dillon, Elizabeth Gaillard
Northern Illinois University, DeKalb, IL, USA

POS37
6:00pm-9:00pm

Transfersomal Chlorophyllin Derivatives: A Novel Model in the Photodynamic Treatment of Malignant Brain Tumors
Nada Attia, Nabila Hamdi, Samar Mansour, Mahmoud Abdel-Kader
German University in Cairo, Cairo, Egypt

POS38
6:00pm-9:00pm

Compositional Studies of Human Retinal Lipofuscin: Wet Versus Dry Age Related Macular Degeneration
Jennifer Tournear, James Dillon, Elizabeth Gaillard
Northern Illinois University, Dekalb, IL, USA

POS39
6:00pm-9:00pm

Photoacoustic Monitoring of Photosensitizer Photobleaching Rate to Predict Photodynamic Therapy Response
Srivalleesha Mallidi, Tayyaba Hasan
Massachusetts General Hospital, Boston, MA, USA

POS40
6:00pm-9:00pm

Kinetics of Photosynthetic Response to Ultraviolet and Visible Light in Synechococcus WH8102 (CYANOBACTERIA)
Glaucia Fragoso[1], Patrick Neale[1], Todd Kana[2], Alicia Pritchard[1]
[1]Smithsonian Environmental Research Center, Edgewater, Maryland, USA, [2]University of Maryland Center for Environmental Sciencefor, Cambridge, Maryland, USA

POS41
6:00pm-9:00pm

Treating Pancreatic Cancer with Nano-PDT and Liposomal Irinotecan
Huang Chiao Huang, Srivalleesha Mallidi, Imran Rizvi, Zhiming Mai, Chun Te Chiang, Joyce Liu, Dmitriy Timerman, Tayyaba Hasan
Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

POS42
6:00pm-9:00pm

Ultraviolet B Sensitivity of BALB/c 3T3 Cells. Increased UVB Exposure Can Be Used in the 3T3 Neutral Red Uptake Phototoxicity Test for the Evaluation of UVB-absorbing Test Materials
Mary Dougherty, Mark Schwartz, Douglas Learn
Charles River Laboratories Preclinical Services, Horsham, PA, USA

POS43
6:00pm-9:00pm

Detection of Singlet Oxygen Using Photomultiplier-tube to Evaluate Photodynamic Therapy
In-Wook Kim[1], Ju Hee Kim[1], Jae Myung Park[1], Zhiming Mai[2], Tayyaba Hasan[2], Myung-Gyu Choi[1]
[1]Catholic-Harvard Wellman Photomedicine Center, Division of Gastroenterology, Medical School, The Catholic University of Korea, Seoul, Republic of Korea, [2]Wellman Center for Photomedicine, Department of Dermatology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

POS44
6:00pm-9:00pm

ATP-binding Cassette Sub Family G Member 2 Inhibition Effect on Photodynamic Therapy Efficacy in Colon Cancer
Ju Hee Kim[1], In-Wook Kim[1], Jae Myung Park[1], Zhiming Mai[2], Myung-Gyu Choi[1]
[1]Catholic-Harvard Wellman Photomedicin Center, Division of Gastroenterlology,The Catholic University of Korea, School of Medicine, Seoul, Republic of Korea, [2]Wellman Center for Photomedicine,Department of Dermatology,Massachusetts General Hospital, Harvard Medical School, Boston/MA, USA

POS45
6:00pm-9:00pm

Photoluminescent Metal Complex Probes: A Tale of Metals, Light and Time
Angel Marti, Nathan Cook, Kewei Huang, Avishek Saha
Rice University, Houston, USA

POS46
6:00pm-9:00pm

Dose Construction Parameters for Photodynamic Targeting of Multifocal Nodules in a 3D Tumor Model

Imran Rizvi[1], Sriram Anbil[1], Nermina Alagic[1], Jonathan Celli[2], Lei Zak Zheng[1], Akilan Palanisami[1], Michael Glidden[1], Brian Pogue[3], Tayyaba Hasan[1]
[1]Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA, [2]Department of Physics, University of Massachusetts Boston, Boston, MA, USA, [3]Thayer School of Engineering, Dartmouth College, Hanover, NH, USA

POS47
6:00pm-9:00pm

Impact of Physical Forces on 3D Ovarian Cancer Biology: Targeting Epithelial-Mesenchymal Transition, Cellular Heterogeneity and Biomarker Modulation Induced by Flow

Imran Rizvi[1], Umut Gurkan[2], Savas Tasoglu[2], Nermina Alagic[1], Lawrence Mensah[1], Zhiming Mai[1], Jonathan Celli[3], Michael Glidden[3], Sriram Anbil[1], Utkan Demirci[2], Tayyaba Hasan[1]
[1]Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA, [2]Bio-Acoustic-MEMS Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA, [3]Department of Physics, University of Massachusetts, Boston, Boston, MA, USA

POS48
6:00pm-9:00pm

Classification of Neocortical Neurons

Naureen Ghani, Rafael Yuste
Columbia University, New York, USA

POS49
6:00pm-9:00pm

Examining the Use of Liposomal Conjugated Lapatinib and Photodynamic Therapy (PDT) for the Treatment of Malignant Glioma.

Carl Fisher[1], Carloyn Nui[2], Obraid Girgis[3], Tayyaba Hasan[3], Lothar Lilge[2]
[1]University of Toronto, Toronto, Ontario, Canada, [2]University Health Network, Toronto, Ontario, Canada, [3]Welman Laboratory of Photomedicine, Massachusetts General Hospital, Boston, MA, USA

POS50
6:00pm-9:00pm

Elicitation of Tumour-free Long-term Survival and Long-lasting Antitumor Memory with Novel Non-immunosuppressive Near-infrared PDT.

Kamola Kasimova[1], Yaxal Arena[1], Sherri McFarland[3], Arkady Mandel[1], Lothar Lilge[2]
[1]Theralase Inc., Toronto, Ontario, Canada, [2]University Health Network, Toronto, Ontario, Canada, [3]Acadia University, Wolfville, Nova Scotia, Canada

POS51
6:00pm-9:00pm

Reversing the Cancerous Glycolytic Phenotype with Dichloroacetate In Vitro and its Effects on Photodynamic Therapy.

Victoria Kuta[1], Carl Fisher[2], Lothar Lilge[1]
[1]University Health Network, Toronto, Ontario, Canada, [2]University of Toronto, Toronto, Ontario, Canada

POS52
6:00pm-9:00pm

Altered Expression of PKCs Leads to Different Response of Human Glioma Cells (U87 MG) on Photo-activated Hypericin and Switch Apoptosis to Necrosis

Zuzana Nadova[1], Jaroslava Joniova[1], Franck Sureau[3], Pavol Miskovsky[2]
[1]Department of Biophysics, Safarik University, Kosice, Slovakia, [2]Centre for Interdisciplinary Biosciences, Safarik University, Kosice, Slovakia, [3]Laboratoire Jean Perrin, P. et M. Curie University, Paris, France

POS53
6:00pm-9:00pm

Kinetics of Incorporation/Redistribution of Photosensitizer Hypericin to/from High-density Lipoproteins

Jaroslava Joniova[1], Luboslava Buriankova[2], Diana Buzova[3], Pavol Miskovsky[4], Daniel Jancura[1]
[1]Department of Biophysics, Safarik University, Kosice, Slovakia, [2]Institute of Physics, Charles University, Prague, Czech Republic, [3]Department of Adaptation Biotechnologies, Czech Globe, Drasov, Czech Republic, [4]Centre for Interdisciplinary Biosciences, Kosice, Slovakia

POS54
6:00pm-9:00pm

Role of a Helix B Lysine Residue in the Photoactive Site in Channelrhodopsins

Hai Li, Govorunova Elena, Sineshchekov Oleg, Spudich John
University of Texas Health Science Center at Houston, Houston, TX, USA

POS55
6:00pm-9:00pm

Light Mediated Toxic Effect of ZN Phthalocyanines on Hela Cells a Comparison Using dppc Liposomes and bsa as Delivery System

Ana María Edwards[1], Angélica María Garcia[2], Emilio Alarcón[3], Eduardo Lissi[4]
[1]Pontificia Universidad Católica de Chile, Santiago, Chile, [2]Universidad Pedagógica y Tecnológica de Colombia, Tunja, Colombia, [3]University of Ottawa, Ottawa, Canada, [4]Universidad de Santiago de Chile, Santiago, Chile

POS56
6:00pm-9:00pm

Comparative Characterization of Solar Radiation-induced DNA Lesions Between Ex Vivo Human Skin and In Vitro Human Hair Follicle Derived Epidermis Model

Daniel Bacqueville[1], Thierry Douki[2], Laure Duprat[1], Hélène Dromigny[1], Valérie Perier[1], Sandrine Bessou-Touya[1], Hélène Duplan[1]
[1]Service Pharmacologie tissulaire et Pharmacocinétique cutanée, Département Pharmacologie in vitro, Centre R&D Pierre Fabre, Toulouse, France, [2]Service de Chimie Inorganique et Biologique, Institut Nanosciences et Cryogénie UMR E3 CE/UJF, CEA, Grenoble, France

POS57
6:00pm-9:00pm

Novel Targets for Vitamin D in Melanoma Prevention, Growth and Metastasis.

Katie M Dixon[1], Nicole Painter[1], Artur Shariev[1], Shivashni S Deo[1], Stephen J Assinder[1], Anthony W Norman[2], Rebecca S Mason[1]
[1]Disciplines of Anatomy & Histology and Physiology, Bosch Institute, The University of Sydney, Sydney, NSW, Australia, [2]Department of Biochemistry, University of California, Riverside, CA, USA

POS58
6:00pm-9:00pm

On the Natural Function(s) of Green Fluorescent Protein (GFP) in Marine Non-bioluminescent Organisms

Dimitri Deheyn
Scripps Institution of Oceanography, UCSD, La Jolla, CA, USA

POS59
6:00pm-9:00pm

Development of Folate-Targeted Photodynamic Therapy Agents Using Protein and PEG Carriers

Ken Olsen, RoJenia Jones, Sana Hira, Katherine Mathewson, Kyle Sullivan, Laura Donahue, David Crumrine, Stefan Kanzok, Rodney Dale
Loyola University Chicago, Chicago, IL, USA

POS60
6:00pm-9:00pm

Harnessing of Novel Visible and Near-infrared Light Photoactivated, Type II/Type I, Tunable, Metal-based, Small Molecule, Coordination Complexes in PDT.

Kamola Kasimova[2], Yaxal Arena[2], Arkady Mandel[2], Pavel Kaspler[2], Sherri MaFarland[3], Lothar Lilge[1]
[1]University Health Network, Toronto, Ontario, Canada, [2]Theralase Inc., Toronto, Ontario, Canada, [3]Arkadia University, Wolfville, Nova Scotia, Canada

POS61
6:00pm-9:00pm

Photodynamic Therapy and Inflammatory Breast Cancer

neha aggarwal, david kessel, bonnie sloane
wayne state university school of medicine, detroit, MI, USA

Tuesday, June 17

Registration
June 17, 7:30am - 2:30pm **Penny Lane**

New Investigator Award Lecture
June 17, 8:30am - 9:30am **The Edge** **Chair: Frances Noonan**

Angel Marti/Kaushal Rege

ASP Research Award Lecture
June 17, 9:30am - 10:30am **The Edge** **Chair: Frances Noonan**

TUES1
9:30am-10:30am

Photochemistry in Nanotechnology: Bridging the Gap between Nanomaterials and Nanomedicine
Juan Scaiano
University of Ottawa, Ottawa, Ontario, Canada

Break with Exhibitors
June 17, 10:30am - 11:00am **Legends**

Business Meeting (With Refreshments)
June 17, 10:30am - 12:00pm **The Edge**

Grant Writing Workshop With Lunch (pre-registration required)
June 17, 12:00pm - 2:00pm **Encore**

Lunch
June 17, 12:00pm - 1:00pm

Theoretical and Clinical Validation of the Utility of Dosimetry: Pretreatment Planning and Online Treatment Monitoring
June 17, 1:00pm - 2:30pm **Celebrate** **Chair: Lothar Lilge & Stephen Kanick**

TUES2
1:00pm-1:30pm

Challenges for PDT Dosimetry in Small Animal Models.
Emma Henderson[1], Lothar Lilge[2]
[1]University of Toronto, Toronto, Ontario, Canada, [2]University Health Network, Toronto, Ontario, Canada

TUES3
1:30pm-2:00pm

Towards PDT Treatment Planning.
Jeff Cassidy[1], Vaughn Betz[1], Lothar Lilge[2]
[1]University of Toronto, Toronto, Ontario, Canada, [2]University Health Network, Toronto, Ontario, Canada

TUES4
2:00pm-2:30pm

Optical Measurements Prior to PDT Treatments of Actinic Keratosis are Predictive of Patient-specific Response: Our Pilot Clinical Experience
Stephen Kanick[1], Scott Davis[1], Yan Zhao[1], Tayyaba Hasan[2], Edward Maytin[3], M. Shane Chapman[4], Brian Pogue[1]
[1]Thayer School of Engineering, Dartmouth College, Hanover, NH, USA, [2]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, USA, [3]Biomedical Engineering, Cleveland Clinic, Cleveland, OH, USA, [4]Department of Surgery, Section of Dermatology, Dartmouth Hitchcock Medical Center, Lebanon, NH, USA

Platform Session - Targeted Photosensitization

June 17, 1:00pm - 2:30pm **The Edge** **Chair: Kimberley Samkoe**

TUES5
1:00pm-1:15pm
LUZ11: A Fluorinated Sulfonamide Bacteriochlorin in Clinical Trials for Head and Neck Cancers
Luis Arnaut[1], Luis Rocha[2], Janusz Dabrowski[3], Mariette Pereira[1], Ligia Gomes-da-Silva[1]
[1]University of Coimbra, Coimbra, Portugal, [2]Luzitin SA, Coimbra, Portugal, [3]Jagiellonian University, Krakow, Poland

TUES6
1:15pm-1:30pm
Photodynamic Therapy with Long Duration, Ultra low Level (Nanowatt Range) Light can Kill Mesothelioma Cancer Cells In Vitro
Joseph Friedberg, Michael Tenuto, Warren Naselsky, Theresa Busch, Arash Darafsheh, Christopher Murray, Ann-Marie Chacko, Taejong Paik, Daniel Pryma, Jarod Finlay
University of Pennsylvania, Philadelphia, PA, USA

TUES7
1:30pm-1:45pm
Porphysome Nanotechnology: Explore New Frontiers of Cancer Imaging and Therapy
Gang Zheng[1]
[1]University of Toronto, Toronto, Ontario, Canada, [2]Ontario Cancer Institute, Toronto, Ontario, Canada

TUES8
1:45pm-2:00pm
Receptor Concentration Imaging (RCI) Can Quantify Available Epidermal Growth Factor Status After Photodynamic Therapy in Pancreatic Cancer
Kimberley Samkoe[1], Kenneth Tichauer[3], Jason Gunn[2], Tayyaba Hasan[4], Brian Pogue[2]
[1]Geisel School of Medicine, Lebanon, NH, USA, [2]Thayer School of Engineering, Hanover, NH, USA, [3]Illinois Institute of Technology, Chicago, IL, USA, [4]Wellman Center for Photobiology, Boston, MA, USA

TUES9
2:00pm-2:15pm
Her2/Neu Oncogene Transformation Enhances 5-aminolevulinic Acid-mediated Protoporphyrin IX Production and Mitochondrial Accumulation
Xue Yang[1], Kenneth Myers[1], Chenguang Wang[2], Bin Chen[1]
[1]University of the Sciences, Philadelphia, PA, USA, [2]Thomas Jefferson University, Philadelphia, PA, USA

TUES10
2:15pm-2:30pm
Combination of TSPO Targeted PDT and Differentiation-inducing Agent: Image (PET and fluorescence) – Guided Therapy for Breast Cancers, Especially for TNBC (Triple Negative Breast Cancers)
Yihui Chen, Jerry Glickson
University of Pennsylvania, Philadelphia, USA

Platform Session - Photosensitizers and Contrast Agents

June 17, 1:00pm - 2:30pm **Imagine** **Chair: Cristina Mari & David Ohayon**

TUES11
1:00pm-1:15pm
Time-dependent Intracellular Association of Photosensitizers with Organelles Modulates the Efficacy of Photodynamic Therapy
Rebecca Gilson, Rui Tang, Pinaki Sarder, Samuel Achilefu
Washington University in St Louis, St Louis, Mo, USA

TUES12
1:15pm-1:30pm
Discovering Ru(II) Complexes as Potent Tool in Photodynamic Therapy
Cristina Mari[1], Vanessa Pierroz[2], Riccardo Rubbiani[1], Malay Patra[1], Stefano Ferrari[2], Gilles Gasser[1]
[1]University of Zurich, Department of Chemistry, Zurich, Switzerland, [2]University of Zurich, Institute of Molecular Cancer Research, Zurich, Switzerland

TUES13 1:30pm-1:45pm	Development of Porphyrin-Phospholipid Liposomes Permeabilized by Near Infrared Light *Kevin Carter[1], Shuai Shao[1], Matthew Hoopes[2], Dandan Luo[1], Bilal Ahsan[3], Vladimir Grigoryants[4], Wentao Song[1], Haoyuan Huang[1], Guojian Zhang[1], Ravindra Pandey[5], Jumin Geng[1], Blaine Pfeifer[1], Charles Scholes[4], Joaquin Ortega[3], Mikko Karttunen[2], Jonathan Lovell[1]* *[1]University at Buffalo, Buffalo, New York, USA, [2]University of Waterloo, Waterloo, Ontario, Canada, [3]McMaster University, Hamilton, Ontario, Canada, [4]University at Albany, Albany, New York, USA, [5]Roswell Park Cancer Institute, Buffalo, New York, USA*
TUES14 1:45pm-2:00pm	BODIPY as Fluorescent Photosensitizers in Near IR Region *Ryan Watley[1], Samuel Awuah[1], Sushanta Das[2], Francis D'Souza[2], Youngjae You[1]* *[1]University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA, [2]University of North Texas, Denton, TX, USA*
TUES15 2:00pm-2:15pm	Shining Light on the Dark Side of Imaging: Exploring Photoacoustic and Non-Linear Optical Properties of Molecular Contrast Agents Based on Curcumin and BODIPY Chromophores. *Mathieu Frenette[1], Maryam Hatamimoslehabadi[2], Stephanie Bellinger-Buckley[1], Samir Laoui[2], Seema Bag[1], Olivier Dantiste[2], Jonathan Rochford[1], Chandra Yelleswarapu[2]* *[1]Departement of Chemistry, University of Massachusetts Boston, Boston, MA, USA, [2]Departement of Physics, University of Massachusetts Boston, Boston, MA, USA*
TUES16 2:15pm-2:30pm	Biophotonics: A Novel Approach to the Treatment and Regeneration of Wounds. *Emmanuelle Devemy, David Burroughes, David Ohayon, Eric DesRosiers* *KLOX Technologies Inc., Laval, Quebec, Canada*

Networking Time/Free Afternoon

June 17, 2:30pm - 7:15pm

Banquet and Awards Ceremony

June 17, 7:15pm - 10:00pm **Harbor House (off-site)**

Bring entrance ticket. Drink tickets will be handed out at the door.

Wednesday, June 18

Registration
June 18, 8:00am - 5:30pm **Penny Lane**

Kendric C. Smith Innovations Lecture
June 18, 9:00am - 9:45am **The Edge**

WED1
9:00am-9:45am

Optical Spectroscopy and Tomography of Oxygen Delivery: From Macro to Micro and Back
David Boas
Massachusetts General Hospital, Boston, MA, USA

Plenary Lecture : Nobel Laureate, Roger Tsien
June 18, 9:45am - 10:45am **The Edge** **Chair: Jonathan Lovell & Tayyaba Hasan**

"Cells in health and disease, seen mostly in pretty colors"

Break with Exhibitors
June 18, 10:45am - 11:00am **Legends**

ASP President's Lecture: Beth Gaillard
June 18, 11:00am - 12:00pm **The Edge** **Chair: Don Forbes**

Mentor's Lunch
June 18, 12:00pm - 1:30pm **Encore**

Lunch
June 18, 12:00pm - 2:00pm

Interrogating Disease with Light: Preclinical and Clinical Progress
June 18, 2:00pm - 3:35pm **The Edge** **Chair: Norbert Lange & Srivalleesha Mallidi**

WED2
2:00pm-2:20pm

Quantitative Functional Assessment of Tumor Microenvironment Using Contrast Enhanced Ultrasound and Photoacoustic Imaging
Melissa Yin[1], Mina Lakshman[3], F. Stuart Foster[2]
[1]Sunnybrook Research Institute, Toronto, Ontario, Canada, [2]University of Toronto, Toronto, Ontario, Canada, [3]VisualSonics Inc., Toronto, Ontario, Canada

WED3
2:20pm-2:35pm

Nanoprobes for Photoacoustic Imaging and Phototherapy
Ghayathri Balasundaram[1], Chris Jun Hui Ho[1], Kai Li[3], Amalina Attia[1], Kienvoon Kong[1], Bin Liu[3], Malini Olivo[1]
[1]Biooptical Imaging Group, Singapore Bioimaging Consortium, Singapore, Singapore, [2]School of Physics, National University of Ireland, Galway, Galway, Ireland, [3]Institute of Materials Research and Engineering, Singapore, Singapore, [4]Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore, Singapore, Singapore, [5]nstitute for Biological and Medical Imaging, Helmholtz Center, Munich, Germany

WED4
2:35pm-2:55pm

Identifying Photodynamic Therapy Non-responders Using Photoacoustic Imaging
Srivalleesha Mallidi[1], Kohei Watanabe[2], Dmitriy Timerman[1], Tayyaba Hasan[1]
[1]Massachusetts General Hospital, Boston, MA, USA, [2]Canon USA Inc, Boston, MA, USA

WED5
2:55pm-3:15pm

Silent Probes for Optical Imaging: an Overview
Norbert Lange
University of Geneva, Geneva, Switzerland

WED6
3:15pm-3:35pm

Image Guided Surgery using Near Infrared Fluorescent Light. From Bench to Bedside.
Alexander Vahrmeijer
Leiden University Medical Center, Leiden, The Netherlands

Vascular Effects of PDT & Interaction with Molecular-targeted Agents

June 18, 2:00pm - 3:35pm **Imagine** **Chair: Bin Chen & Patrycja Nowak-Sliwinska**

WED7
2:00pm-2:20pm

Improving Therapeutic Response to PDT through Targeting Tumor Blood Vessels at the Molecular Level
Theresa Busch[1], Shannon Gallagher-Colombo[1], Manon te Dorsthorst[2], Joann Miller[1], Shirron Carter[1]
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]University of Groningen, Groningen, The Netherlands

WED8
2:20pm-2:40pm

Photoactivation of Sunitinib as Anti-tumor Strategy
Patrycja Nowak-Sliwinska[1], Andrea Weiss[1], Judy R. van Beijnum[2], Grzegorz Szewczyk[3], Tadeusz Sarna[3], Arjan W. Griffioen[2]
[1]Institute of Chemical Sciences and Engineering, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland, [2]Angiogenesis Laboratory, Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands, [3]Department of Biophysics, Jagiellonian University, Krakow, Poland

WED9
2:40pm-2:55pm

Outshining Drug Resistance with Light: How Adding Erlotinib to Photodynamic Therapy Can Improve Therapeutic Response in Non-small Cell Lung Cancer
Shannon Gallagher-Colombo, Rensa Chen, Joann Miller, Shirron Carter, Keith Cengel, Theresa Busch
University of Pennsylvania, Philadelphia, PA, USA

WED10
2:55pm-3:15pm

Anti-angiogenic Treatment at Vascular Normalizing Doses Enhances Chemotherapy and Photodynamic Therapy Effects in a Preclinical Model of Human Ovarian Carcinoma
Andrea Weiss, Debora Bonvin, Robert Berndsen, Patrycja Nowak-Sliwinska
Institute of Chemical Sciences and Engineering, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland

WED11
3:15pm-3:35pm

Combination of Photodynamic Therapy and Cancer Molecular Targeted Agents
Babasola Fateye, Daniel Kraus, Bin Chen
University of the Sciences, Philadelphia, PA, USA

Sunscreens

June 18, 2:00pm - 3:30pm **Celebrate** **Chair: Henry Lim & Frances Noonan**

WED12
2:00pm-2:25pm

An Ideal Sunscreen – How to Achieve It
Uli Osterwalder[1], Bernd Herzog[2]
[1]BASF PCN GmbH, Duesseldorf, Germany, [2]BASF Grenzach GmbH, Grenzach-Whylen, Germany

WED13
2:25pm-2:50pm

The Role of Botanicals and Antioxidants in Sun Protection
Mary Matsui
The Estee Lauder Companies, Melville, NY, USA

WED14
2:50pm-3:05pm

Changes to the Stratum Corneum After Narrow-Band UVB (311nm) Phototherapy in Polymorphic Light Eruption Patients
Emma J. Pond[1], Catherine A. O'Neill[1], Lesley E. Rhodes[2], Neil K. Gibbs[1]
[1]Centre for Dermatology, Institute of Inflammation & Repair, Manchester Academic Health Sciences Centre, University of Manchester, Manchester, UK, [2]Salford Royal Hospital, Manchester Academic Health Sciences Centre, University of Manchester, Manchester, UK

WED15
3:05pm-3:30pm

Controversies on Photoprotection
Henry Lim
Henry Ford Hospital, Detroit, MI, USA

Break with Exhibitors
June 18, 3:30pm - 4:00pm **Legends**

Light, Biology and Mechanics: Optical Methods in Tissue Mechanics and Mechanobiology
June 18, 4:00pm - 5:30pm **The Edge** **Chair: Giuliano Scarcelli & Jonathan Celli**

WED16
4:00pm-4:20pm
Mapping Microscopic Viscosity in Cells Using Molecular Rotors
Marina Kuimova
Imperial College London, London, UK

WED17
4:20pm-4:35pm
Predominant Photogeneration of Singlet Oxygen or Free Radicals by Selected Nanoparticle Photosensitizers
Anna Kozinska[1], Andrzej Zadlo[1], Przemyslaw Labuz[1], Michael Hamblin[2], Tadeusz Sarna[1]
[1]Jagiellonian University, Krakow, Poland, [2]The Wellman Center for Photomedicine, Boston, MA, USA

WED18
4:35pm-4:55pm
Photomechanical Responses of Photoreceptors
Roger Hardie, Kristian Franze
University of Cambridge, Cambridge, UK

WED19
4:55pm-5:10pm
On the Unique Light Production From the Marine Worm Chaetopterus: Where Do We Stand?
Dimitri Deheyn
Scripps Institution of Oceanography, UCSD, La Jolla, CA, USA

WED20
5:10pm-5:30pm
Biomechanical Imaging with Brillouin Microscopy
giuliano scarcelli
Wellman Center Photomedicine, Harvard Medical School, Cambridge, MA, USA

UV & Melanoma / Pigment Cell Photobiology
June 18, 4:00pm - 5:35pm **Celebrate** **Chair: Frances Noonan & Edward De Fabo**

WED21
4:00pm-4:20pm
Melanoma, UV and Melanin- Clues From Mouse Models
Frances Noonan, Edward De Fabo
The George Washington University, Washington, DC, USA

WED22
4:20pm-4:40pm
HGF/SF Does Not Affect Melanogenesis But Increases the Number of Extra-follicular Melanocytes in Mouse Skin
Agnieszka Wolnicka-Glubisz
Jagiellonian University, Krakow, Poland

WED23
4:40pm-5:00pm
“The Two Faces of Melanin – Protective and Anti-protective”.
Julian Menter[1]
[1]Morehouse School of Medicine, Atlanta,GA 30310-1495, USA, [2]Morehouse School of Medicine, Atlanta, GA 30310-1495, USA

WED24
5:00pm-5:20pm
Tanning Lamps and Health
Henry W. Lim
Dermatology, Henry Ford Hospital, Detroit, MI, USA

WED25
5:20pm-5:35pm
A 3-year Follow-up of Sun Behavior in Patients with Cutaneous Malignant Melanoma Based on Ultraviolet Radiation Measurements and Sun Diary Data
Luise Winkel Idorn, Pameli Datta, Jakob Heydenreich, Peter Alshede Philipsen, Hans Christian Wulf
Dermatological Research Department, Bispebjerg University Hospital, Copenhagen, Denmark

Distribution of UV Irradiance in the Environment: Models, Measurements, and Applications

June 18, 4:00pm - 5:40pm **Imagine** **Chair: John Streicher & Joanna Turner**

WED26 4:00pm-4:20pm	Measurements in the Built Environment: UV Reflection in Small Scale Systems and What it Means for Outdoor Workers *Joanna Turner, Alfio V Parisi* *University of Southern Queensland, Toowoomba, Queensland, Australia*
WED27 4:20pm-4:40pm	Horizon Sky Radiance – The Relevance for Ocular UV Dosimetry *David Sliney[1], Stephen Wengraitis[2], John Streicher[3]* *[1]The Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA, [2]US Army Public Health Command, Aberdeen Proving Ground, MD, USA, [3]National Oceanic and Atmospheric Administration, Research Triangle Park, NC, USA*
WED28 4:40pm-5:00pm	Observed and Predicted Levels of Ultraviolet Radiation at the Earth's Surface *Germar Bernhard, Charles Booth* *Biospherical Instruments Inc., San Diego, CA, USA*
WED29 5:00pm-5:20pm	Cell Killing and Transformation Induced by Polychromatic UV Light: an Integrated Theory *John Sutherland* *East Carolina University, Greenville, NC, USA*
WED30 5:20pm-5:40pm	Development and Applications of a Radiance Model *John Streicher* *US Environmental Protection Agency, RTP, NC, USA*

Thursday, June 19

Registration

June 19, 8:00am - 11:00am **Penny Lane**

Nitric Oxide as a PDT Modulator

June 19, 8:30am - 10:05am **Imagine** **Chair: Albert Girotti**

THUR1
8:30am-8:50am

Role of NO Induced By Repeated Treatments With Pba/PDT in Prostate Cancer Cells
Valentina Rapozzi[1], Emilia Della Pietra[1], Daniela Cesselli[1], Benjamin Bonavida[2], Luigi E Xodo[1]
[1]Department of Medical and Biological Sciences, Udine, Italy, [2]Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, Jonsson Comprehensive Cancer Center, University of California Los Angeles, Los Angeles, USA

THUR2
8:50am-9:10am

Pro-Survival Signaling by NOS2-Derived NO in Photodynamically-Stressed Cancer Cells
Albert Girotti, Reshma Bhowmick, Jon Fahey
Medical College of Wisconsin, Milwaukee, WI, USA

THUR3
9:10am-9:25am

Combination of Nitric Oxide Therapy, Anti-oxidative Therapy, Low Level Laser Therapy, Plasma Rich Platelet Therapy and Stem Cell Therapy as a Novel Therapeutic Application to Manage the Pain and Treat Many Clinical Conditions
Salaheldin Halasa[1]
[1]Beroyal, Easton,MD, USA, [2]Cutting edge treatment center, Eason , MD, USA

THUR4
9:25am-9:45am

Revisiting Early Studies on the Impact of Nitric Oxide on PDT Response
Mladen Korbelik
British Columbia Cancer Agency, Vancouver, BC, Canada

THUR5
9:45am-10:05am

Role of Nitric Oxide and Other Soluble Mediators in the Acute Inflammatory Response to ALA-PDT in Human Skin
Mark Farrar[1], Rebecca Brooke[1], Rachel Watson[1], Peter Friedmann[2], Geraldine Clough[2], Lesley Rhodes[1]
[1]University of Manchester, Manchester, UK, [2]University of Southampton, Southampton, UK

Bidirectionally Informed Photobiology: Developing Relevant Preclinical Tools

June 19, 8:30am - 10:05am **The Edge** **Chair: Keith Cengel & Imran Rizvi**

Introduction-Keith Cengel 8:30am-8:35am

THUR6
8:35am-9:00am

Estimating Receptor Concentration in Solid Tumors Noninvasively Using Multi-tracer Fluorescence Tomography
Scott Davis[1], Kimberley Samkoe[1], Kenneth Tichauer[2], Kristian Sexton[1], Tayyaba Hasan[3], Brian Pogue[1]
[1]Dartmouth College, Hanover, NH, USA, [2]Illinois Institute of Technology, Chicago, IL, USA, [3]Harvard Medical School, Boston, MA, USA

THUR7
9:00am-9:15am

Photodynamic Therapy May Mitigate the Risk of Surgical Tract Site Tumor Seeding for Malignant Pleural Mesothelioma
Charles Simone, Andrew Barsky, John Buckley, Melissa Culligan, Stephen Hahn, Joseph Friedberg, Keith Cengel
University of Pennsylvania, Philadelphia, PA, USA

THUR8
9:15am-9:40am

Optogenetically Engineered T Cells for Cancer Immunotherapy
Yuexin Xu, Young-min Hyun, Scott Gerber, Edith Lord, Minsoo Kim
University of Rochester Medical Center, Rochester, NY, USA

THUR9
9:40am-10:05am

Targeting Physical and Stromal Determinants of Ovarian Cancer Biology in Bioengineered Models to Inform PDT-based Combination Regimens
Imran Rizvi[1], Umut Gurkan[2], Tri Dinh[1], Lawrence Mensah[1], Jonathan Celli[3], Savas Tasoglu[2], Nermina Alagic[1], Zhiming Mai[1], Brian Pogue[4], Utkan Demirci[2], Tayyaba Hasan[1]
[1]Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA, [2]Bio-Acoustic-MEMS Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA, [3]Department of Physics, University of Massachusetts Boston, Boston, MA, USA, [4]Thayer School of Engineering at Dartmouth College, Hanover, NH, USA

DNA Structure, Photoproducts and Mutagenesis

June 19, 8:30am - 10:15am **Celebrate** **Chair: Regen Drouin**

THUR10
8:30am-9:00am

Repair of DNA Photolesions in Chromatin
John Hinz
Washington State University, Pullman, WA, USA

THUR11
9:00am-9:15am

UV-induced Psoralen Photoadducts and Their Papid Detection by Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)
Francis P Gasparro[1], Michael Crockett[1], Alexandru Buhimschi[2], Irina Buhimschi[3]
[1]Hamden Hall Country Day School, Hamden, CT, USA, [2]Yale University, New Haven, CT, USA, [3]Nationwide Childrens Hospital, Columbus, OH, USA

THUR12
9:15am-9:45am

Why do Solar-UV Signature Mutations Occur Preferentially at TCG Context?
Hironobu Ikehata
Tohoku University, Sendai, Japan

THUR13
9:45am-10:15am

Compared to UVC, UVB Irradiation Generates More Cyclobutane Pyrimidine Dimers in Dipyrimidine Sites Potentially More Frequently Mutated in Skin Cancer.
Nathalie Bastien[1], Jean-Philippe Therrien[2], Régen Drouin[1]
[1]Universite de Sherbrooke, Sherbrooke, Quebec, Canada, [2]Stiefel, a GSK company, Research Triangle Park, NC, USA

Break with Exhibitors

June 19, 10:00am - 10:30am **Abbey Road**

Photocleavable Materials in Health Sciences

June 19, 10:30am - 12:10pm **Imagine** **Chair: Adah Almutairi**

THUR14
10:30am-10:55am

New Approaches for Developing Near-Infrared Light-Controllable Drug Carriers
Yue Zhao
University of Sherbrooke, Sherbrooke, Canada

THUR15
10:55am-11:20am

Biocompatible (Light)Responsive Polymer Layers to Manipulate Cells.
christophe Tribet[1]
[1]Ecole Normale Supérieure, Paris, France, [2]CNRS, Paris, France, [3]UPMC, Paris, France

THUR16
11:20am-11:45am

Controlling Cellular Proteins with Light
Arnaud Gautier
Ecole Normale Supérieure, Paris, France

THUR17
11:45am-12:10pm

Efficient Upconversion of 800 nm Near Infrared via Novel Core-shell Lanthanide-doped Nanocrystals
Noah Johnson, Sha He, Adah Almutairi
UC San Diego, La Jolla, CA, USA

Dosimetry and Treatment Monitoring in Photobiology: Hands On Demonstrations

June 19, 10:30am - 12:00pm **The Edge** **Chair: Keith Cengel & Imran Rizvi**

THUR18
10:30am-10:55am
Newly Cloned GFP from Rhacostoma Jellyfish and a Novel Spot Test for BPA
William Ward[1], Michael Tota[1]
[1]Center for Research & Education in Bioluminescence & Biotechnology (CREBB), New Brunswick, NJ, USA, [2]Brighter Ideas, Inc., North Brunswick, NJ, USA

THUR19
10:55am-11:15am
CLIPT for Progression of Breast Cancer
Gary Rogers
Rogers Sciences Inc., Beverly, MA, USA

THUR20
11:15am-11:35am
Biostatistical Considerations in Designing Clinical Trials
Mary Putt
University of Pennsylvania, Philadelphia, PA, USA

THUR21
11:35am-12:00pm
A Spreadsheet for Detection of Possible Data Fabrication in Numerical Data Sets of the Type Frequently Encountered in Cell and Radiation Biology Survival Studies
Helene Hill[1], Joel Pitt[2]
[1]Rutgers NJ Medical School, Newark, NJ, USA, [2]Renaissance Associates, Princeton, NJ, USA

Impact of Climate, Environment and Personal Factors on UVR Exposure and its Health Consequences

June 19, 10:30am - 12:10pm **Celebrate** **Chair: Antony Young**

THUR22
10:30am-10:55am
A Climate Model to Predict Population Exposure to UVR in Coming Decades Based on Personal UV Measurements
Peter Philipsen
Department of Dermatology, Copenhagen University Hospital, Bispebjerg, Copenhagen, Denmark

THUR23
10:55am-11:20am
Body Modelling of UVR Exposure Under Different Solar Environments
Alois Schmalwieser[1], Antony Young & team[2], Hans Christian Wulf & team[3], Paul Eriksen & team[3]
[1]University of Veterinary Medicine, Vienna, Austria, [2]Kings College, London, UK

THUR24
11:20am-11:45am
Sun and Ski Holidays Improve Vitamin D Status, But Are Associated With High Levels of DNA Damage
Bibi Petersen[1], Hans Christian Wulf[1], Margarita Triguero-Mas[2], Peter Alshede Philipsen[1], Elisabeth Thieden[1], Peter Olsen[1], Jakob Heydenreich[1], Payam Dadvand[2], Xavier Basagaña[2], Tove Sandberg-Liljendahl[3], Graham Harrison[4], Dan Segerbäck[3], Alois Schmalwieser[5], Mark J Nieuwenhuijsen[2], Antony R Young[4]
[1]Bispebjerg Hospital, Copenhagen, Denmark, [2]CREAL, Barcelona, Spain, [3]Karolinska Institute, Novum, Sweden, [4]Kings College London, London, UK, [5]University of Veterinary Medicine, Vienna, Austria

THUR25
11:45am-12:10pm
Skin Colour has no Effect on Vitamin D Photosynthesis
Antony Young
St. John's Institute of Dermatology, London, UK

ASP Council Meeting & Luncheon

June 19, 12:30pm - 4:00pm **The Edge**

A B S T R A C T S

SUN1

Biosensing of Molecules, Cells, and Tissue Using Metallic Nanoparticles and SERS

Duncan Graham

University of Strathclyde, Glasgow, UK

Metallic nanoparticles offer many opportunities in terms of detection including light scattering, surface plasmon resonance and surface enhanced Raman scattering (SERS). We are interested in the optical properties of metal nanoparticles and their potential application in a range of different biological studies. We can make use of the optical properties of nanoparticles in two ways.

1. The nanoparticle can act as an extrinsic label for a specific biomolecular target in the same way as a fluorescent label is used. The advantage of using the nanoparticle is its optical brightness (typically several orders of magnitude more than fluorophores) and the lack of background vibrational signals. Functionalisation of the nanoparticle with a specific targeting species such as an antibody or peptide aptamer allows this approach to be used in a wide range of studies including cell, tissue and *in vivo* analysis.

2. Nanoparticles can be designed to contain a specific recognition probe designed to cause a change in the aggregation status of the nanoparticles resulting in a discernible optical change when it interacts with its biomolecular target. This allows separation free analysis of specific biomolecular interactions and can be applied to a range of different probe/target interactions such as DNA-DNA, peptide-protein and sugar-protein.

We have been making use of nanoparticles in both of these approaches in conjunction with SERS which is an advanced vibrational spectroscopy. To demonstrate the applicability of the two different approaches examples will be given on the use of nanoparticles for cell imaging in two and three-dimensions, imaging of nanoparticles at centimetre depths through tissue and also their ability to report on biological molecules *in vitro* and *in vivo*. A further property of nanoparticles is their ability to heat up with electromagnetic radiation which will be discussed in relation to hollow gold nanoparticles and their ability to be synthesized to cause localized heating with specific wavelengths of light.

SUN2

Bio-Optics: Enabling Photobiology in the Dark

S. H. Andy Yun

Harvard Medical School, Cambridge, USA

Molecular absorption of photons can trigger a variety of photochemical events useful for therapies. Despite the growing biomedical applications of light-based techniques, a major common challenge has been the difficulty of delivering the activation light deep into the target tissue. Owing to its intrinsic absorption and scattering, the penetration depth of visible or near-infrared light is less than several mm's in tissue. Here, we show that non-radiative resonance energy transfer from bioluminescence molecules to photosensitizers can induce strong cytotoxicity to kill cancer cells in mice in deep tissues that are not accessible by conventional external illumination. This approach based on internal light source may be an effective approach enabling deep-tissue photodynamic therapy and potentially other techniques based on photochemistry and photobiology.

SUN3

Spiders, Silk and Light

David Kaplan

Tufts University, Medford, MA, USA

Silk fibers have a long and important history in textiles and as medical sutures. We have been extending the utility of this unique protein into new medical arenas, exploiting the mechanical, thermal, versatile material formats and optical features due to new understanding of structure-function relationships via different processing routes. With this fundamental insight into self-assembly of silk proteins, tunable material structures and features, including optical systems based on silk biomaterials, have been developed. Importantly, these new systems are fully degradable and compatible in vivo. Further benefits from these new systems include all aqueous processing, tunable degradation rates and facile functionalization with bioactive components. The opportunity to generate implantable medical devices with this broad range of optical and electronic functions, while avoiding the need to remove the devices with a second surgery opens up new horizons in next generation device designs and functions.

SUN4

Dark Materials for Molecular Imaging

Zhen Cheng

Stanford University, Stanford, USA

A variety of molecular platforms including small molecules, peptides, aptamers and nanoparticles have been explored for molecular imaging of diseases. Melanin is a natural dark pigment that can be found in most organisms. It is an amorphous, irregular polymer and composed by mixtures of two different but biogenetically related pigments, eumelanins and phenomelanins. Melanin biosynthesis is an essential metabolic pathway regulated by tyrosinase in cells. In malignant melanoma, melanin formation is highly increased because tyrosinase activity is significantly elevated. Therefore it can serve as a promising molecular target for melanotic melanoma imaging. Imaging probes that either are involved in the melanin biosynthesis pathway or have high affinities with melanin could be developed for melanin targeted imaging. In this lecture, we will present our research on developing benzamide analogs for melanin targeted imaging. More interestingly, melanin can serve as a target for multimodality imaging of diseases including PET, MRI and photoacoustic imaging (PAI). Tyrosinase, the key enzyme in melanin production, was thus explored as a novel reporter gene for PET/MRI/PAI trimodality imaging. Our recent research progress on this direction will be presented in this talk as well.

SUN5

Fluorescent and Singlet Oxygen-Activatable Conjugate of Photosensitizer and Anticancer Drug, Overcoming the Problems of Heterogeneity of PDT by Bystander Effect

Youngjae You, Moses Bio, Pallavi Rajaputra, Gregory Nkepang

OUHSC College of Pharmacy, Oklahoma City, OK, USA

In photodynamic therapy (PDT), heterogeneity of the key variables can cause an incomplete ablation of tumor, resulting in tumor relapse. All the key factors of PDT are heterogeneous such as tumor itself, photosensitizer, light, and oxygen. The incomplete ablation may be in part due to the limited diffusion distance of singlet oxygen, which makes singlet oxygen hard to cause direct bystander effect. To overcome this problem, we developed unique conjugate system composed of photosensitizer and anticancer drug linked by singlet oxygen cleavable linker. Our hypothesis is that the conjugates first damage tumor by PDT effect (i.e., singlet oxygen) during the illumination and at the same time releases the anticancer drug. Then, the released drugs kill surviving cancer cells after the illumination, via bystander effect. The major value of this approach is to take advantage of chemotherapy without the concern for its systemic side effects because extremely low active drug will be needed for killing residual cancer cells. We developed our own singlet oxygen cleavable linker and termed the cleavage of this linker “photo-unclick chemistry”. We prepared conjugates of photosensitizer (core-modified porphyrin or phthalocyanine) and CA4 (combretastatin A-4, anticancer drug). The conjugates showed excellent bystander effect in vitro which photosensitizer was not able to cause the bystander effect. The conjugates also showed superior antitumor effect than their corresponding non-cleavable conjugates. In particular, the conjugate of phthalocyanine and CA4 was optically imaged in live mice to provide information of its distribution in real time. We believe that this new conjugate system could be readily adapted to PDT clinical settings because the dose of anticancer drugs for this regime is far below their toxic dose.

SUN6

Image-Guided Cancer NanoTheranostics with Hollow Gold Nanospheres and CuS Nanoparticles

Chun Li

UT MD Anderson Cancer Center, Houston, TX, USA

The development of biocompatible nanoparticles for molecular imaging and targeted cancer therapy is an area of considerable current interest across a number of disciplines. The premise is that nanoparticles possess unique structural and functional properties that are not available from either small-molecular-weight molecules or bulk materials. However, successful delivery of nanomaterials to the tumor sites requires overcoming many biological barriers, including extravazation from tumor vasculature and dispersion of nanoparticles from perivascular area. In my presentation, I will discuss our experiences towards enhanced delivery of nanoparticles to solid tumors, the development of multi-functional nanoplatforms for image-guided multimodal therapy, and the use of radiation and near-infrared laser as external energy source to facilitate tumor delivery of anticancer drugs mediated by nanoparticles. My discussion will be exemplified by three classes of nanomaterials: water-soluble polymer-drug conjugates, hollow gold nanospheres, and semiconductor nanoparticles.

SUN7

Tumor-targeted, activatable photoimmunotherapy for selective destruction of cancer micrometastases

Bryan Spring[1], Adnan Abu-Yousif[1], Akilan Palanisami[1], Imran Rizvi[1], Xiang Zheng[1], Zhiming Mai[1], Sriram Anbil[1], R. Bryan Sears[1], Lawrence Mensah[1], Ruth Goldschmidt[1], S. Sibel Erdem[1], Esther Oliva[2], Tayyaba Hasan[1]

[1]Harvard Medical School, Boston, MA, USA, [2]Massachusetts General Hospital, Boston, MA, USA

Drug-resistant micrometastases that escape surgical resection and chemotherapy often go undetected until the emergence of lethal recurrent disease. Here, we introduce a platform for treating microscopic tumors selectively using an activatable immunoconjugate comprising a self-quenching, near infrared chromophore (benzoporphyrin derivative, BPD) loaded onto a cancer cell-targeting antibody (cetuximab). This unique approach—termed "tumor-targeted, activatable photoimmunotherapy (taPIT)"—enables (*i*) activatable photodynamic therapy (PDT); (*ii*) activatable fluorescence contrast; and, (*iii*) inhibition of a prominent treatment escape mechanism—survival signalling via epidermal growth factor receptor (EGFR) activation. taPIT mitigates phototoxicity to sensitive organs (e.g., the bowel) to enable 20–50× the photocytotoxic dose compared to the maximum tolerated PDT dose using "always-on" immunoconjugates or unconjugated BPD. Furthermore, a single cycle of taPIT with platinum and taxol chemotherapy achieves 97% reduction of micrometastatic burden—compared to 3% for chemotherapy alone—in a mouse model of intrinsically chemoresistant, micrometastatic ovarian cancer.

SUN8

In Vivo Rapid Cancer Detection and Therapy Based on Rationally Designed Activatable Fluorescence and Photosensitizing Probes

Yasuteru Urano

The University of Tokyo, Tokyo, Japan

Fluorescence imaging is one of the most powerful techniques currently available for continuous observation of dynamic intracellular processes in living cells. Suitable fluorescence probes are naturally of critical importance for fluorescence imaging, but only a very limited range of biomolecules can currently be visualized because of the lack of flexible design strategies for small molecule-based fluorescence probes. We have succeeded to construct several versatile rational design strategies for novel activatable fluorescence (fluorogenic) probes, and recently we found that hydroxymethyl rhodamine green (HMRG) was strongly fluorescent in aqueous solution at pH 7.4, while mono-amidated HMRG derivatives were colorless and non-fluorescent due to the preferred spirocyclized structure.

Based on above findings, we have developed various novel aminopeptidase-sensitive probes which were applicable for living cell system, including gGlu-HMRG, a novel HMRG-based "activatable" fluorescence probe for gamma-glutamyltranspeptidase (GGT). We could establish a novel and highly activatable strategy for sensitive and fast-responding fluorescence imaging of tiny tumors in vivo by spraying gGlu-HMRG onto tissue surfaces that are suspected of harboring tumors, creating high signal contrast between the tumor and the background within 1 min.

We have also developed an activatable photosensitizer capable of specifically inducing death of GGT-overexpressing cells in response to photoirradiation. Using a seleno-rhodamine scaffold, we designed and synthesized gGlu-HMSeR, which takes a non-phototoxic spirocyclic structure due to the presence of the gamma-glutamyl moiety. However, GGT efficiently converts gGlu-HMSeR to phototoxic HMSeR, which exists predominantly in xanthene form. This structural change results in drastic recovery of visible wavelength absorption and the ability to generate singlet oxygen. When gGlu-HMSeR was applied to cancer cells-inoculated chick chorioallantoic membrane (CAM), photoirradiation induced specific cancer cell death without any damages to the normal cells.

1. Urano Y, et al., Sci. Transl. Med., 3, 110ra119 (2011). 2. Sakabe M, et al. J. Am. Chem. Soc., 135, 409-414 (2013). 3. Urano Y, et al., Nat. Med., 15, 104-109 (2009).

SUN9

Novel cellular activities targeting UV damage recognition in chromatin

Ling Zhang, Abigail Lubin, Hua Chen, Leah Nemzow, Feng Gong

University of Miami, Miami, FL, USA

DNA damage induced by UV irradiation is repaired by the nucleotide excision repair (NER) pathway. How NER operates in chromatin is not completely understood. Recently we and others have shown that chromatin remodeling activities facilitate NER in chromatin. BRG1 is a catalytic subunit of the human SWI/SNF chromatin remodeling complex. Inactivation of BRG1 sensitizes mammalian cells to various DNA damaging agents, including ultraviolet (UV) and

ionizing radiation. It was proposed that chromatin remodeling activities are utilized to increase the accessibility of the NER proteins and checkpoint factors to the damaged DNA. Indeed, using a micropore UV radiation technique, we demonstrated that recruitment of the UV damage recognition protein XPC, as well as the checkpoint factor BRCA1, to sites of UV lesions is disrupted when BRG1 is depleted in mammalian cells. We note that BRCA1 contributes to UV damage response by promoting photoproduct excision, triggering post-UV checkpoint activation and post-replicative repair. These findings suggest that the SWI/SNF chromatin remodeling complex plays a role in the repair of UV-induced DNA damage by facilitating UV damage recognition. Unpublished data on novel deubiquitinating activities targeting UV damage recognition will also be discussed.

SUN10

Absence of UV-induced cancer in the human cornea; a comparative study of UV-induced pyrimidine dimers repair and cell death in human corneal epithelium and epidermis

Justin D. Mallet, Marie-Catherine Drigeard Desgarnier, Sébastien P. Gendron, Patrick J. Rochette

Axe Médecine Régénératrice, Centre de Recherche FRQS du CHU de Québec, Hôpital du Saint-Sacrement and Centre LOEX de l'Université Laval, Québec, Qc, Canada

The human eye and skin are both exposed to sun's ultraviolet (UV) light. Exposure to solar UV wavelengths has a well-defined genotoxic effect on skin cells. Absorption of UV radiations by DNA leads to the formation of the predominant UV-induced DNA damage, the cyclobutane pyrimidine dimer (CPD). CPD are considered the most pro-mutagenic UV-induced DNA adduct and have a demonstrated role in the initiation of sun-related skin cancers. We have shown that UV radiations induce a large amount of CPD in the cornea and mainly in its foremost layer, the epithelium. It is thus unexpected that despite the UV-induction of mutagenic CPD in the cornea, no sun-related cancer has ever been reported in this ocular structure.

We have analyzed and compared the response to genotoxic stress for cell strains derived from the skin epidermis (NHEK) and the corneal epithelium (HCEC). The transcriptome analysis by microarray, even though confirming great differences in gene signature between cell types, shows no important difference in the expression of stress response genes. In accordance to these results, we have found a similar sensitivity to UVB-induce cell death in both cell types. Nonetheless, our results clearly demonstrate that UVB-induced CPD are repaired significantly faster in HCEC (45% of initial CPD are repaired 12hrs post-UVB treatment) as opposed to NHEK (12% in 12hrs) in both *in vitro* and *ex vivo* models. We further analyzed the implication of nucleotide excision repair (NER), the sole responsible of human CPD removal, in this preferential repair. Our results show that the level of DDB2, responsible of CPD recognition in NER, is 1.8 times higher in HCEC as opposed to NHEK. At the transcription level, qPCR analyses revealed a 2 fold increase of DDB2 transcript in NHEK when compared to HCEC. This suggests a more efficient stabilization mechanism for DDB2 present in HCEC that is possibly responsible for the proficient repair of CPD found in this cell type.

Our results indicate there is no involvement of UV-induced cell-death sensitivity in the capacity of corneal epithelial cells to avoid UV-induced tumorigenesis, but it is rather, at least in part, due to a fast repair of CPD. Furthermore, we suggest that a greater stability of DDB2 in HCEC may be responsible for this highly efficient CPD repair.

SUN11

E2F1 and RB Direct histone Acetylation at Sites of DNA Damage

David Johnson, Renier Velez-Cruz, Swarnalatha Manickavinayaham, Anup Biswas, David Mitchell

University of Texas MD Anderson Cancer Center, Science Park, Smithville, Texas, USA

The E2F1 transcription factor regulates the expression of genes involved in cell proliferation, apoptosis and differentiation. In addition, E2F1 accumulates at sites of DNA damage and directly stimulates DNA repair in a process involving E2F1 phosphorylation by the ATM or ATR kinases. At sites of ultraviolet (UV) radiation–induced DNA damage, E2F1 recruits the GCN5 histone acetylatransferase (HAT) and mediates the acetylation of histone H3 lysine 9 (H3K9ac). This correlates with relaxation of chromatin structure, increased recruitment of nucleotide excision repair factors to sites of damage, and enhanced DNA repair efficiency. In contrast to UV-induced DNA damage, E2F1 is not required for GCN5 recruitment or the induction of H3K9ac at sites of DNA double-strand breaks (DSBs). Instead, E2F1 is involved in the recruitment of other HATs and the induction of different histone acetylation marks at DSBs. E2F1 also recruits the retinoblastoma (RB) tumor suppressor to DSBs where it participates in HAT recruitment and chromatin remodeling to facilitate DNA repair. Mutation of the conserved ATM/ATR phosphorylation site in E2F1 (serine 29 in mice) has little impact on the expression of E2F target genes but prevents E2F1 association with damaged DNA and reduces DNA repair efficiency. Moreover, E2F1 serine 29 mutant

mice display increased sensitivity to UV-induced skin carcinogenesis and are hypersensitive to ionizing radiation. These findings thus link transcription-independent functions for E2F1 in modifying chromatin structure at sites of DNA damage with radiation sensitivity and tumor suppression.

SUN12

Impact of irradiating skin diploid fibroblasts with chronic low dose of UVB on nucleotide excision repair

Marie-Catherine Drigeard Desgarnier[1], Régen Drouin[2], Patrick Rochette[1]

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Cyclobutane pyrimidine dimers (CPD) are the main photoproducts induced by UVB. In human, these damage must be efficiently repaired by nucleotide excision repair (NER) in order to avoid mutations. Although the two NER pathways (i.e. transcription-coupled NER; TCNER and global genome NER; GGNER) has been extensively studied, it has never been tested whether conditioning cells with chronic low UVB doses would have an impact on NER activation. We hypothesized that irradiating cells with chronic sublethal low doses (CLD) of UVB stimulates the NER pathway, which leads to a more efficient repair of acute UVB-induced CPD. Moreover, we suspect this effect will be more important in genes implicated in genome integrity maintenance, like p53 gene.

Human diploid dermal fibroblasts were subjected to the CLD regime, which constitute of 100 J/m² UVB every 12 hours for 7.5 days (15 irradiations, total 1500 J/m²). Twelve hours following the last chronic irradiation, cells were irradiated with an acute UVB dose (400 J/m²) and DNA was harvested at 0, 6, 12 and 24h post irradiation. Our results show that accumulated CPD from CLD regime are unrepaired 24h following the last irradiation. On the other hand, newly formed CPD from the acute dose are repaired significantly faster in cells with the CLD treatment. Moreover, we precisely analyzed CPD repair at a single-nucleotide resolution on p53 gene using ligation-mediated PCR (LMPCR). While genome-wide results show an enhancement of repair for newly formed CPD in CLD treated cells, the LMPCR analysis shows that CPD repair on the non-transcribed strand of an active gene (i.e. p53) is greatly enhanced by the CLD treatment.

In order to analyze the consequences of unrepaired DNA lesions from the CLD treatment at the chromosome level, we used immunocytofluorescence technique. We found that CPD accumulate on the chromosome at a particular hotspot still under investigation. We also show that CPD are, in some metaphases, found only in one of the two sister chromatids, indicating that accumulated CPD does not prevent DNA replication.

Taken together, our results indicate that NER can be stimulated by a CLD treatment. On the other hand, the CLD regime leads to the accumulation of CPD that are tolerated in the genome.

SUN13

Retinoblastoma Protein Influences Susceptibility to DNA Damage via Chromatin Regulation

Ashby Morrison

Stanford University, Stanford, CA, USA

Cancer results from the attainment of multiple genetic alterations, which produce a sustained proliferative and survival advantage. It is known that DNA lesions caused by exposure to carcinogens often initiate these mutations. Determinants of lesion acquisition include the DNA sequence and its chromatin environment, which is exceptionally dynamic in coordination with varied DNA-templated processes. States of relaxed and condensed chromatin are mediated by chromatin modifiers, including enzymes that post-translationally modify histones and alter nucleosome positioning. Relaxed chromatin is more vulnerable to DNA lesion acquisition when exposed to genotoxic agents, compared to condensed chromatin. Interestingly, factors that directly influence genome maintenance and cancer development, such as the retinoblastoma (RB) tumor suppressor, increase global chromatin relaxation when disrupted, through mechanisms that include cell cycle control, transcriptional regulation, and heterochromatin formation.

Here we demonstrate that manipulation of both the RB tumor suppressor pathway and histone modifications has a dramatic affect on susceptibility to carcinogen-induced DNA damage. Moreover, markers of open chromatin are abundant in cancer cells and correlate with prognostic markers. These results reveal that genome stability pathways can function through chromatin-mediated mechanisms to alter lesion acquisition following exposure to carcinogens. Furthermore, they suggest mechanisms by which precancerous cells can acquire mutations necessary for malignant transformation.

SUN14

Antimicrobial blue light against skin and soft tissue infections

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Skin and soft tissue infections (SSTI) are the second most common infections encountered in clinical practice and affect millions of individuals annually in the United States. Management of SSTI has been significantly complicated by the increasing emergence of multidrug-resistant pathogens. In this study, we investigated the effectiveness and safety of antimicrobial blue light (415 nm) against SSTI using burn infections in mice as the model.

Pseudomonas aeruginosa, *Acinetobacter baumannii*, and *Candida albicans* were studied. All strains of the pathogens are bioluminescent, allowing real-time monitoring of the extent of infection in vivo by bioluminescence imaging. The susceptibility of the pathogens to blue light inactivation was compared in vitro with that of human keratinocytes. Repeated cycles of sub-lethal inactivation of the pathogens by blue light were carried out to investigate the potential resistance of these pathogens to blue light inactivation. Mouse models of 3rd degree burn infected with the pathogens were developed. A single exposure of blue light was delivered at 30 min after inoculation to each infected mouse burn. TUNEL assay was performed to evaluate potential blue light induced DNA damages in skin cells in vivo.

All pathogens were significantly more susceptible (tens of fold) to blue light inactivation than human keratinocytes. Transmission electron microscopy revealed blue light-mediated ultrastructural damage in pathogen cells. Fluorescence spectroscopy suggested the presence of endogenous porphyrins inside the pathogen cells. A single exposure of blue light at 55.8 J/cm^2 significantly reduced the bacterial/fungal burden in mouse burns (16-100 fold), and saved the lives of mice in the event of potentially lethal *P. aeruginosa* infections. No elevated bacterial/fungal resistance to blue light inactivation was observed after 10 cycles of sub-lethal inactivation of the pathogens. No significant DNA damage was detected in mouse skin after a blue light exposure of 195 J/cm^2.

In conclusion, antimicrobial blue light is potentially an effective and safe approach against SSTI, including those caused by multidrug-resistant pathogens.

SUN15

Photodynamic Control of Malaria Vector and Other Parasites in Infested African Swamps

Mahmoud Abdel Kader

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According to the latest WHO malaria report, 2013, an estimate of 627,000 malaria deaths worldwide in 2012, 90% from sub-Saharan Africa where 77% were children under the age of 5. Malaria vector control is usually achieved by Indoor Residual Spraying (IRS) using DDT and/or by the use of nets embedded with Permethrin.

In this work, we present successful field implementation of using a photodynamic modality to control vector-borne diseases, such as Malaria, Filaria, Dengue fever, Schistosomiasis and fasioliasis vectors in infested epidemic swamps in Uganda, Ethiopia and Sudan.

Field trials were performed using chlorophyll derivatives as a photosensitizer which was approved by the FDA as food additives. It was added to the infested swamps to be uptaken by the mosquito larvae. The accumulated chlorophyll derivatives inside the larvae body induce, upon sunlight exposure, oxidative stress, which cuts off the life cycle of the parasite and results in organism death.

Small scale field implementation was done in Kasangati and Namanve cities of Wakiso, a district in Uganda, by applying chlorophyll derivatives as a sunlight active photosensitizer to infected swamps and sand pits (4gm/ m²). The results revealed 85% to 100% mortality of larvae population was obtained at different concentrations of chlorophyll derivatives (0-100 µm). The formulated photosensitizer used, achieved target selectivity where all other biological beneficiary organisms (which were present in the same treated swamps) were not affected.

Using the same technique of treatment, a successful integrated control of all different stages of the life cycle of Schistosomiasis (eggs, Miracidium, and cercaria) was achieved with high efficiency. In case of snail vector control, intermediate host of Schistosomiasis (Biomphalaria alexandrina) mortality percentage ranged from 80% to 90%. As for fasciolia snail vector control (Lymnae natalensis), the efficiency of photosensitization process on the snail eggs' hatchability has been investigated. The results revealed that the mortality rate percentage varies from 70% to 80% according to the different environmental parameters.

In conclusion, the field results shows promising success in controlling vector- borne diseases by cutting the parasite's life cycle without new generation, or re-infestation.

SUN16

Photodynamic inactivation of plant-pathogenic fungi - So what is stopping us?

Henrique D. de Menezes[1], Gabriela B. Rodrigues[1], Simone de Pádua Teixeira[1], Nelson S. Massola Jr[2], Luciano Bachmann[1], Mark Wainwright[3], Gilberto U. L. Braga[1]

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The control of plant-pathogenic fungi faces some of the problems that have been observed in the related clinical area, including the selection of antifungal-tolerant strains and the relatively few classes of currently available and effective fungicides. Antimicrobial photo treatment (APT) is a promising antifungal alternative that can be used to control localized mycoses or to kill fungi in the environment. The process is based on the use of a photosensitizer (PS) that preferentially accumulates in the target fungal cell. PS exposure to light of an appropriate wavelength initiates photochemical processes that kill the fungal cells without significant damage to the host. We demonstrated that treatments with coumarins, furocoumarins and phenothiazinium photosensitizers in combination with solar radiation were highly effective in killing conidia of the plant-phatogenic fungus *Colletotrichum acutatum*. APT with NMBN (50 µM) and S137 (10 µM) resulted in a reduction of approximately 5 logs in the survival of the conidia. APT with the furocoumarin 8-MOP (50 µM) and with a mixture of two coumarins (7-methoxycoumarin and citropten) (12.5 mg mL^{-1}) extracted from Tahiti acid lime led to a reduction of approximately 4 and 3 logs in conidial survival, respectively. All PS penetrated the conidia and accumulated in cytoplasmic vesicles such as lipid bodies. No damage to orange tree leaves was observed after APT with the photosensitizers. APT efficacy to kill plant-pathogenic fungi and the lack of damage to the host are two essential prerequisites for the use of APT in the field. So what is stopping us to explore the potential of APT to control phytopathogens?

SUN17

Carbon Flux Modulates the Sensitivity of the Pathogenic Fungus *Candida albicans* to PDT.

Constantine Haidaris

University of Rochester Medical Center, Rochester, NY, USA

Most infections caused by the pathogenic fungus *Candida albicans* are superficial, and are thus attractive targets for photodynamic therapy (PDT). *C. albicans* rapidly adjusts its central metabolism to facilitate its survival and growth in response to nutritional conditions its host niche. This metabolic flexibility also allows the fungus to adapt to environmental stresses. We investigated the connection between central carbon metabolism in *C. albicans* and the response to oxidative stress induced by PDT *in vitro*. When comparing carbon sources, distinct responses were observed depending on the specific carbon source and time of exposure. When subjected to the same PDT conditions, cells grown to early stationary phase in medium using several different alternative carbon sources resulted in a 2-3 $\log_{10}$ greater reduction of *C. albicans* colony forming units compared to cells grown using glucose. Surprisingly, regardless of how the cells were grown initially, PDT conditions resulting in a 2-3 $\log_{10}$ reduction of *C. albicans* CFU could be completely blocked if respiratory substrates such as lactate, pyruvate or acetate were present during irradiation. In contrast, glucose, glycerol or ethanol did not block killing. Selected amino acids, including glutamine, also protected *C. albicans* against PDT. The rapidity of the protective response, within minutes of exposure to the selected carbon source, suggested that metabolic pathways are in place to act as a mechanism of immediate protection against PDT. The carbon sources providing protection against PDT indicated the tricarboxylic acid (TCA) cycle and the gamma-glutamyl cycle responsible for glutathione (GSH) synthesis were involved in the protective response. The TCA and gamma-glutamyl cycles are inter-connected via the TCA cycle intermediate 2-oxoglutarate and glutamine. Glutamate synthase deaminates glutamine in *C. albicans* to yield glutamate for synthesis of GSH, as does the reaction of glutamine and 2-oxoglutarate with NADPH. Glutamine is also used to generate 2-oxoglutarate via a glutamate intermediate. Furthermore, 2-oxoglutarate and GSH are ROS scavengers. Our results strongly suggest that carbon flux between the TCA and gamma-glutamyl cycles is used by *C. albicans* to rapidly counter oxidative stress. Given that the protective carbon sources are readily available to the fungus in the human host, this rapid response may have implications for the efficacy of PDT against *C. albicans in vivo*.

SUN18

UVB radiation induces both beneficial and deleterious effects in a localized skin infection with *Mycobacterium ulcerans* in the hairless guinea pigs

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Mycobacterium ulcerans (MU) is an emerging human pathogen responsible for a severe skin condition known as Buruli Ulcer Disease. It is considered as the third most frequently occurring mycobacterial infection in humans after *M*. *tuberculosis (Mtb)* and *M*. *leprae*. Previous studies from our laboratory demonstrated that UVB radiation (UVR) suppressed the skin test response to purified protein derivative (PPD), increased the colony forming units (CFU) in the lungs and upregulated IL-10 mRNA expression after aerosol infection with *Mtb* in guinea pigs (GP). Using a localized skin infection with *MU* in the hairless GP (Crl:IAF(HA)-hrBR), we examined whether exposure to UVR (5.6 kJ/m^2) induces such deleterious effects after infection ($1x10^6$ CFU) through the UV-irradiated site (Local UV model) or at a distant un-irradiated site ((Systemic UV model). UVR induced a significant reduction in the lesion size at the site of infection and the skin test response to *MU* antigen was suppressed after 28 days of infection in both the UV-irradiated groups when compared to the un-irradiated *MU*-infected controls. Surprisingly, exposure to UVR significantly reduced the CFUs in the skin whether the infection was given at the site of UVR or at a distant un-irradiated site. However, IL-10 mRNA expression was significantly increased in the lymph node cells after stimulation with heat-killed *MU* or whole cell lysate in the GPs infected through the UV-irradiated site while the expression of IL-4 mRNA expression was significantly increased in both groups of UV-irradiated animals. Thus, our previous reports along with the current findings suggest that UVR has a beneficial effect when the infection is localized *(MU)* while it induces deleterious effects as the infection (*Mtb*) disseminates from the site to other organs. These results seem to be consistent with the effect of UVR in humans as exposure to natural sunlight improved the lesions in patients with skin tuberculosis but it exacerbated pulmonary TB infection as reported in early 1900s. Furthermore, GP serves as a good small animal model for distinguishing the effects of UVR in a localized or disseminated infection with mycobacteria. *Supported by NIH grant R21-AI79463 and Texas A&M HSC Bridge funding (AJ)*.

SUN19

Bad Bugs – New Photosensitizers – No "ESKAPE" against antimicrobial PDT

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The CDC estimates that in the United States, more than two million people are sickened every year with antibiotic-resistant infections, with at least 23,000 dying as a result. Bacteria are very good in developing resistance against antibiotics in a short time. So far bacteria have adapted to a point where they pose serious clinical challenges for humans worldwide. The leading bacteria are ***E****nterococcus faecium*, ***S****taphylococcus aureus*, ***K****lebsiella pneumonia*, ***A****cinetobacter baumannii*, ***P****seudomonas aeruginosa* and ***E****nterobacter* species which are called the "ESKAPE" pathogens, because they currently cause the majority of hospital infections and therefore effectively "escape" the effects of antibiotics.

These species are superbugs, because they are multi-resistant towards more than one different class of antibiotics. In recent years topical application of antimicrobial agents has become unpopular because of the worst development of resistance. Furthermore the Infectious Diseases Society of America (IDSA) highlights that over the past several years, the number of new antibacterial drugs approved continues to decrease.

Therefore new approaches like antimicrobial PDT (APDT) will become more important in the future as antimicrobial resistance is expected to continue to increase.

Firstly this talk gives a snapshot about the antibiotic resistance threats based on the CDC report 2013. Secondly this talk summarises the potential candidates of new photosensitizers which are useful for antimicrobial PDT. Thirdly APDT is a localized process which may prevent that a localized infection becomes worst systemic (sepsis). Taken together the most promising clinical applications of APDT are (i) decolonization of pathogens on skin, (ii) treatments of the oral cavity like parodontitis, endodontitis and mucosis and (iii) superinfected burn wounds, because these are relatively accessible for photosensitizer application and illumination.

SUN20

A Lipidomic Approach to Identify Minute Differences Among *Staphylococcus aureus* Strains. Possible Role in Antimicrobial Photoinactivation.

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Background: Light- and photosensitiser-based antimicrobial photodynamic inactivation (PDI) is a very promising approach to control microbial infections. However, huge differences in vulnerability to PDI exists among clinical isolates belonging to the same species. It is currently of interest to explore how the phenotypic, and genotypic features of a microorganism affect its response to photosensitizer-based photoinactivation. We focused our attempts on the analysis of bacterial cellular lipid content, due to the fact that certain photosensitizers are hydrophobic molecules efficiently interacting with cellular membranes. To understand the mechanisms governing the variations in a strain-dependent response to PDI, specifically the possible role of lipids in the observed phenomenon, we developed the mass spectrometry-based approach (RP-LC-Q-TOF-MS) to build a comprehensive lipid profile one of the representative of so called alarm pathogens, *S. aureus*. The developed method appeared to be able to decipher even the minute differences in lipidome of our model bacterium *S. aureus*. The method allowed to identify, in qualitative as well as a semi-quantitative manner, the differences in lipid profile of the two strains differing with antimicrobial resistance pattern, and two isogenic *S. aureus* strains differing with a single protein status (active vs. inactive). *Staphylococcus. aureus* strain with inactivated heme transporter (HrtA), is significantly more prone to porphyrin-based photokilling in comparison to the wild type strain. Moreover, the observed differences cannot be explained by the differences in accumulation of the photosensitizer. We first identified, via non-targeted lipid profiling, the differences in lipid profiles of the two analyzed strains, and second, the observed differences were confirmed by advanced statistical methods. Further, the lipids of interest were identified and their possible role regarding photoinactivation is discussed.

SUN21

Polysaccharides and photosensitizers: new materials and surfaces for antimicrobial photodynamic therapy

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Nosocomial infections have become a major concern, complicated by the emergence of multidrug-resistant microbial strains such as the so-called superbugs belonging to *Staphylococcus aureus* and *Escherichia coli* bacterial species. Hospital equipments, including polymeric materials and textiles, are potential vectors for microbial dissemination. As a consequence, interest has grown in the preparation of materials with antibacterial properties and numerous research works on antimicrobial surfaces have been published.[1] Photosensitizers (PS) such as porphyrins have been intensively studied for their photobactericidal effects in Photodynamic Antimicrobial ChemoTherapy (PACT).[2] In connection with our research program on development of biomaterial from natural polymers with new properties. We have developed a new cellulosic material bearing a covalent linker between polymeric surface and new antimicrobial molecules. Indeed, cellulose is an excellent starting material for developing a more sustainable material from renewable resources. Then, we have developed antimicrobial plastic films based on cellulose esterified by porphyrins and lauric acid or chloroacetate.[3] Antimicrobial textiles or papers have been obtained from a cotton fabric or filter paper by grafting of hydrophilic porphyrins on cellulose using click chemistry reaction or 2,4,6-trichloro-1,3,5-triazine in aqueous medium.[4,5] In parallel, we have developed new photosensitizers -nanomaterials like iron oxide magnetic nanoparticles coating by dextran for potential application in antimicrobial photodynamic therapy. Antimicrobial activity of porphyrin-cellulose materials was tested under visible light illumination against *Staphylococcus aureus* and *Escherichia coli*.

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SUN22

Porphyrins In the Photodynamic Inactivation of Microorganisms Beyond the Medical Scope

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In spite the discovery of light-activated antimicrobial agents was reported in the 1900s only more recently research work towards the use of photodynamic process as an alternative to more conventional methods of inactivation and/or destruction of micro(organisms) is being developed. It is well-known that the photoprocess causes cell death through irreversible oxidative damage by reactive oxygen species produced by the interaction between a photosensitizing compound and a light source.

With greater emphasis on the environmental area, the photodynamic inactivation (PDI) has been tested successfully in insect eradication or in water disinfection and more recently, it is considered the possible use in aquaculture waters or to the control of food-borne pathogens. Other potential applications of PDI in industrial and hospital settings have been considered.

In the last decade, scientific research in this area has gained importance due to great developments in the field of materials chemistry but also because of the serious problem of the increasing number of bacterial species resistant to common antibiotics. In fact, the design of antimicrobial surfaces or self-cleaning materials is a very appealing idea from the economic, social and public health standpoints. Thus, PDI of microorganisms, being a non-antibiotic approach, is a promising alternative.

In this communication will be discussed some examples of the efforts made in the last decade in the investigation of PDI of (micro)organisms with potential applications beyond the medical field, focusing on porphyrins as photosensitizing agents.

Acknowledgments

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SUN23

Unblinded by the Light: Photobiomodulation in Retinal Disease

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Retinitis pigmentosa (RP) is an inherited retinal degeneration and the most common cause of inherited blindness in the developed world. There is no prevention or treatment for RP. Considerable evidence supports a key role for photoreceptor mitochondrial dysfunction and oxidative damage in the pathogenesis of photoreceptor death in RP. Photobiomodulation (PBM) using low-intensity far-red to near-infrared (NIR) light has been shown to act on mitochondria-mediated signaling pathways to preserve mitochondrial function, attenuate oxidative stress, stimulate the production of cytoprotective factors and prevent neuronal death in experimental and clinical studies. Our research sought to answer the following questions: *Will PBM protect against photoreceptor death and preserve retinal function in a well-established rat model of retinitis pigmentosa, the P23H rat? If so, does PBM act by promoting mitochondrial integrity and function and activating neuroprotective pathways in the retina?* Our results show that photobiomodulation attenuates photoreceptor death and preserves retinal function in the P23H rat model of retinitis pigmentosa.. Specifically we have shown that (1) PBM reduces photoreceptor cell death and stimulates cytoprotection in early states of P23H retinal dystrophy, (2) PBM has no adverse effects on retinal structure or function in non-dystrophic control rats, (3) PBM protects against photoreceptor cell death when administered early or late in the process of retinal degeneration in the P23H rat, (4) mitochondria are dysfunctional in the P23H retina and (5) PBM restores mitochondrial function in the P23H retina to control values. These results have direct clinical implications for many types of retinal degenerative disease, including age-related macular degeneration and diabetic retinopathy.

SUN24

Effects of blue LED-light on hemodynamic parameters of human skin *in vitro* and *in vivo*

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Insufficient hemodynamics of limbs can cause chronic wounds and neuropathy. Local hemodynamic disorders as well as chronic wounds can be significantly improved by exogenous application of nitric oxide (NO). Recent studies suggest that local blood flow can be significantly enhanced by non-enzymatic NO generation via blue LED light (453 nm) decomposing cutaneous photo-labile NO derivates like nitrite. Thus usage of blue light offers an opportunity for a local therapeutic approach of hemodynamic disorders.

In the present study, we non-invasively measured *in vivo* the impact of different irradiation algorithms of blue LED light on local skin perfusion treating ten healthy volunteers by micro-light guide spectrophotometry. For risk evaluation, potential toxic, corrosive, or irritative effects of the light treatments were assessed *in vitro* with human skin specimens or artificial human skin samples. We compared unpulsed and pulsed irradiation algorithms on untreated or nitrite-incubated skin: unplused blue LED light with irradiance averages of 34 mW/cm^2 (S1) and 58 mW/cm^2 (S2) versus pulsed light with irradiance averages of 50 mW/cm^2 and irradiance peaks of 100 mW/cm^2 (S3) and 200 mW/cm^2 (S4). NO and its derivates (nitrosylated thiols) were quantified by chemiluminescence detection.

Irradiation with unpulsed or pulsed blue LED light with doses up to 52 J/cm^2 neither caused any necrotic nor apoptotic events in human skin specimen with or without prior nitrite treatment. Nevertheless, blue LED light with small doses (≤ 52 J/cm^2) already induced a release of NO in untreated skin specimen. Beyond that we detected an increase of blood flow in healthy volunteers with similar light doses correlating with the height of irradiance peaks (blood flow increase: S1<S2<S3<S4). Blue LED light with irradiance peaks of 200 mW/cm^2 (S4) doubled the blood flow in 6 to 8 mm skin depth and tripled the superficial flow (1 to 2 mm depth). However, the increasing surface skin temperature of volunteers during irradiation correlated with the average of irradiance, thus the higher and longer lasting enhancement of blood flow by pulsed light irradiation could be temperature independent.

The observed effects suggest that blue LED light is an effective enhancer of cutaneous blood flow. Furthermore, the therapy efficiency might be even enhanced by using high irradiances in a pulsed modus.

SUN25

Can near-infrared light induce the brain to heal itself?

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Low-level laser (or light) therapy (LLLT) is attracting growing interest to treat both stroke and traumatic brain injury (TBI). The fact that near-infrared light can penetrate into the brain allows non-invasive treatment to be carried out with a low likelihood of treatment-related adverse events. It is proposed that red and NIR light is absorbed by chromophores in the mitochondria of cells leading to changes in gene transcription and upregulation of proteins involved in cell survival, antioxidant production, collagen synthesis, reduction of chronic inflammation and cell migration and proliferation.

We developed two different models of TBI in mice; a closed head weight drop and an open skull controlled cortical impact (CCI). Transcranial laser therapy consisting of a single exposure 4-hours post TBI to 36 J/cm^2 of 810-nm laser was delivered to both the closed head and CCI models and significantly improved neurological severity score in TBI up to 4-weeks post TBI. Pulsing the laser at 10 Hz gave even better improvement that CW or 100 Hz. We then examined the effect of 0, 1, 3, and 14 daily 810-nm laser treatments in the CCI model. 1 laser Tx gave a significant improvement while 3 laser Tx was even better. Surprisingly 14 laser Tx was no better than no treatment. Histological studies at necropsy suggested that the cortical lesion was repaired by neural progenitor (stem) cells from the subgranular layer of the dentate gyrus of the hjppocampus and the subventricular zone of the lateral ventricle, possibly stimulated by the laser. Brain derived neurotrophic factor (BDNF) was increased in the hippocampus and double-cortin and TUJ-1 which are markers of migrating neuroprogenitor cells were upregulated at 7 days. Synapsin1 expression was increased at 28 days. These data suggest that transcranial laser therapy is a promising treatment for acute (and chronic TBI) and may have much wider applications to neurodegenerative and psychiatric diseases. The lack of side-effects and paucity of alternative treatments for brain diseases encourages early clinical trials.

SUN26

Near infrared light-induced protection of heart during reperfusion

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Far red/near-infrared light (NIR) promotes a wide range of biological effects including tissue protection but how NIR is capable of acutely protecting myocardium against ischemia and reperfusion injury *in vivo* is not known. We tested the hypothesis that NIR exposure immediately before and during early reperfusion protects rabbit and mouse myocardium against infarction through mechanisms that are nitric oxide (NO)-dependent but nitric oxide synthase (NOS) independent. Particularly we were interested to probe whether NIR elicits protection in a diabetic model were other cardioprotective interventions such as pre- and postconditioning fail. Rabbits or mice subjected to a 30 min left anterior descending coronary artery occlusion and reperfusion received no NIR (control) or continuous NIR (beginning 1 min before and ending 4 min after reperfusion; 660 nm, 50 mW/cm^2 at epicardial surface). NIR reduced infarct size dose dependently. Importantly, NIR-induced protection was preserved in a diabetic mouse model (db/db) and during acute hyperglycemia, as well as in endothelial $NOS^{-/-}$ mice and in wild type mice treated with NOS inhibitor L-NAME. In *in vitro* experiments R/NIR light liberates NO from HbNO and MbNO in a wavelength (660-950 nm) and dose-dependent manner. Irradiation at 660 nm yields the highest release of NO, while at higher wavelengths a dramatic decrease of NO release can be observed. Similar wavelength dependence was observed in protection of mice against cardiac ischemia and reperfusion injury *in vivo*. NIR-induced NO release from deoxymyoglobin in the presence of nitrite mildly inhibits respiration of isolated mitochondria after hypoxia. A hemoglobin based oxygen carrier (HBOC) was also tested whether it enhanced NIR-induced protection. In summary, NIR applied during reperfusion protects the myocardium against infarction in an NO dependent, but NOS-independent mechanisms, whereby mitochondria may be a target of NIR-induced NO, leading to reduced reactive oxygen species generation during reperfusion. This unique mechanism preserves protection even during diabetes where other protective strategies fail.

SUN27

Induction of Regulatory T cells by 670nm Light in a model of Autoimmunity

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) characterized by an immune-mediated attack on CNS axons. While early disease is due to autoimmunity, disease progression is due to increased oxidative stress and death of the axon. Current therapies are only partially effective because they only target the immune response and do not protect against oxidative stress. In addition to destructive autoimmunity, a role of protective regulatory T cells in preventing disease is recently recognized. Experimental Autoimmune Encephalomyelitis (EAE) is the primary animal model for MS, sharing clinical and histopathologic similarities with MS. The EAE model has been instrumental in developing therapeutic strategies. Previous data from our lab demonstrated the therapeutic efficacy Low Level Light Therapy (LLLT) with 670nm light in the amelioration of EAE through down-regulation of pro-inflammatory mediators, up-regulation of anti-inflammatory cytokines, and protection of CNS cells from apoptotic cell death. A clear understanding of the mechanism by 670 nm light LLLT is critical to the approval of this novel therapeutic strategy for the treatment of MS. Data will be presented that demonstrates the induction of protective regulatory T cells by 670 nm light. These cells are expected to play a direct role in the protection against clinical disease afforded by 670 nm light.

SUN28

Learning from Nature - Supramolecular Photocatalysis Mediated By Cucurbiturils

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Supramolecular container compounds present an ideal opportunity to investigate catalytic process that mimic the action of enzymes in biological systems. Cucurbiturils are a family of molecular container compounds with shapes similar to that of pumpkins that feature a cavity similar to that of cyclodextrins. CB's processes superior molecular recognition properties with very high binding affinities to organic and organometallic compounds. Additionally, these container compounds are water-soluble and are optically transparent above 230 nm. But to successfully employ CBs as nano-reaction vessels for manipulating synthetic transformations in particular photochemical transformations, it is critical to employ CBs in catalytic amounts in order to overcome a fundamental bottleneck *viz*., solubility of CB[8] in high amounts (>0.2 mM) that is typically employed for synthetic reactions. The presentation will focus employing water-soluble nano-containers known as Cucurbit[8]uril (CB[8]) in catalytic amounts to control photochemical reactions. The [2+2] photodimerization of coumarin derivatives will be presented as a model system. The presentation will focus on the plausible reasoning for the observed product selectivity,

kinetic/thermodynamic aspects and photophysics of encapsulated guest molecules leading to supramolecular catalysis.

SUN29

The use of metallic nanoparticles to enhance the production of singlet oxygen.

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In the past decade the metal enhancement effect has been investigated for its unique influence on fluorescence emission, and increase in fluorescence intensity is readily observed for a fluorophore in close proximity to a metal nanoparticle. On the contrary, studies involving enhancement of singlet oxygen production by metal nanoparticles are relatively scarce and so far only stationary silver island films have been proven to be adequate to do so. The application of metal enhancement effect in photodynamic therapy is therefore limited since the metal colloids are anchored onto glass or polymeric substrates in the case of silver island films. In the study presented herein, we have engineered novel nanoparticles based on a core-shell approach on which a photosensitizer has been covalently tethered to the nanoparticle shell. As a proof-of-concept, we developed a silver nanoparticle coated with a silica shell decorated with Rose Bengal. These nanoparticles were not only able to generate singlet oxygen, but its production was greatly amplified. To further the investigation, we engineered similar nanoparticles where the core has been replaced by gold. These nanoparticles proved to be more stable under high chlorine concentration while keeping a high singlet oxygen production. All these new nanoparticles open the doors to new nanotechnologies to be used in photodynamic therapy.

SUN30

Photosensitization in drug - cucurbit[n]uril - protein ternary complexes

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Cucurbit[n]urils (CB[n]s) are macrocyclic molecules composed of 5-10 units of glycoluril cycled by methylene bridges, which can bind a great variety of organic molecules [1]. These macrocycles show high binding constants with positively charged molecules and some recognition properties [1]. Previous complexation studies with some molecules have shown that their photophysical and/or photochemical properties are generally improved [2]. Additionally, CB[n] complexes can cross the cellular membrane [3] and some *in vivo* studies show no detrimental effects on drug activity. In this sense, the use of CB[n]s constitute a great promise for their application in biological samples. An important point that needs to be addressed is the interaction between these complexes and proteins, which are the main constituents of cells. Previous studies by other authors [4] and our group have shown that ternary drug-CB[n]-protein interactions are possible, and therefore, it remains to be investigated as how these ternary interactions will affect their photoactivity. In the current study, we assessed the photosensitization potential of some photoactive molecules when complexed with CB[7] or CB[8] and serum albumins. Initial studies showed protein fragmentation is more important than crosslinking. Oxidation of tryptophan residues in the protein was also observed. During the course of irradiation, no photobleaching of the drugs was observed. The generation of reactive oxygen species and the general mechanism for photosensitization is further analyzed.

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SUN31

Moving Metal-Based Photosensitizers for Photodynamic Therapy from Concept to Reality

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Platinum-based drugs for cancer therapy have enjoyed widespread acceptance owing to the success of Cisplatin. Despite its dose-limiting toxicity and deplorable side effects, Cisplatin represents one of the most important classes of anticancer drugs to date and continues to generate worldwide annual sales in the billions of dollars. Attempts have been made to introduce other metal-containing compounds as improvements over the platinum drugs, and ruthenium as an example has garnered considerable attention. Ru(III)-based prodrugs that can be activated selectively by reduction, thereby reducing systemic toxicity, have shown some promise in human clinical studies, and have set the stage for considering

ruthenium in other targeted approaches to cancer therapy. We are particularly interested in the use of Ru(II)-based photosensitizers as agents for photodynamic therapy (PDT), another avenue for reducing toxicity toward healthy tissue by exploiting light for spatial and temporal selectivity. PDT has not reached its full potential and is far from being a mainstream cancer treatment, owing at least in part to the drawbacks associated with clinically approved organic photosensitizers. Ru(II) coordination complexes exhibit excellent photophysical and photochemical properties for light-triggered therapy, and their modular architecture offers the ability to fine-tune the chemical characteristics of this class of compounds further. Others have recognized the advantages that Ru(II) complexes offer for PDT, and the number of published examples is enormous —yet none has progressed to clinical evaluation. Herein we shall discuss our efforts toward making Ru(II)- and other metal-based clinical agents for PDT a reality.

SUN32

Reactions of Singlet Oxygen with Metal Thiolates

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Singlet oxygen undergoes a variety of reactions with Co(III) and Pt(II) thiolato complexes. Superficially, these reactions appear to be similar to the photooxidation of organic sulfides. The major product is usually the corresponding sulfenato complexes, although sulfinato adducts are sometimes formed as well. On the other hand, prior studies with bis-thiolato complexes by the groups of H.B. Gray, M. Darensbourg, and K. Schanze have exclusively obtained sulfinato complexes and, in one case, elimination products. The mechanistic factors that determine these different outcomes are presently not well understood. We have found that in contrast with the photooxidation of organic sulfides, the rate of initial reaction of the metal-thiolato complex with singlet oxygen (k_T) appears to be affected by protic solvents and acids: the nucleophilicity of the thiolate moiety is reduced by addition of acids or in protic solvents, leading to significantly lower k_T values in protic solvents compared to aprotic solvents. The primary peroxidic intermediate in these reactions appears to be less nucleophilic than the persulfoxide formed during the photooxidation of organic sulfides, as trapping attempts with triphenyl phosphite have been unsuccessful. Our product studies with both Co and Pt complexes indicate that sulfenato complexes are formed exclusively if there is a proton on the a-Carbon attached to the sulfur atom, while both sulfinato and sulfinato complexes are obtained when this proton is not present. Possible mechanistic implications of these observations will be discussed.

SUN33

Tools for high resolution optical imaging of neuronal, glial, vascular, and metabolic activity for neuroscience studies *in vivo*

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The ability to descend to the single-cell and single-capillary levels *in vivo* and observe firing of individual neurons, vasodilation, and infusion of O_2 into the tissue - all while directly controlling neuronal activity - has long been a dream of neuroscientists interested in understanding activity of cerebrocortical circuits including neurovascular and neurometabolic physoiology. However, in contrast to the detailed and elegant mechanistic studies in isolated tissue, *in vivo* reports have, in the main, focused simply on correlations between the “observables”, limited by the available methods. This “too hard to do” status quo for mechanistic studies *in vivo* is starting to change, due to rapid developments in optical microscopy. In fact, already today, a versatile suite of optical tools is available for high-resolution, high-sensitivity measurements of neuroglial, vascular, and metabolic parameters in deep tissue and local, cell-type specific manipulations of neuronal activity. We consider the current state of the art of a number of key optical microscopy technologies that now power mechanistic *in vivo* neuroscience studies.

SUN34

Bright Porphyrin Phosphors and Click-Assembled Dendrimers: A Modular Platform for Tissue Oxygen Tension Imaging

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Hypoxia is known to play a major role in the poor prognosis and survival of patients with cancer, and is thought to help drive the development of aggressive and metastatic cellular populations. Hypoxia gives rise to a host of therapeutic resistance mechanisms, including cellular quiescence, upregulation of anti-apoptotic factors, and increased expression of DNA repair enzymes that allow cancer cells to survive treatment with chemotherapeutic agents. Due to the irregular growth of tumors and their leaky, unorganized vasculature, the distribution of oxygen throughout

tumorous tissue can be highly heterogeneous on the microscale. It is not entirely understood how this complex oxygenation landscape impact therapeutic response on the cellular and tumor-levels. This lack of knowledge is partially due to the fact that current oxygen imaging tools are focused on providing either large-scale tissue oxygenation measurements or have been designed to report blood oxygenation levels. Our research has been focused on developing a platform for real-time, cellular-level imaging of oxygen tension deep within tumors. First, we have developed a set of click-chemistry compatible, bright planar porphyrin molecular oxygen sensors based on near-infrared phosphorescence quenching. These meso-unsubstituted molecules have considerably higher phosphorescence quantum yield than existing commercial probes, enabling rapid oxygen tension sensing and image acquisition. Second, we have developed a simple, but extensible, click-chemistry based scheme that allows for the rapid growth of custom dendrimer layers surrounding these new porphyrin sensors that not only provide an extended oxygen sensing dynamic range, but are also designed to enable cellular uptake even in highly acidic tumor compartments. These new sensors have been tested in a three-dimensional in vitro model of ovarian cancer where model tumors grow and develop into large (>300 um diameter) nodules that become both hypoxic and acidic. Studies have shown that oxygen sensors built with our sequential click dendrimers readily penetrate throughout large nodules and report oxygenation changes within the in vitro models.

SUN35

Engineering of bacterial phytochromes for *in vivo* imaging.

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Fluorescence imaging is a powerful and widely used technique for biological research. However, lack of genetically-encoded probes for *in vivo* imaging is the major limitation in this field. Mammalian tissues are relatively transparent in a near-infrared optical window of 650-900 nm due to significant decrease in hemoglobin and melanin absorbance and still low water absorbance. Therefore, probes with fluorescence spectra within the near-infrared range are preferable for imaging in mammalian tissues and in whole animals. On the basis of bacterial phytochromes we have engineered three types of near-infrared fluorescence probes, which utilize present in mammalian tissues heme-derived biliverdin as a chromophore. These probes include several spectrally distinct permanently fluorescent proteins (iRFP670, iRFP682, iRFP702, iRFP713 and iRFP720), fluorescent proteins that are photoactivatable from low to high brightness (PAiRFP1 and PAiRFP2) and bimolecular fluorescence complementation probe that reports on protein-protein interactions (iSplit). The designed near-infrared proteins were imaged in tumor models in living animals using fluorescence and photoacoustic techniques. The multicolor whole-body imaging aided by the developed near-infrared probes should become common approaches in cell and developmental biology, in studies of cancer and pathogen invasion and in biomedicine.

SUN36

Two-photon Microscopy with Continuous Wave Laser Sources and Upconverting Nanoprobes

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Lanthanide-based upconverting nanoparticles (UCNPs) form a class of imaging agents with unique non-linear optical properties. However, utilization of UCNPs in biomedical arena has been hampered by the lack of robust methods of their solubilization and surface functionalization. Here we show that non-covalent modification of UCNPs with polyanionic porphyrin-dendrimers converts them into stable, water-soluble, non-toxic imaging probes. UCNP-to-porphyrin excitation energy transfer enables analyte-sensitive detection by upconverted luminescence. As an example we demonstrate that UCNP/porphyrin-dendrimers make up ratiometric pH nanosensors for physiological pH range. Exceptionally high apparent multiphoton absorption cross-sections of dendritic UCNPs combined with their excellent bio-compatibility make them directly suitable for physiological imaging. Using a low power continuous wave (CW) laser for excitation we performed mapping of mouse cortical vasculature with micron-scale resolution down to 400 mm under the brain surface, setting the first precedent of true *in vivo* two-photon microscopy with CW sources.

SUN37

Direct measurement of local oxygen concentration in the bone marrow of live animals by two-photon phosphorescence lifetime microscopy

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Bone marrow is a highly vascularized tissue. Blood vessels make up approximately 25% of the bone marrow by volume. Yet hematopoietic stem cells are thought to reside in hypoxic niches within the bone marrow. We ask how such a highly vascularized tissue

can harbor hypoxic niches. Using a metalloporphyrin-based oxygen sensor and two-photon phosphorescence lifetime microscopy, we measured the local oxygen concentration directly in the bone marrow of live mice. Our measurements uncovered a unique hypoxic landscape established by the simultaneous presence of high vascularity (oxygen supply) and high cellularity (oxygen consumption).

SUN38

Near infrared laser-tissue welding using plasmonic nanocomposite as a photothermal converter

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Each year, over half a million surgical procedures are performed in the United States to resect diseased colorectal tissues. Standard suturing and stapling techniques involve piercing healthy tissues and can cause anastomosis leakage leading to life-threatening bacterial infections. In this study, we develop a plasmonic nanocomposite for photothermal welding of ruptured intestinal tissue to achieve a fluid-tight sealing. Nanocomposites were prepared *via* self-assembly and phase separation of gold nanorods and elastin-like-polypeptides. Our results demonstrated that the nanocomposite are biocompatible and supported cell proliferation with minimum toxicity. The mechanical properties of the nanocomposite can be easily adjusted *via* changing the gold to peptide ratio in the nanocomposite. Upon laser-activation, heat generated from the gold nanorods facilitated nanocomposite-tissue fusion, enhanced the tensile strength and leaking pressure of the tissue, and created a fluid-tight seal to prevent bacteria leakage. Along with the unique ability to control-release therapeutic agents, plasmonic nanocomposites possess enormous translational potential in the closure and repair of colorectal tissues and others.

SUN39

Nuclear delivery of photo immunoconjugates

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The nuclear protein Ki-67 is highly expressed in all proliferating cells and antibodies against Ki-67 are widely used as prognostic tools in tumor diagnosis. In earlier studies we have shown, that photodynamic inactivation of the Ki-67 protein leads to an effective elimination of proliferating tumor cells. This was the first time that PDT was directed against a key marker for cell proliferation. We successfully targeted Ki-67 via a liposomal formulation of fluorescent-labeled antibodies. However an effective reproducible nuclear transport of the photo immunoconjugates into the nucleus remains a challenge. Here, we apply nanotechnology-based strategies to overcome this hurdle. Antibodies against Ki-67 were linked to a NLS (nuclear localization signal) peptide for enhanced nuclear import. After liposomal delivery of these conjugates into the cell, the antibodies were efficiently and reliable transported to the nuclei of the cells. Our new approach allows reliable light inactivation of the nuclear protein Ki-67 with a selectivity on the molecular scale. With different antibodies it may be used to target other biological relevant proteins in the cell nucleus.

SUN40

Photoimmunotherapy; basis, applications and beyond

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Photo-immunotherapy (PIT) is a newly developed, molecularly-targeted cancer photo-therapy based on conjugating a near infrared silica-phthalocyanine dye, IR700, to a monoclonal antibody (MAb) thereby targeting cancer-specific cell-surface molecules. When exposed to NIR light, the conjugate induces a highly-selective necrotic cell death only in receptor-positive MAb-IR700-bound cancer cells. Necrosis occurs as early as 1 minute after exposure to NIR light and results in irreversible morphologic changes including cellular swelling, bleb formation, and rupture of vesicles due to membrane damage. Meanwhile, immediately adjacent receptor-negative cells are unharmed. Due to the concentration gradient of MAb-IR700 leaking from vessels, PIT first causes necrosis in perivascular cancer cells resulting in dramatically enhanced vascular permeability with enhanced nano-particle delivery to cancer tissue, an effect termed “super-enhanced permeability and retention (SUPR)”. The combination of PIT and SUPR effects can effectively treat a variety of solid cancers including inhomogeneous cancers and cancer stem-like cells by employing different targeting molecules (including but not limited to MAbs) and nano-sized anti-cancer drugs. In this presentation, preclinical examples of successful PIT, employing a variety of single and multi-target-PIT, combined with nano-sized cancer reagents will be discussed. The combination of PIT and nano-sized systemic therapies is especially well adapted for real world heterogeneous tumors containing both receptor positive and receptor negative cells. The basis of PIT

and implications for further clinical translation will be also discussed.

SUN41

Nanobody-photosensitizer conjugates for targeted photodynamic therapy

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Targeted delivery of photosensitizers (PS) via monoclonal antibodies has been extensively investigated to improve tumor selectivity in photodynamic therapy (PDT)[1]. However, antibody-PS conjugates have a characteristic long blood half-life and, due to their size, a relatively poor tumor penetration, which result in long photosensitivity in patients and limited therapeutic efficacy.

In an attempt to target PS specifically and homogenously to tumors and to accelerate PS clearance, we have developed new conjugates consisting of nanobodies (NB) targeting the epidermal growth factor receptor (EGFR) and a traceable PS (IRDye700DX)[2]. NBs are the smallest naturally derived antigen binding domain with great potential in cancer imaging and therapy[3] and the selected PS is a silicon-phthalocyanine derivative that is relatively hydrophilic and has been shown to induce PDT[4].

The developed fluorescent NB-PS conjugates bind specifically to EGFR and allow the distinction of cell lines with different expression levels of EGFR. Results show that these conjugates specifically induce cell death of EGFR overexpressing cells in low nanomolar concentrations, while PS alone or the NB-PS conjugates in absence of light induce no toxicity. Delivery of PS using internalizing biparatopic NB-PS conjugates results in even more pronounced phototoxicities.

Altogether, EGFR targeted NB-PS conjugates are specific and potent, enabling the combination of molecular imaging with cancer therapy which can have a significant impact in the field of targeted PDT.

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SUN42

***In vivo* evaluation of nanoliposomal photochemotherapy for pancreatic cancer**

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Pancreatic cancer (PanCa) is one of the most deadliest and difficult to treat tumors. Standard radiation and chemotherapies are either ineffective or highly toxic, and tumors often develop compensatory signaling mechanisms to sustain their survival and growth. Recognizing the genetic complexity and heterogeneity of PanCa, we develop nanoconstructs that co-delivers two promising *in-clinical-trial* regimens: photodynamic therapy (PDT) and irinotecan, to provide meaningful improvements *via* interactive mechanisms that target multiple survival pathways. Our results demonstrated a threefold mechanistic interaction: irinotecan aids in reducing the tumor hypoxia to a PDT-favorable condition, PDT destroys efflux transporters increasing the intracellular irinotecan concentration, and PDT blocks the irinotecan-induced survivin expression. In orthotopic PanCa xenograft models, a single, low-dose combination drastically reduced the primary tumor volume, without acute systemic toxicity. In summary, this novel and clinically feasible photochemotherapy combines two fundamentally different, but cooperative, modalities to enhance cancer treatment outcome, allowing for non-overlapping side effects and dose-reduction, and may facilitate rapid clinical translation.

SUN43

Ultrafast Spectroscopy of DNA: Connecting Excited States and Photoproducts

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Damage to the genome by UV light has driven interest in excited electronic states of DNA for decades. Although the intensity of solar UV radiation reaching earth's surface is strongly attenuated by stratospheric ozone, excitation of DNA is efficient on account of the strong $p^* \leftarrow p$ transitions of the nucleobases. In order to minimize photochemical damage, DNA excited states should decay to the electronic ground state rapidly and with high quantum efficiency. Low fluorescence quantum yields and low photoproduct quantum yields provide indirect evidence that this is the case, but direct observation of the rapid events behind the

nonradiative deactivation of excited states has only become possible within the past decade using ultrafast laser spectroscopy. Upon UV excitation, excited states of individual nucleobases deactivate to the electronic ground state in hundreds of femtoseconds. Similar ultrafast nonradiative decay pathways are responsible for the photostability of sunscreen molecules. Surprisingly, the lifetimes of excited states of single- and double-stranded oligonucleotides are generally much longer. This raises the intriguing question of whether the advantages conferred by a molecular architecture like the double helix come at the cost of reduced photostability. Current evidence suggests that the environment in a DNA strand doesn't just prolong the lifetime of excited states localized on single bases, but instead creates new classes of excitations and new photophysical pathways not found in base monomers. Recent experiments reveal a deep connection between nucleic acid structure (i.e. helical conformation, base sequence, and single- vs. double-stranded character) and nonradiative decay mechanisms. Time-resolved vibrational spectroscopy has been used to show that UV excitation efficiently induces electron transfer between stacked nucleobases. These events and possible photochemical consequences will be discussed in the presentation.

DNA has remarkable and unexpected photophysical properties that are only now coming into focus. Excited states of single DNA and RNA bases generally decay by internal conversion to the electronic ground state in hundreds of femtoseconds. Ultrashort excited-state lifetimes are thought to enhance DNA's photostability and inhibit photodamage. Surprisingly, excited states in single- and double-stranded DNAs frequently decay orders of magnitude more slowly. The nature of long-lived excited states in DNA and RNA base multimers is still uncertain and the subject of on-going experimental and theoretical efforts. Using transient absorption spectroscopy with femtosecond UV/vis and mid-IR laser pulses, we are systematically studying long-lived excited states in a wide variety of DNA and RNA model systems ranging from simple dinucleosides to single and multi-strands with significant base-sequence disorder and different helix conformations. The results in concert with computational studies are providing new insights into how structural motifs such as base sequence, helical conformation, p-p stacking, and base pairing govern the evolution and decay of excited states created by UV light.

SUN44

Repair of the (6-4) photoproduct by its DNA photolyase: Experimental evidence for a two-photon mechanism

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UV irradiation induces two major types of harmful crosslinks between adjacent pyrimidines in DNA: cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6-4)pyrimidone photoproducts (6-4PPs). In many organisms, these lesions are repaired by photolyases, flavoproteins that require light for their catalytic action. The repair mechanism involves electron transfer to the lesion from the photoexcited state of the fully reduced flavin cofactor $FADH^-$, splitting of the intra-dimer bond(s) and return of the excess electron to the flavin.

For repair of the CPD by CPD photolyase, recent time-resolved studies [1, 2] have established this reaction sequence in detail. Overall, CPD repair is complete within ~1 ns after absorption of a single photon by the enzyme; the repair quantum yield is 50-100%.

Repair of the 6-4PP by (6-4) photolyase is known to have a much lower quantum yield (3-11%), and the repair mechanism is not established experimentally. Theoretical predictions are strongly controversial, including a singular suggestion that repair requires not only one but two successive photo-induced electron transfers from $FADH^-$ to the DNA lesion [3].

We have tried to distinguish experimentally between a one- and a two-photon process for repair of the 6-4PP by quantifying repair during series of single turnover flashes given to dark adapted samples. Our results [4] strongly support a two photon process. The DNA based intermediate formed by the first photoreaction has spectral features consistent with an oxetane-bridged dimer; in the absence of a second excitation, it decays back to the original 6-4PP in ~100 s. The quantum yields of the 1^{st} and 2^{nd} photoreaction were estimated to be ~7% and ~80%, respectively. The long lifetime of the intermediate should allow achieving a reasonable overall repair yield in a two photon process under natural sunlight.

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SUN45

Photochemistry of G-quadruplex forming sequences in telomeric and promoter DNA

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While the major photoproducts of duplex DNA, the *cis-syn* cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts, are well known and studied, very little is known about the photochemistry of higher order structures of DNA that have been found to form in telomeric and promoter DNA. Many years ago, evidence that *Tetrahymena* and *Oxytricha* telomeric DNA could be photo-crosslinked by 254 nm light was used to support the existence and conformation of G-quadruplexes (Williamson et al., *Cell* **1989** *59*, 871). It wasn't until the accidental discovery of UVB induced *cis-anti* CPD formation between two remote T's in a single stranded DNA molecule in acidic water (Su et al., *J. Am. Chem. Soc.*, **2008** *130*, 11328) that the photochemistry of telomeric DNA was reinvestigated. *Anti*-CPDs had previously only been detected in photo-irradiated dessicated DNA or ethanolic solutions of DNA (Douki et al. *Nucleic Acids Res*. **2003** *31*, 3134) and had not been detected to any great extent in native DNA. Irradiation of G-quadruplex forming human telomeric DNA sequences with UVB light, however, produced *anti* cyclobutane pyrimidine dimers between loops 1 and 3 at neutral pH in the presence of K^+, the major intracellular cation, but not in the presence of Na^+ (Su et al., *Proc. Natl. Acad. Sci. U.S.A.* **2009** *106*, 12861-12866). What was puzzling was that non-photoreactive hybrid G-quadruplex conformations are favoured in K^+ solution, whereas a potentially photoreactive basket conformation is favored in Na^+ solution. A proposed explanation that loops 1 and 3 are too far apart in the basket conformation in Na^+ solution but close enough in a two G-tetrad basket-like form 3 conformation that has been more recently been found to exist in K^+ solution was later shown not to be viable, suggesting that some other conformation was involved (Smith et al., *Nucl. Acids Res*. **2014** in press). In this talk we will describe further experiments that indicate that a reverse-Hoogsteen base paired hairpin, rather than a G-quadruplex is involved in the formation of *trans,anti*-CPDs in human telomeric DNA, though *anti* CPDs may also be forming in G-quadruplexes. We will also show the formation of *anti*-CPDs in potential G-quadruplex forming sequences in promoter DNA, which suggests that *anti*-CPD's, while minor photoproducts , may well form in biologically important DNA sequences *in vivo* and hence may have important biological effects.

SUN46

Unraveling the Potential of Sulfur-Substituted DNA and RNA Bases as UVA Photosensitizers

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Substitution of oxygen by a sulfur atom in the natural DNA and RNA bases gives rise to a family of derivatives commonly known as the thiobases. Upon excitation with UV radiation, the natural bases are able to quickly and efficiently dissipate the imparted energy as heat to their surroundings. Thiobases, on the other hand, relax into a long-lived triplet excited state in quantum yields that approach unity. This finding has both fundamental and biological relevance because the triplet state plays a foremost role in determining the efficacy of a sensitizer in damaging DNA and proteins within the cell. In fact, several thiopurine derivatives have been used since the 1960's as pro-drugs for cancer treatment and as immunosuppressants. However, the metabolization of these pro-drugs results in the incorporation of 6-thioguanosine into DNA; and ultimately, prolonged treatment of patients with these thiobases results in up to a 200-fold increase in the incidence of skin cancer. Our group has recently focused its efforts on gaining a deeper understanding of the time-resolved photochemical properties of several DNA and RNA thiobase derivatives upon UVB and UVA excitation. In particular, I will present our results on how the site of sulfur substitution and the degree of substitution impact the population of the reactive triplet state in these thiobase derivatives. Unraveling the excited-state dynamics of the thiobases is important to the discovery of thiobase derivatives with desirable phototherapeutic properties.

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SUN47

Impact of the methylation site of cytosine on the formation of bipyrimidine photoproducts

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Interaction between DNA and UV photons results in formation of mutagenic dimeric photoproducts involving adjacent pyrimidine bases, thymine (T) and cytosine (C). Two main types of dimeric photoproducts are produced, namely the cyclobutane pyrimidine dimers (CPDs) and the pyrimidine (6-4) pyrimidone photoproducts (64PPs). The yield and the ratio between CPD and 64PP greatly depend on the bipyrimidine dinucleotide considered.

Methylation of cytosine is another parameter to take into account. In cells, 5-methylcytosine (m5C) is endogenously produced in both prokaryotic and eukaryotic cells as an epigenetic mechanism controlling gene expression. The DNA of some bacteria also contains a second methylated cytosine: N4-methylcytosine (N4mC).

In the present work, we compared the photochemistry of trinucleotides upon exposure to either UVC or UVB radiation. The selected sequence was T-X-G (with X being a pyrimidine) because methylation in mammalian cells occurs mostly in CpG island which are also known to be mutational hotspots. Formation of the photoproducts was monitored by HPLC combined to tandem mass spectrometry. For this purpose, this assay used routinely in group for T and C dimers was extended to T-m5C and T-N4mC photoproducts.

The main conclusion of this study is that the methylation of cytosine greatly impacted the formation of CPDs and 64PPs. As already observed in double-stranded DNA, m5C is more photoreactive than C. A drastic effect was also observed on the ratio between CPD and 64PP, while methylation at C5 favors CPD, methylation at N4 promotes the formation of 64PP. Our results also confirm that methylation greatly reduces the deamination rate.

MON1

Mechanism Of Action Of Prohibitin In Regulation Of UVB-induced Apoptosis

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Prohibitin (PHB) plays a role in regulation of ultraviolet B light (UVB)-induced apoptosis of human keratinocytes, HaCaT cells. The regulatory function of PHB appears to be associated with its lipid raft translocation. However, the detailed mechanism for PHB-mediated apoptosis of these keratinocytes upon UVB irradiation is not clear. In this presentation, we demonstrate that upon UVB irradiation PHB is translocated from the non-raft membrane to the lipid rafts, which is correlated with a release of both Akt and Raf from membrane. Overexpression of Akt and/or Raf impedes UVB-induced lipid raft translocation of PHB. Immunoprecipitation analysis indicates that UVB alters the interactions among PHB, Akt, and Raf. Reduced expression of PHB leads to a decreased phosphorylation of Akt and ERK, as well as a decreased activity of Akt, and increased apoptosis of the cells upon UVB irradiation. These results suggest that PHB regulates UVB-induced apoptosis of keratinocytes via a mechanism that involves detachment from Akt and Raf on the plasma membrane, and sequential lipid raft translocation.

MON2

Effects of the pharmacological inhibition of macrophage inhibitory factor on ultraviolet light induced inflammation and tumor development.

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Macrophage Migration Inhibitory Factor (MIF) is a homotrimeric proinflammatory cytokine implicated in chronic inflammatory diseases and malignancies including cutaneous squamous cell carcinomas (SCC). The role of MIF in the development and progression of ultraviolet-B (UVB) light induced inflammation and ACC has been demonstrated using knock-out and transgenic mouse models. To determine if MIF inhibition could reduce ultraviolet-B light (UVB)-induced inflammation and squamous carcinogenesis, we utilized a MIF inhibitor (MIFi) that disrupts homotrimerization. To examine the effect of on acute UVB-induced skin changes, we systemically treated Skh-1 hairless mice with MIFi for 5 days prior to UVB exposure. In addition to decreasing skin thickness and myeloperoxidase activity, MIFi pre-treatment increased keratinocyte apoptosis and p53 expression, decreased proliferation and phospho-histone H2A.X, and enhanced repair of cyclobutane pyrimidine dimers (CPD). To examine the effect of MIFi on squamous carcinogenesis, we exposed mice to UVB for 10 weeks, followed by MIFi treatment for 8 weeks. MIFi decreased the density of UVB-associated p53 foci in non-tumor bearing skin to approximately 50% while also decreasing the epidermal Ki67 proliferation index. In addition to slowing the rate of tumor development, MIFi decreased the average tumor burden per mouse. While MIFI-treated mice developed only papillomas, 28.6% of papillomas in vehicle-treated mice progressed to SCC. Thus, MIF inhibition is a promising strategy for prevention of the deleterious cutaneous effects of acute and chronic UVB exposure

MON3

Dual role of SIRT1 in UVB-induced skin tumorigenesis

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The protein deacetylase SIRT1 regulates various pathways in metabolism, aging and cancer. However,

the role of SIRT1 in skin cancer remains unclear. Here, using mice with targeted deletions of SIRT1 in their epidermis in both resistant B6 and sensitive SKH1 hairless backgrounds, we show that the role of SIRT1 in skin cancer development induced by ultraviolet B (UVB) radiation is dependent on its gene dose. Keratinocyte-specific heterozygous deletion of SIRT1 promotes UVB-induced skin tumorigenesis, whereas homozygous deletion of SIRT1 suppresses skin tumor development but sensitizes the B6 mice to chronic solar injury. In mouse skin, SIRT1 is haploinsufficient for UVB-induced DNA damage repair and expression of xeroderma pigmentosum C (XPC), a protein critical for repairing UVB-induced DNA damage. As compared with normal human skin, down-regulation of SIRT1 is in parallel with down-regulation of XPC in human cutaneous squamous cell carcinoma at both the protein and mRNA levels. In contrast, homozygous SIRT1 deletion in mouse skin augments p53 acetylation and expression of its transcriptional target Noxa, and sensitizes the epidermis to UVB-induced apoptosis *in vivo*, while heterozygous SIRT1 deletion has no such effect. The gene dosage-dependent function of SIRT1 in DNA repair and cell survival is consistent with the dual roles of SIRT1 in UVB-induced skin tumorigenesis. Our results reveal the gene dosage-dependent *in vivo* functions of SIRT1 in skin tumorigenesis and may shed light on the role of SIRT1 in epithelial cancer induced by DNA damage.

MON4

Roles of C/EBP Family Transcription Factors in UV-Induced Carcinogenesis

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The CCAAT/Enhancer Binding Proteins (C/EBPs) are a family of leucine-zipper transcription factors that regulate physiological processes such as energy metabolism, inflammation, cell cycle, development and differentiation of several tissues including skin. Involvement of C/EBPα and C/EBPβ in cancer is known, with a loss of C/EBPα commonly reported in cancer. In response to UVB exposure, C/EBPα knockout (KO) mice are susceptible to tumorigenesis mainly due to defective cell cycle control, whereas, C/EBPβ KO mice are completely refractory to tumor development due to robust apoptotic response following UV exposure. We are interested in one of the newest C/EBP family members, called CHOP (C/EBP Homologous Protein, also known as C/EBPζ, *gadd153* or *ddit3*) for its possible role in carcinogenesis. CHOP KO mice, along with CHOP heterozygous and wild type (WT) mice, were exposed to UVB (progressively increasing doses up to 180 mJ/cm^2 UVB, 3 times a week for 20 weeks). At week 25 the number and size of tumors on CHOP KO mice were significantly higher than in WT controls. Heterozygous mice as expected, had an intermediate response. Since p53 is activated by UVB exposure and p53 mutations are a major cause of skin cancer, we focused on involvement of the p53 pathway in enhanced tumor response. Immunofluorescence (IF) analysis of tumor sections showed an upregulation of p53 in tumors from both CHOP KO and WT mice, but in CHOP KO tumors the majority of p53 was mutated (pAb240). Sequence analysis of tumor cDNA showed missense mutations in exons corresponding to DNA binding domain of p53, confirming p53 mutations in CHOP KO tumors. Concurrently, p21 and MDM2 levels were reduced in the CHOP KO tumors, indicating a defective growth arrest and DNA damage repair process, the primary function of p53 protein. To study the immediate responses to UVB exposure, which might result in enhanced tumor phenotype, CHOP KO and WT mice were exposed to UVB and then skin samples harvested at different times. A decrease in sunburn cells and apoptosis (TUNEL and Caspase-3 cleavage) were observed in CHOP KO skin, relative to WT skin. Results presented here indicate that in addition to C/EBPα and C/EBPβ, a newer member of the family, CHOP also plays an important role in skin carcinogenesis involving p53 mutation and altered apoptotic response.

MON5

UV-induced platelet activating factor activates systemic immune suppression

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UV radiation (UVR) induces both skin cancer and systemic immune suppression, which is recognized as a risk factor for cancer induction. Because UVR is absorbed within the upper layers of the skin, indirect mechanisms must play a role in activating immunosuppression. One essential step in the cascade of events leading to immunosuppression is mast cell migration from the skin to the draining lymph nodes. The molecular signal that triggers mast cell migration is not entirely clear, but our findings indicate that platelet-activating factor (PAF) is involved. Mast cell deficient mice (Kit$^{W\text{-}sh/W\text{-}sh}$) were resistant to the suppressive effect of UVR. Reconstituting Kit$^{W\text{-}sh/W\text{-}sh}$ mice with wild type bone marrow derived mast cells (BMMC) reconstitutes immune suppression. No suppression was found when Kit$^{W\text{-}sh/W\text{-}sh}$ mice were reconstituted with mast cells derived from PAF receptor deficient (PAF-R$^{-/-}$) mice. No mast cell migration was observed in UV-irradiated PAF-R$^{-/-}$

mice. Injecting PAF into wild type mice mimicked the effect of UVR and induced mast cell migration, but not in PAF-$R^{-/-}$ mice. UVR also suppresses germinal center formation and antibody secretion. No immune suppression was found in mast cell deficient mice. Reconstituting mast cell-deficient mice with wild type BMMC reconstitutes UV-induced suppression of germinal center and antibody formation. However when mast cells derived from IL-$10^{-/-}$ mice were used, no suppression of germinal center formation was observed. These data indicate that UV-induced PAF activates mast cell migration from the skin to the lymph node. In the lymph node, the PAF-activated mast cells secrete IL-10, which suppresses adaptive immune responses.

MON6

UV radiation-induced DNA hypermethylation promotes immunosuppression in UV exposed mice

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We have explored the role of epigenetic regulators in UV-induced immunosuppression using *in vivo* animal model. Here, we report that UV-induced suppression of contact hypersensitivity (CHS) response in C3H/HeN mice was associated with increases in the levels of cyclooxygenase-2 (COX-2), prostaglandin (PG) E_2 and PGE_2 receptors in the exposed skin. UV radiation-induced suppression of CHS was inhibited by topical treatment of the skin with celecoxib or indomethacin (inhibitors of COX-2) or AH6809 (an antagonist of EP2). Mice deficient in COX-2 were found to be resistant to UV-induced suppression of CHS. Exposure of wild-type mice with UV radiation resulted in DNA hypermethylation, increased DNA methyltransferase (Dnmt) activity, and elevated levels of Dnmt proteins in epidermal skin samples, and these responses were downregulated on topical treatment of the site of exposure with EP2 antagonist or indomethacin. Topical treatment of UV exposed COX-2-deficient mice with PGE_2 enhanced the UVB-induced suppression of CHS as well as global DNA methylation and elevated the levels of Dnmt activity and Dnmt proteins in the skin. Intraperitoneal injection of 5-aza-2'-deoxycytidine (5-aza-dc), a DNA demethylating agent, restored the CHS response to 2,4-dinitrofluorobenzene in UVB-exposed skin and this was associated with the reduction in global DNA methylation and Dnmt activity compared to the mice which were not treated with 5-Aza-dc. Further, treatment with 5-aza-dc reversed the effect of PGE_2 on UV-induced suppression of CHS response in COX-2-deficient mice. These findings uncover a previously unrecognized role of PGE_2 in UV-induced suppression of CHS and that it is mediated through epigenetic mechanisms involving DNA hypermethylation.

MON7

Photodynamic therapy can induce a non-specific protective immune response against a bacterial infection

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Photodynamic therapy (PDT) for cancer is known to induce an immune response against the tumor, in addition to its well-known direct cell-killing and vascular destructive effects. PDT is becoming increasingly used as a therapy for localized infections. However there has not to date been a convincing report of an immune response being generated against a microbial pathogen after PDT in an animal model. We have studied PDT as a therapy for bacterial arthritis caused by bioluminescent methicillin-resistant *Staphylococcus aureus* infection (MRSA) in the mouse knee. We had previously found that PDT of an infection caused by injection of MRSA (5X10(7) CFU) into the mouse knee followed 3 days later by 1 microg of Photofrin and 635-nm diode laser illumination 5 minutes later using a range of fluences, gave a biphasic dose response in CFU. The greatest reduction of MRSA CFU was seen with a fluence of 20 J/cm^2, whereas lower antibacterial efficacy was observed with fluences that were either lower or higher. We then tested the hypothesis that the host immune response mediated by neutrophils was responsible for most of the beneficial antibacterial effect. We used bioluminescence imaging of luciferase expressing bacteria to follow the progress of the infection in real time. We found similar biphasic results using intra-articular methylene blue (a photosensitizer that was shown to cause least damage to neutrophils in vitro) and red light, and more importantly, that carrying out PDT of the non-infected joint and subsequently injecting bacteria after PDT led to a significant protection from infection. Taken together with substantial data from studies using blocking antibodies we believe that the pre-conditioning PDT regimen recruits and stimulates neutrophils into the soon-to-be infected joint which can then destroy bacteria that are subsequently injected and prevent infection developing. This procedure may be applied prophylactically to patients undergoing high-risk orthopedic surgery.

MON8

Photodynamic therapy induced immune response towards tumor antigens.

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Photodynamic therapy (PDT) is a rapidly developing locally ablating anticancer modality that involves administration of a photosensitizer (PS), illumination with light of a specified wavelength and production of cytotoxic reactive oxygen species. PDT has been successfully applied in the treatment of inoperable cancers. It is a unique treatment modality because it can be used to destroy local tumors while at the same time it can induce systemic immune response. The significance of this statement has been recently confirmed in a clinical setting. PDT has also been shown to reliably destroy local lesions as well as induce strong anti-tumor immune response capable of destroying well established tumors and distant metastases. The immune-based therapies act through a mechanism that is distinct from chemotherapy or radiation therapy, and represent a non-cross-resistant treatment. However the mechanisms of immune escape both at the local and systemic level do exist and are recognized. T-cell recognition of melanoma tumors can be inhibited or suppressed due to the downregulation of MHC class I molecules on tumor cells or by the increased numbers of Treg that accumulate in malignant tumors. These mechanisms will therefore need to be circumvented to fully develop an effective melanoma therapy. It has been recently showed that PDT can lead to the development of strong antigen-specific anti-tumor adaptive immune response against model as well as naturally occurring tumor antigens. Moreover, this response can be further potentiated by epigenetic modification and T regulatory cells depletion. These strategies significantly increased the effectiveness of both treatments and significantly enhanced potential benefits of both therapies while at the same time decreased the side effects.

MON9

Intraoperative Photodynamic Therapy for Malignant Pleural Mesothelioma – Evidence Suggesting a Positive Immunologic Effect

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Purpose: The purpose of this study was to review the results obtained using photodynamic therapy (PDT) as an intraoperative adjuvant for patients with malignant pleural mesothelioma and to determine if there was any evidence suggesting an immune effect resulting from the PDT.

Methods: Patients with malignant pleural mesothelioma were enrolled in a trial combining surgical debulking with intraoperative photodynamic therapy and adjuvant standard pemetrexed-based chemotherapy. Patients received 2mg/kg porfimer sodium 24 hours preoperatively. A macroscopic complete resection was initially accomplished with lung-sacrificing surgery, but in recent years a lung-sparing technique has been employed. After all detectable cancer was removed intraoperative PDT was performed by delivering 630nm light to a measured dose of 60J/cm2, utilizing 8 isotropic light detectors and a dosimetry system to assure even light distribution. Postoperatively patients were given standard chemotherapy, if they had not received it preoperatively, and they were followed every three months.

Results: When a lung-sacrificing technique was employed for surgery, disease free and overall survival results were comparable to what has been reported in other surgical series. When a lung-sparing technique was employed, however, the disease free survival remained comparable to that seen in other series, but the overall survival rates remain among the best results reported in the literature for this cancer. The uncharacteristically long interval between disease free and overall survival observed in these patients is analogous to what is commonly reported in immunotherapy trials – lack of cure but decrease in the rate of tumor progression. Anecdotally, patients who recurred and were subsequently enrolled in an immunotherapy trial demonstrated some of the most robust responses observed in that trial.

Conclusions: The clinical course demonstrated by the patients in this trial is consistent with, and suggestive of, an immune effect. Given that microscopic disease always remains after surgery for this cancer, the possibility exists that PDT is inducing an autologous tumor vaccine effect and that the lung is playing a role in generating this effect.

MON10

Biological photoreceptors as tools in superresolution microscopy and optogenetics applications

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Biological Photoreceptors allow non-invasive activation or deactivation solely by light. As many of these chromoproteins carry chromophores with notable fluorescence quantum yield, photoreceptors can be employed in both superresolution microscopy and optogenetics applications. Besides the well-established protagonists GFP and derivatives and channelrhodopsin, photoreceptors showing a functional separation (sensing/signalling) into individual protein domains have been introduced. In this contribution, photoreceptors are presented that carry an enzyme function in their signaling domain, exemplified for adenylyl cyclases. Genes encoding photo-activated adenylyl cyclases (PAC) have been isolated from several bacteria. The recombinant gene products could be isolated, purified to homogeneity, and spectrally characterized in vitro. Expression of such PAC in frog oocytes allowed electrophysiological determination of the light-regulated cAMP synthesis and activation of a co-expressed cyclic-nucleotide gated channel. Following, expression of a photo activated adenylyl cyclase from the sulfurbacterium *Beggiatoa* sp. (bPAC) in cultures of nerve cells demonstrated its capability to open in a light-regulated manner cAMP-gated channels thereby causing neuronal activity. As a final proof, a photoactivated adenylyl cyclase from the cyanobacterium *Microcoleus chthonoplastes* (mPAC) was expressed in the social amoeba *Dictyostelium discoideum*. Dictyostelids have been established as model organisms for studying cAMP-dependent differentiation, as their entire lifecycle is dictated by the intracellular concentration of cAMP. As endogenous adenylyl cyclases (AC) would interfere in this test experiment, an AC null-mutant was used for demonstrating mPAC function. Despite a small constitutive enzymatic activity (that was not sufficient to provide sufficient signaling for differentiation), the mPAC transformed null-mutant could be rescued, i.e., showing differentiation and fruiting body formation under blue light irradiation. For applications, a combination of such enzyme activities with the fluorescence properties of the chromophore-bearing sensing domain allows precise tempo-spatial detection of the proteins under study. As in some photoreceptor types the chromophore fluorescence can be light regulated (i.e., only one of several stable states shows significant fluorescence), they are excellent tools for superresolution microscopy.

MON11

Increasing the light-sensitivity of LOV2 domain-based optogenetic tools

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Optogenetic control of cellular behavior requires a photoreceptor fused with an output domain, thus a cellular response is coupled to light as signaling entity. The photo-sensitive degron (psd) module consists of the light-reactive LOV2 domain of Arabidopsis thaliana phot1 fused to the murine ornithine decarboxylase-like degradation sequence cODC1. The latter induces proteolysis of the fusion protein in the proteasome. Thus, the abundance of proteins tagged with the psd module can be regulated by blue light if the degradation tag is accessible for the proteasome. To search for psd modules with altered light-sensitivity, we generated variants by site-specific and random mutagenesis. Characterization of the variants in the model organism Saccharomyces cerevisiae coupled to in silico modeling of the behavior showed that we obtained psd modules with increased and decreased light sensitivity. The simulations suggested that most of the analyzed mutations affected the light-response of the LOV2 domain. The mutational approach resulted in improved variants of the psd module and increased the knowledge about the LOV2 domain. It demonstrated that characterization of LOV2 domains with unknown properties is feasible in short time, which will facilitate generation of optogenetic tools based on this photoreceptor domain in the future.

MON12

Molecular properties of channelrhodopsin and their impact on optogenetics

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Microbial rhodopsins are routinely used as light-controlled switches in neurobiology. Their versatile applicability relies on the simplicity of the optogenetic approach. Light-sensitivity is promoted to the host cell encoded in genetic information. However, the signal output is limited by the expression level and the molecular properties of the rhodopsins. The light-sensitivity of the system can set a limit for its usage. Different strategies might be envisaged how to tune and increase the responsiveness on a molecular level in the case of channelrhodopsin-2 (ChR2), a light-gated cation channel from *Chlamydomonas reinhardtii*: 1) increased absorption cross-section and quantum efficiency, 2) increased single channel conductance, 3) increased lifetime of the open state and 4) an amplification system. Here, we follow up the different strategies in a combined biophysical and neurobiological approach. As a first step we have developed the tools to study the different properties to have experimental access to the molecular properties from a spectroscopic and electrophysiological side.

Especially, the development of fusing different rhodopsins into a single entity allows the discrimination between effects on the expression level and an increased single channel current by using one of the rhodopsins as a molecular ruler. In a next step we looked into the light-induced dynamical changes that accompany the photocycle of ChR2. A pronounce movement of transmembrane helix B can be observed and is unique among the microbial rhodopsins. This strategy allows us to map the conformational changes connected to the open state of the channel. We further generated a more calcium permeable mutant (CatCh, L132C) whose action on tuning the light sensitivity mightn be different.

MON13

Channelrhodopsin et al.: photoreceptors for optogenetic applications

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Photoreceptors from archaea, bacteria, and green algae were molecularly identified in recent years. We could show that some of them are ideal tools to manipulate animal cells by illumination. The Channelrhodopsins from the unicellular green alga C. reinhardtii are Light-gated cation channels which allow fast light-induced depolarization (1,2) of the plasma membrane. Mutations led to a slower photocycle and therefore to Channelrhodopsins with higher light sensitivity. Neuronal expression of Channelrhodopsin-2 (ChR2) yields Light-induced action potentials and Light-manipulated behaviour in C. elegans (3). The Light-activated chloride pump halorhodopsin (HR) from the archaeum Natronomonas pharaonis hyperpolarizes the plasma membrane and therefore allows Light-induced silencing of neurons (4). These two antagonistic rhodopsins may even be expressed in the same cell and still specifically be light-activated with 460 nm for ChR2 and 580 nm for HR. Recently we found a ChR2 mutant with increased expression and high light sensitivity (ChR2-XXL) which allows light modulation of deep brain neurons in adult *Drosphila* flies, even without feeding the chromophor all-trans retinal.

We heterologously expressed Photoactivated Adenylyl Cyclases (PAC) from *Euglena gracilis* (5,6) or bacteria (7,8), flavoproteins which quickly elevate cytoplasmic cyclic AMP by illumination with blue light in cultured cells and in living animals or plants. Now we engineered PAC proteins with an increased ratio of activity in the light vs. in the dark.

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MON14

A new cryptochrome-based optogenetic tool for probing protein interaction and function

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The *Arabidopsis* blue light receptor Cryptochrome 2 (CRY2) was previously used as an optogenetic tool, allowing for spatial and temporal control of cellular processes. While screening for CRY2 mutants with altered photo responses, we identified 'CRY2olig', a mutant version of CRY2 that undergoes rapid, robust and reversible clustering in response to blue light. We utilized the clustering property of CRY2olig to develop an assay to monitor protein-protein interactions in real time. In addition, we illustrate the usefulness of CRY2olig in modulating cellular processes by disrupting clathrin mediated endocytosis and inducing actin polymerization with light. We demonstrate that CRY2olig is a powerful genetically-encoded optical tool for probing protein interactions and inducibly perturbing fundamental cellular processes.

MON15

Engineered microbial rhodopsins for all-optical electrophysiology

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We introduce variants of a near infrared indicator and a blue light gated channelrhodopsin actuator which together constitute a tool for all-optical electrophysiology (Optopatch). We used Optopatch to probe neural excitation across spatial scales, from parallel measurements of firing patterns in dozens of

neurons, to detection of back-propagating action potentials in individual dendritic spines. Second, we combined Optopatch with patterned illumination to probe signal propagation across spatial scales, from large-scale network activity, to sub-cellular details of action potential initiation and propagation. Finally, we applied the Optopatch system to detect subtle excitability phenotypes in human stem cell-derived neurons. The Optopatch platform enables electrophysiology with high throughput and high information content without the use of conventional electrodes.

MON16

Use of Hypothermia During PDT Treatment of Malignant Glioma

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Malignant gliomas are invasive and difficult to treat tumors. Of this group of tumors, Glioblastoma Multiforme (GBM) is the most common and the most aggressive. Of proposed GBM treatments, ALA-PpIX mediated Photodynamic Therapy (PDT) has been explored and researched into. Selectivity is given by the fact that tumor tissue shows a large preferential uptake of the photosensitizer than the brain. However, cell responses to PDT vary widely both *in vitro* and *in vivo*. Therefore there is an interest in developing adjuvant therapies to further increase the selectivity of PDT in treating GBM..

As one such adjuvant therapy, our group has examined the effect of hypothermia on PDT treatment *in vitro* and *in vivo*. Specifically, mild hypothermia (32 degrees Celsius, 90 degrees Fahrenheit) led to an increase in the amount of PpIX in tumor cell lines *in vitro* while leading to minor reductions in the LD50, a surrogate for resistance against PDT treatment and in the unit of administered ALA concentration, in those cells. Hypothermia also provided protection of neurons from *in vitro* PDT treatment, with an increase of the LD50 by a factor of approximately 100.

Our group has then moved to an *in vivo* glioma model utilizing RG2 (Rat Glioma 2) tumor cells implanted into the cerebral cortex of rats. Following tumor growth animals were subjected to whole body cooling to a core temperature between 30.5 – 32 degrees Celsius during the 2.5 hours prior to PDT treatment and 2 hours following treatment. Comparing to control animals with a core temperature of 37 degrees Celsius, animals subjected to hypothermia were observed to have 7-10 times higher PpIX concentration inside tumor as measured by quantitative point measurements *in vivo*, which was confirmed by ex vivo PpIX concentration measurement using a tissue solubilization protocol. The final part of the study is the measurement of the acute and long-term effects of hypothermia on tumor growth and animal survival following PDT treatment.

MON17

Cell type-specific optogenetic vision restoration strategies

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In retinal degenerative diseases such as Retinitis pigmentosa (RP) rod photoreceptors, important for night vision, degenerate whereas cones, important for high acuity daytime and color vision, lose their light-sensitive outer segments and become non functional. RP patients progressively lose their vision. I will present that single opto-gene therapeutic interventions to strategically important cell types in blind RP retinas restores light sensitivity.

We delivered Channelrhodopsin-2 or microbial halorhodopsins to RP retinas and restricted the expression to distinct retinal neurons by cell-type-specific promoter elements. Both opto-genes conferred light-sensitivity to former blind retinas. Resensitized retinas were analyzed by molecular biological, imaging and electrophysiological techniques. Furthermore, one approach was successfully translated to *post-mortem ex vivo* human retinas demonstrating its clinical prospects. Currently, the clinical trials for these cell type-specific therapeutic interventions have started (http://www.gensight-biologics.com).

Our results demonstrate that, despite the diverse genetic origin of RP, the targeted expression of opto-genes to retinal neurons can restore significant functionality to the visual system following degenerative changes.

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MON18

Combination Therapy Incorporating PDT

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Photodynamic therapy (PDT) generates reactive oxygen species and these molecules can mediate direct cytotoxicity to malignant cells within a tumor. PDT mediated oxidative stress can also exert direct and indirect effects on a variety of non-malignant components of the tumor microenvironment. An expanding number of studies are being performed to examine mechanisms of how PDT modulates the tumor microenvironment as well as to define the relevance of these responses on tumor treatment outcomes. This presentation will describe the interactive role that PDT and components of the tumor microenvironment have on the expression and function of growth factors, mediators of inflammation, angiogenic modulators and pro-survival molecules. A primary focus will be on the clinical opportunities of using PDT to treat retinoblastoma. The lecture will include specific information on hypoxia-inducible factor 1α, vascular endothelial growth factor, cyclooxygenase-2, matrix metalloproteinases, and the anti-apoptotic protein survivin. Data will also be presented on clinically relevant drugs that can enhance PDT responsiveness.

MON19

Novel ways of targeting the tumor vasculature

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Angiogenesis inhibitors are firmly established in the clinical management of cancer. Their effect on patient survival, however, is rather limited, probably due to the induction of resistance to these compounds. To avoid resistance, drugs should not be targeted to tumor produced growth factors, but should instead directly target the tumor endothelial cells. To achieve this selective markers of the tumor endothelium are to be identified. Since angiogenesis is not limited to pathologies, careful evaluation of putative therapeutic targets is warranted to prevent side effects associated with impaired physiological angiogenesis. We performed yeast 2-hybrid screening techniques using existing angiogenesis inhibitors as bait, and genomic transcriptome subtraction techniques using RNA from angiogenic endothelial cells isolated from both malignant and non-malignant tissues and of resting endothelial cells. We identified a series of genes that show specific overexpression in tumor endothelium but not in angiogenic endothelium of normal tissues, creating a therapeutic window for tumor vasculature specific targeting. Antibody targeting of four cell-surface expressed or secreted products (vimentin, galectin-1, HMGB1 and IGFBP7) inhibited angiogenesis *in vitro* and *in vivo*. Targeting vimentin, shown to be expressed at the surface of the tumor vasculature, was shown to significantly inhibit tumor growth in a preclinical mouse model, by inhibition of angiogenesis. Next to targeting of these markers for direct therapeutic use, it is possible to use ligands or antibodies directed to these markers for targeted delivery of drugs or tracers for imaging. A therapeutic ligand of galectin-1 was successfully used for delivery of a fluorescent dye and of a gadolinium-based tracer for visualization of tumor angiogenesis by fluorescence microscopy and by magnetic resonance imaging. Specific delivery of photosensitizers is expected to enhance selectivity of phototherapy.

MON20

Studying the Effects of Photodynamic Therapy on Tumor Oxygenation and Blood Flow Toward Better Treatment

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The vasoactive and oxygen consumptive effects of photodynamic therapy (PDT) substantially impact tumor microenvironment during the course of light delivery. Both tumor blood flow and oxygen concentration can change rapidly during illumination, and the magnitude and time course of these changes will be determined by treatment-related factors such as photosensitizer type, drug-light interval and illumination fluence rate. In murine studies, measurements of tumor oxygenation and blood flow during PDT can be used to predict long-term outcome. Approaches that "prime" the tumor vasculature to treatment with PDT can be used to increase the sensitivity of tumor vessels to PDT damage, accompanied by changes in local hemodynamics during treatment. For example, increasing the basement membrane composition of tumor blood vessels can increase the uniformity of PDT vascular response and significantly improve therapeutic outcome. The value in modulating tumor

microenvironment during PDT will ultimately be tested in clinical applications for which present day technology importantly facilitates the noninvasive measurement of tumor physiologic and hemodynamic properties. PDT effect on the hemodynamics and oxygenation of treated tissue has been measured in patients enrolled in several of our clinical trials, including those treated for the mesothelioma-involved thoracic cavity and for pre-malignant/early stage cancer of the head and neck. Detection of large PDT-induced changes in tumor microenvironment in the clinical setting points toward the possibility that real time adjustment of PDT dose based on its physiologic effects would be valuable in the treatment of patients.

MON21

Evaluating the roles of stromal rheology and heterotypic cross-talk in the pancreatic tumor microenvironment to inform PDT treatment strategies

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Cancer of the pancreas is well known to be among the most lethal of human malignancies, and is associated with a particular abundance of rigid, fibrotic stroma that plays multiple tumor promoting roles. Through interactions with tumor-associated fibroblasts, the biophysical properties and noted rigidity of the stromal microenvironment may limit drug penetration and contribute to mechanosensitive regulation of growth and therapeutic response. Here we describe the use of custom in vitro 3D tumor and tumor-fibroblast co-culture models with varying stromal composition combined with particle tracking microrheology (PTM) to monitor local changes in the mechanical microenvironment correlated heterotypic signalng events and therapeutic intervention. We use this integrated platform combined with previously described imaging-based tools for quantitative treatment assessment to evaluate rheology-informed PDT and chemotherapy strategies for this disease.

MON22

Treatment planning and microenvironment in PDT of head and neck cancer

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The quantification of photosensitizer fluorescence in-vivo is complicated by the influence of tissue optical properties on the collected fluorescence signal. The quantitation of intrinsic photosensitizer concentrations during PDT in tumor tissue and in the surrounding normal tissue has important implications for the therapeutic response following PDT and illustrates the importance of the tumor microenvironment. We present a series of translational pre-clinical investigating the use of Chlorin based photosensitizes in pre-clinical models. Intrinsic fluorescence spectroscopy, confocal fluorescence microscopy and intra-vital microscopy are used to monitor PDT in oral squamous cell carcinoma in the mouse tongue and surrounding normal tissues. The macroscopic and microscopic photosensitizer pharmacokinetics are determined at various time points after PDT. Photosensitizer concentration, decreases for all time points investigated, but shows significantly more Bremachlorin present compared to ce6 at long incubation times and suggest that long incubation times could be explored as an optimum treatment protocol for Bremachlorin-based PDT. The PDT response of tissues using these Chlorin formulations was investigated in dorsal window chambers on the back of mice and treated with PDT after a drug-light intervals of 5 or 24h. Acute vascular effects were analyzed using transmitted therapeutic illumination during PDT. 2 h after illumination, vascular leakage and stasis was investigated by intra-vital confocal microscopy. 48 hours after treatment, chambers were inspected for microscopic vascular damage such as hemorrhage and tissue was removed and sectioned for histological analysis of PDT-related cell damage. Most acute vascular effects were observed during Bremachlorin PDT with a 24h drug-light interval, followed by ce6 PDT 5h after injection. Leakage and stasis were more present in Bremachlorin treated animals independent of incubation time. The PDT-induced histological responses were most severe in Bremachlorin PDT with a 24h drug-light interval. PDT-related damage to tumor cells was, although not significant, related to the onset of acute vascular effects during PDT illumination. We conclude that for both ce6 and Bremachlorin PDT with a 24h drug-light interval was most effective in inducing damage to tumor cells. This is in contrast with the short incubation times that have previously been utilized in Ce6 based studies.

MON23

Genome-wide repair kinetics of UVC induced CPDs and correlation to epi-genetic chromatin states

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The induction and persistence of the two major DNA photo lesions cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproduct are thought to be influenced by the chromatin state in which they occur.

Here we present genome wide distribution profiles for CPDs and 6-4-PP obtained by a modified immuno-precipitation technique combined with high-throughput sequencing. We studied the kinetics of induction and removal of CPDs and 6-4-PP after a single exposure of UV-C (254 nm). We used different genetic backgrounds including a nucleotide excision repair proficient cell line, a CS-B as well as a XPC deficient cell lines.

Correlations between induced damages and epigenetic states of the chromatin like e.g. histone H3K27me3 or H3K4me3 or DNA methylation or GC content and transcription will be presented. Also structural influences of epigenetic marks and chromatin structures will be related to their specific repair kinetics. The influence of the different genetic backgrounds on the preferential repair of the photoproducts will be discussed in the light of gene expression.

MON24

Acute exposure to solar UV drives the cutaneous formation of photodamage-associated protein epitopes that are prevalent in melanoma and nonmelanoma skin cancer

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The molecular pathogenesis of cutaneous photoaging and photocarcinogenesis involves oxidative stress induced by solar ultraviolet light (UV) exposure. Under conditions of increased photooxidative stress, lipid peroxidation generates electrophilic reactive carbonyl species capable of propagating tissue damage through the formation of photodamage-associated protein epitopes (PAPEs). Utilizing immunohistochemical detection in single cell, ex-vivo tissue, and tissue microarray (TMA) format we have undertaken a comprehensive assessment of cutaneous PAPE content. In a panel of cultured human skin cells (immortalized HaCaT keratinocytes, primary keratinocytes and epidermal melanocytes, primary and metastatic cutaneous melanoma), we observed that acute exposure to subapoptogenic doses of simulated full spectrum solar UV is sufficient to drive the formation of specific PAPEs including N^{ε}-(carboxymethyl)lysine (CML)-, dihydropyridine-lysine (DHP)-, and malondialdehyde (MDA)-epitopes. Importantly, in photo-naive human skin exposed to acute solar insult *ex-vivo,* PAPEs were significantly elevated with accumulation localized primarily to the epidermal compartment. Subsequent analysis focused on PAPE detection in both melanoma and nonmelanoma skin cancer (NMSC) performed in TMA format. In squamous cell carcinoma tissue, MDA-, DHP-, and CML-epitopes were increased more than three-fold as compared to adjacent normal tissue. Similarly, within primary cutaneous and metastatic melanoma tissue, PAPE content was elevated as compared to benign nevi; however, immunohistochemical intensity scores displayed variability as a function of disease stage and anatomical location. Taken together, these data demonstrate the occurrence of photodamage-associated protein epitopes in both solar UV-exposed healthy human skin and NMSC and melanoma skin cancer tissue. Our ongoing research efforts focus on exploring the functional role of these epitopes in skin photocarcinogenesis.

MON25

UV-induced Epigenetic Alterations in Human keratinocytes - From DNA Damage Induction to Skin Cancer

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UV-radiation which reaches Earth level (UVB: 280 – 315 nm; UVA: 315 – 400 nm) is a known carcinogen to humans (Group 1, according to IARC) and is accepted to be the main environmental risk factor for the induction of skin cancer (basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant (MM)). UV-radiation induces specific mutations in the genome which lead to skin cancer. However, beside this known effects, there is increasing evidence over the last years that UV-radiation is also able to introduce epigenetic changes, both on the DNA-level (e.g. DNA methylation) as well as on the chromatin level (histone modifications like acetylation and methylation of terminal amino acid residues of certain histones). These modifications are strongly involved in regulation of gene expression, chromatin organization and cell fate (e.g. transitions of epidermal stem cell to differentiated keratinocytes). Additionally, post-transcriptional regulation of gene expression by miRNAs controls networks of cellular pathways involved in DNA damage response as well as induction and progression of skin cancer.

We, and others, have recently been able to show that UV-radiation is able to introduce epigenetic changes in the genome of human skin cells. Interestingly the type of epigenetic changes is dependent on radiation quality (UVB vs. UVA) and on exposure patterns of UV-radiation (acute vs. chronic exposure). We show that chronic UVA irradiation of human keratinoctytes is able to silence the expression of tumor suppressor p16 via CpG island promoter hypermethylation and specific histone methylation patterns. These changes together with UV-induced chromosome aberrations render HaCaT cells tumorigenic in the nude mice. Mice derived human SCC cells then show different epigenetic patterns, still silencing p16 expression.

Most interestingly, by using micro array analysis, we have also been able, to show that UV-radiation cause differential expression of miRNAs in human primary keratinocyte. Certain miRNAs are only changed in expression after UVA-irradiation, others only after UVB and a third group in response to UVA and UVB. Target gene analysis of UV-regulated miRNAs shows gene networks and pathways prominently involved in photocarcinogenesis and human skin cancer development. In SCC cell lines we were furthermore able to show that a so called “tumor suppressor miRNA”, itself, is epigenetically regulated in their expression.

We discuss our results in connection to a better understanding of UV-induced skin cancer development and to their use to identify biomarkers for risk assessment of UV-radiation and/or skin cancer as well as disease progression.

MON26

Enhancing photodynamic therapy by regulating ABCG2 expression and activity in cancer cell side populations.

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An increased side population phenotype in tumors from treated cancers, characterized by expression of the protein transporter ABCG2 is an indication of resistance to therapy, and contains stem-like cancer cells which may be responsible for recurrence of the tumor. Increased ABCG2 expression can be mediated by various transcription factors which bind to and regulate promoter sites on the ABCG2 gene and are attractive targets for therapy. Hedgehog (Hh) pathway driven cancers such as basal cell carcinomas (BCC) and some squamous cell carcinomas (SCC) arise because of mutations in the smoothened (SMO) or patched 1 (Ptch1) receptors which promote growth and proliferation through the GLI transcription factors. ABCG2 contains binding sites for GLI. Some tumors recur after treatment with aminolevulinic acid-induced photodynamic therapy (ALA-PDT), a light activated cancer treatment. One reason is because of the efflux of protoporphyrin IX (PpIX) (the photoactive product of ALA) by ABCG2. Vismodegib, a SMO antagonist FDA approved for treatment of advanced BCC inhibits Hh signaling mediated growth by and ABCG2 expression by decreasing GLI activity and also inhibits ABCG2 efflux activity directly, increasing PpIX levels and hence cellular phototoxicity. However, Vismodegib requires long term use for effective inhibition of tumor growth leading to adverse side effects, including alopecia, nausea, fatigue, weight loss and muscle spasms; a deterrent for clinical treatment. However, short term use prior to PDT may enable effective treatment by a double attack on ABCG2: downregulation and inhibition of activity. Alternatively, calcitriol and cholecalciferol are two forms of vitamin D3 that inhibit Hh-pathway signalling by a non canonical pathway by a mechanism similar to Vismodegib, leading to downregulation of ABCG2. Calcitriol or cholecalciferol at high (but non-toxic) concentrations decrease side populations and increase PDT efficacy by ABCG2 downregulation within ABCG2 expressing side populations, thus targeting PDT-resistant tumor cells. Successful optimization of such non-toxic preconditioning regimens followed by PDT could lead to widespread adoption of pre-conditioned PDT as a clinical treatment for cancers with ALA-PDT mediated PpIX or other ABCG2 substrate photosensitizers. Supported by NIH CA055792 and CA16056 Core support grant and the Castellani Foundation.

MON27

In vivo imaging of normal stem cells

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Hematopoietic stem cells (HSCs) reside in the adult bone marrow and are responsible for blood cell formation (hematopoiesis) throughout life. Clinical success of bone marrow transplantation depends on successful homing and engraftment of HSC in the recipient bone marrow. We describe optical techniques for tracking HSC in the bone marrow of live mice, and for characterizing the bone marrow microenvironment.

MON28

Specific and Efficient Targeting of Cancer Stem Cells by Photochemical Internalization

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Cancer stem cells (CSCs) are highly malignant cells that have acquired characteristics associated with normal stem cell biology. Hence, in contrast to differentiated cancer cells with restricted proliferative potential, CSCs are proposed to be immortal cells having the capacity to self-renew, differentiate into phenotypically diverse cancer cells and initiate and thereby recapitulate the original human tumor histology in immunodeficient mice models. CSCs persist in tumors as a distinct clone or multiple clones and are suggested to be the drivers of metastasis and relapse of tumor after therapy. Photochemical internalization (PCI) is an efficient and specific drug and gene delivery technology established in our lab. PCI is based on photodynamic therapy (PDT). Briefly, PCI using Amphinex/TPCS2a as photosensitizer induces endosomal or lysosomal membrane rupture and escape of drugs sequestered in these organelles into the cytosol of the targeted cell. In this presentation we demonstrate that PCI of therapeutics targeting different CSC markers (e.g. CD133, CD44, CSPG4 and CD271) provides high selectivity and potent cytotoxicity establishing PCI as a potential rational for elimination of CSCs.

MON29

Stem Cells, T Cells, and Selective Targeting with PDT—The Role of the Amide-thioamide 'Switch' in Rhodamine Photosensitizers in P-gp Expressing Cells

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P-glycoprotein (P-gp) and several other ABC transporters protect cells from xenobiotices by forcibly expelling the xenobiotics from the cell membrane. Multidrug resistance in cancer cells is often caused by overexpression of the ABC transporters including P-gp. Stem cells and T cells also express P-gp, which serve to protect these cells, as well. We have examined several small libraries of rhodamine compounds for their ability to be transported by P-gp as well as for their ability to hinder transport by P-gp. One correlation of structure/function that seems quite promising is the single atom change from an amide to a thioamide on aryl/heteroaryl groups in the 9-position of the rhodamine. The amide functionality stimulates ATPase activity in P-gp and, consequently, leads to rapid transport from P-gp expressing cells. Incorporation of the thioamide functionality – a single "O" to "S" atom change – gives rhodamines that are transported extremely slowly from P-gp expressing cells and that, on net, inhibit P-gp transport. We have synthesized a library of selenorhodamines with amide and thioamide functionality. The selenorhodamines generate singlet oxygen efficiently upon irradiation and have absorption maxima at wavelengths > 600 nm.. As positively charged, delocalized cations, the selenorhodamines localize in the mitochondria of cells. The thioamide-containing selenorhodamines function as efficient photosensitizers in P-gp-expressing cancer cells. The use of the amide/thioamide switch also suggests a strategy for protecting P-gp-expressing stem cells or resting T-cells while targeting activated cells in which mitochondrial potential has increased.

MON30

LCL521, sphingolipid metabolism modulator, is a potent enhancer of antitumor effect of photodynamic therapy

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Higher sensitivity of cancer cells than normal cells to lethal effects of increased endogenous ceramide levels induced by exogenous ceramide or sphingolipid (SL)-modulating drugs has motivated the development of various compounds of this type as prospective anti-cancer agents. Our earlier studies have shown that SLs are important participants in response to photodynamic therapy (PDT) at cellular and tumor levels. We have demonstrated that cure-rates of PDT-treated tumors can be significantly elevated by adjuvant treatment with various SL-modulating agents, including C6- and C16-ceramide analogues (LCL29 and LCL30, respectively) and acid ceramidase inhibitor B13 analogue (LCL85). Currently the most potent clinically relevant inhibitor of acid ceramidase is LCL521, a lysosomotropic N,N-dimethylglycine ester prodrug of B13. This water soluble drug was very well tolerated in mice at the dose of 75 mg/kg, which after single administration immediately after Temoporfin-PDT elevated cure-rates of SCCVII tumors compared to PDT alone. However, this result obtained with immunocompetent C3H/HeN tumor hosts was not matched with SCCVII tumors growing in immunodeficient NOD-scid mice where LCL521 treatment produced no significant improvement of PDT-mediated tumor cures. This finding suggests that the therapeutic gain obtained with LCL521 is rooted in interacting with host immune responses associated with tumor PDT. Further evidence supporting this mechanistic implication includes the impact of LCL521

on the expression of FOXP3 and BACH2 genes that are critical for the activity of regulatory T cells. It is therefore becoming increasingly clear that SL-modulating drugs can affect tumor response to PDT at multiple levels, including the amplification of PDT-induced apoptotic cell death and boosting PDT-elicited anti-tumor immune responses.

MON31

Ceramide-generating drugs enhance cancer cell killing after PDT

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Because photodynamic therapy (PDT) is not always effective as a single anticancer treatment modality, PDT is combined with other anticancer agents for improved efficacy. Apoptosis, an important mechanism for cancer cell killing, is induced by PDT. Bioactive sphingolipids, such as ceramide, enhance cancer cell killing by inducing apoptosis. We have shown that combining PDT with a ceramide analogue enhances not only total cellular ceramide accumulation but also overall cell killing. Here we present our ongoing findings using novel combinations of PDT with ceramide-generating drugs that promote killing of human head and neck squamous cell carcinoma cells via apoptosis. The data suggest potential translational significance of the combinations for cancer treatment.

MON32

Histone Acetyltransferase p300 Involves in Autophagy induced by Photodynamic Therapy and Is a Target to Improve PDT Response

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Photodynamic therapy (PDT) elicits cell death within treated tumors and induces the expression of angiogenic and/or prosurvival factors within the tumor microenvironment. Previously, we have shown that 5-aminolevulinic acid (ALA)-mediated PDT can trigger autophagy (Ref. 1). Furthermore, we have reported that PDT-induced upregulation of histone acetyltransferase p300 (p300HAT) involves in the increased expression of cyclooxygenase-2 (COX-2) (Ref. 2). Based on these results, we hypothesized that p300HAT might play an important role in PDT mediated cell death. To address this hypothesis, we first examined the treatment responses following exposure to PDT and the combination of PDT plus p300HAT inhibitor, anacardic acid (AA) in human A375 melanoma cells and mice colon adenocarcinoma C-26 cells. We found that PDT-induced cytotoxicity increases in the presence of AA or p300HAT shRNA. This increased cytotoxicity correlated with the reduced autophagy and increased apoptotic cell death. Furthermore, inhibition or knockdown of p300HAT inhibited PDT-induced upregulation of Cox-2, bcl-2 and survivin. Finally, we showed that the combination of PDT plus AA showed significant tumor reduction 3 days post PDT compared to the mice received 50% DMSO or PDT only. These results show that targeting p300HAT may enhance PDT responsiveness, which involves the manipulation of the antiapoptotic pathway maintained by p300HAT.

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MON33

Combination of Oral Vitamin D_3 with Photodynamic Therapy Enhances Tumor Cell Death in a Murine Model of Cutaneous Squamous Cell Carcinoma

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Non-melanoma skin cancers (NMSC) are among the most common human cancers. Unlike conventional surgical excision that often results in unsightly scars, photodynamic therapy (PDT) is a non-scarring and repeatable treatment alternative for NMSC. Aminolevulinate (ALA)-based PDT is a new and increasingly popular regimen in dermatology. ALA, a pro-drug, is selectively taken up by tumor cells and metabolized to protoporphyrin IX (PpIX) that induces cell death when activated by light. However, ALA-PDT as currently performed is not effective for deep tumors, mainly due to insufficient uptake of ALA and non-uniform production of PpIX. We recently showed that adding a differentiation-inducing pretreatment (e.g. methotrexate, calcitriol or fluorouracil) prior to PDT, in a combination regimen (cPDT) improves PpIX

accumulation and light-induced cell death in tumors. However, with calcitriol (the active form of vitamin D_3), cPDT poses a risk of toxicity (hypercalcemia) in humans. In this study, we test a possible strategy to circumvent this problem. Using a murine model of NMSC (subcutaneously implanted A431 cells), we show that vitamin D_3 delivered in its natural dietary form (cholecalciferol) can be used instead of calcitriol as a PDT enhancer. Short-term (10 day) dietary supplementation with cholecalciferol significantly enhances the PpIX levels (3-4 fold by confocal microscopy) and cell death (20-fold by TUNEL) in tumors. These vitamin D_3 effects are tumor-specific since no PpIX elevations nor enhancement of cell death were seen in normal skin. Serum measurements indicate only modest increases in hydroxylated metabolic forms of vitamin D_3 and a negligible risk of hypercalcemia. In summary, 10 days of a high cholecalciferol diet can serve as an adjuvant to ALA-PDT, similar in efficacy to calcitriol injections but with lower risk. These findings suggest that a cPDT approach with oral cholecalciferol is appropriate to consider for a clinical trial to treat NMSC in humans.

MON34

The tryptophan photoproduct and endogenous AhR-ligand 6-formylindolo[3,2-b]carbazole (FICZ) is a nanomolar UVA- and visible light-activated photosensitizer in epidermal keratinocytes and reconstructed human skin

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Endogenous UVA-chromophores may act as sensitizers of photooxidative stress underlying cutaneous photoaging and photocarcinogenesis, but the molecular identity of non-DNA key chromophores displaying UVA-driven photodyamic activity in human skin remains largely undefined. Here we report that 6-formylindolo[3,2-b]carbazole (FICZ), a tryptophan photoproduct and endogenous high affinity aryl hydrocarbon receptor (AhR) agonist, displays activity as a nanomolar photosensitizer potentiating UVA- and visible light-induced oxidative stress irrespective of AhR ligand activity. In human HaCaT and primary epidermal keratinocytes, photodynamic induction of apoptosis was elicited by the combined action of solar simulated UVA (3.3 J/cm^2) and FICZ (10 nM), whereas exposure to the isolated action of UVA or FICZ did not impair cell viability. Likewise, in a human epidermal tissue reconstruct (EpiDerm^{TM}, MatTek) sunburn cell formation and proteolytic activation of caspase 3 were detectable only upon combined exposure to FICZ and UVA. Apoptotic elimination of skin cells was also observed upon FICZ photoexcitation using a blue light source (LED 460 nm). Interestingly, indolo[3,2-b]carbazole (ICZ), a FICZ-related chromophore devoid of the 6-carbaldehyde-substituent, did not display photodynamic activity suggesting the crucial involvement of carbonyl group excited state reactivity in FICZ photocytotoxicity. FICZ photosensitization was associated with upregulation of intracellular oxidative stress counteracted by inclusion of singlet oxygen quenchers (NaN_3, DABCO). Furthermore, FX174-plasmid cleavage and cellular comet assays revealed introduction of formamidopyrimidine-DNA glycosylase (FPG)-sensitive DNA lesions suppressible by NaN_3 inclusion, indicative of genotoxic effects downstream of FICZ/UVA-induced photooxidative stress. Array analysis revealed pronounced potentiation of cellular heat shock (*HSPA6*, *HSPA1A*), ER stress (*DDIT3*), and oxidative stress (*TXNRD1*, *HMOX1*, *AKR1C2*, *SPINK1*) response gene expression upon combined exposure to UVA and FICZ, a finding further substantiated by immunoblot analysis (p-eIF2α, p-p38 MAPK, HO-1). Taken together, our data demonstrate that the endogenous AhR ligand FICZ displays nanomolar photodynamic activity representing a novel mechanism of UVA-induced photooxidative stress potentially operative in human skin. [NCI-R03CA167580, R21CA166926]

MON35

Life and UV in Yellowstone: As if Boiling Acid and Arsenic Were Not Enough

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Yellowstone National Park is home to over 14,000 geothermal features that include mud pots, geysers, flowing hot springs and geothermally heated soils. The chemistry of these features range extensively (e.g. pH 1-10) and are home to a dazzling array of microbial diversity. Over the last decade, we have invested significant efforts in studying a specific feature known as Dragon Spring in the Norris Geyser Basin. This is an NSF Microbial Observatory that focuses on the study of chemolithotrophic microbial communities in acidic environments. Within the outflow channel, the bacterium *Hydrogenobaculum* dominates the microbial community (> 95% of metagenome or 16S rDNA clone libraries) along the temperature and geochemical gradients that offer a continuum of niche opportunities. This organism utilizes H_2 and H_2S as energy sources

and CO_2 as its carbon source, and prefers a pH of ~ 3.0-4.0 and temperatures of ~ 55-65 °C.

Of particular interest to us is the intense microdiversity in the *Hydrogenobaculum* gene sequences cloned from this hot spring environment. We have been investigating the potential basis for this diversity, with special interest in UV-B as a source of mutational input. We have measured UV-B as high as 1.7 $J \cdot m^2 \cdot s$ during the 14 h photoperiods of June and July. And, using herring sperm DNA as a target, we have documented DNA damage rates to be roughly the equivalent of every gene in the *Hydrogenobaculum* genome being damaged every hour. We will present data that is consistent with the hypothesis that UV-B may be an active mutagen capable of contributing to microbial genetic diversity over short time frames. We acknowledge support from NSF and NASA.

MON36

Enhanced cold resistance of zoysiagrass cultures through overexpression of wild type and Ser599Ala-mutant phytochrome A genes

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Zoysia japonica, one of the most popular turf grasses used for home lawns, parks, golf courses and sports fields, is a warm season grass. It suffers from cold stress, as the grass usually wilts and browns by late autumn. In a study to confer the cold resistance to the zoysiagrass, we introduced phytochrome A (*PHYA*)genes to the plant. The control and transgenic cell cultures of *Zoysia japonica* over-expressing wild type or S599A *PHYA* genes were challenged with cold stresses by the exposure to low-temperature (0.5, 2 or 3.5°C) for 15 days. The survival rate and packed cell volume (PCV) of all the cell lines were then measured under light and shade conditions. The cell cultures of non-transgenic and control plants exhibited significant decline in PCV and displayed increased ROS activities (peroxidase and superoxide dismutase) under both light and shade conditions, whereas under shade conditions, PCV and ROS activities were higher than under light conditions. In addition, the green callus cultures were placed in MS shoot induction media and subjected to a low-temperature (2°C for 25 days). The transgenic callus cultures of transgenic plants over-expressing either wild type or mutant *PHYA* gene displayed dramatically higher survival rates (89 to 90%) than wild type and control plants (12%) in prolonged cold storage under dark conditions (20°C for 50 days). Based on these findings, we developed *Zoysia japonica* with *PHYA* transgene and demonstrated that they remain green until early winter, confirming that overexpression of *PHYA* transgene confers cold tolerance to the turf grass.

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MON37

Extreme resistance of *Geodermatophilus obscurus* and *Hymenobacter gelipurpurascens* to UV-C irradiation

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Desiccation resistant organisms are usually radiation resistant and have a high intracellular ratio of Mn/Fe. Desiccation, ultraviolet radiation and Mn salts are environmental parameters readily present in desert environments. UV-C radiation (λ=200-280 nm) has been used as a model for ionizing radiation resistance due to the similarities of bacterial survival between UV-C and gamma irradiation. Both types of radiation induce similar damages to the DNA and similar enzymatic machinery is used to repair UV-C and gamma radiation damage. In our research we are looking for the most resistant organisms to better understand their molecular mechanisms for different applications in biotechnology, synthetic biology and astrobiology. Early results show a high diversity of UV-resistant microorganisms present in manganese deposits in the Sonoran Desert, AZ, USA and the Atacama Desert, Chile. The isolates MN04-01 (*Geodermatophilus obscurus*) and AT01-02 (*Hymenobacter gelipurpurascens*) were more resistant to UV-C than *Deinococcus radiodurans*, with LD_{10} (dose that kills 90% of the population) values of 1,380±354 for *G*. *obscurus*, 826±42 for *H*. *gelipurpurascens* and 597±41 for *D*. *radiodurans*. Their intracellular ratio of Mn/Fe were determined by ICP-MS as 0.1719 for *G*. *obscurus*, 0.1425 for *D*. *radiodurans* and 0.0350 for *H*. *gelipurpurascens*. Our preliminary observations indicate the production of pigments by *G*. *obscurus* that strongly absorb UV-C radiation and can protect cells of *Escherichia coli* as shown by survival curves experiments. Moreover, viral-like particles were found in association with *H*. *gelipurpurascens*, as evidenced by scanning electron microscopy (SEM) analysis. These two isolates

represent good biological models for photobiology studies involving pigment characterization and analysis of DNA damage and repair. The study of microorganisms highly resistant to radiation in desert soils containing manganese indicates that this type of environment harbours valuable biological resources with potential applications that could benefit earth and space exploration programs.

MON38

The fine structure of DNA damage in marine microbial communities; geographical and temporal distribution along a latitudinal transect in the Pacific Ocean

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Induction of DNA damage by solar UV radiation is lethal and mutagenic in microorganisms. To better define the nature of these DNA photoproducts, consisting mostly of pyrimidine dimers, in marine bacterioplankton and eukaryotes, a study was performed during a cruise along a latitudinal transect in the Pacific Ocean. The frequency of all possible cyclobutane pyrimidine dimers, pyrimidine (6-4) pyrimidone photoproducts and their related Dewar valence isomers was determined by HPLC-mass spectrometry. Studied samples were bacterioplankton and eukaryotic size fractions isolated from sea water either collected before sunrise or exposed to ambient sunlight from sunrise to sunset (12 h). Isolated DNA dosimeters were also exposed comparative purposes. A first major result was the observation in all samples of large amounts of Dewar photoproducts, a class of photoproducts rarely considered outside photochemical studies. In addition, comparison between the frequency of DNA damage and UV measurements showed that residual photoproducts present in the genome of microorganisms resulted from long-term accumulation possibly due to reduced DNA repair kinetics. Finally, considerations on the ratio between the different classes of photoproducts suggests that photoenzymatic repair is a crucial DNA repair mechanism used by marine microorganisms occupying surface seawater in the open ocean

MON39

The role of autophagy-related proteins [ATGs] in the efficacy of photodynamic therapy

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The ability of comparatively low light fluences to initiate death pathways in photosensitized cells often involves an apoptotic process that can be elicited by any mechanism leading to appearance of cytochrome c in the cytoplasm. Autophagy often fills a cytoprotective role and impairment of autophagy can promote photokilling. In 2012 [Autophagy, 8, 1-9] we demonstrated a new role for the autophagy-related protein ATG7. This protein was required for photodamage to permeabilize the lysosomal membrane sufficiently to permit release of the proteases that trigger apoptosis. We have now found a new role for another such protein: ATG5, in the context of PDT. When tumor cells in 2D or 3D cultures were given a low-level PDT dose directed at lysosomes, we found that this markedly potentiated photokilling by subsequent mitochondrial photodamage. This effect was absent in an ATG5 knockdown and was inhibited by cysteine protease antagonists. Low-level lysosomal photodamage also amplified the pro-apoptotic effects the Bcl-2 antagonist ABT-737. It was reported in 2006 [Nature, Cell Biol 8, 1124-1132] that calpain could cleave ATG5 to a form that amplified any pro-apoptotic signal The sequential PDT protocol outlined above appears capable of potentiating the efficacy of many pro-apoptotic signals and may prove useful in promoting the photokilling by otherwise inadequate PDT doses, e.g., where the light fluence might otherwise be limiting. Crosstalk between autophagy and apoptosis has been the subject of prior reports, e.g., Cell Death Differ. 14, 1247–1250, 2007. We have now provided two examples where ATGs can affect photokilling, depending on the interaction between these proteins and biologic systems.

MON40

Increased PDT efficacy when associated with nitroglycerin. **A study on retinoblastoma xenografted on mice**.

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Purposes: The aim of the study was to assess the efficacy of a treatment protocol that combines photodynamic therapy (PDT) and nitroglycerin (NG) on human retinoblastoma tumors xenografted on nude mice. PDT uses of a non-mutagen photosensitizing agent (PS : glycoconjugated *meso* substituted porphyrin derivative) activated by exposure to red light. Absorption of light initiates the photochemical reactions leading to the generation of cytotoxic products responsible for the therapeutic effects. In vivo follow-up of the therapeutical effects was performed by

sodium MRI. Vasculature damage and necrosis or apoptosis decrease cell density and increase the local sodium concentration [1]. Sodium magnetic resonance imaging (^{23}Na MRI) directly monitors variations of sodium concentrations in a non-invasive way, it can be used to follow-up the tumor response to therapy [2]. NG is known to dilate vessels and enhance the permeability and retention of macromolecules in solid tumors [3].

Methods: PDT (650 nm) targeting both blood vessels and cancer cells was followed by ^{23}Na/^{1}H MRI. NG ointment (0.2 mg) was applied 1 hour before the first PS *i.v*. injection. The first dose of PS was followed by a second dose, separated by a 3h interval. This time lapse allowed the first dose of PS to penetrate into tumor cells. Ten minutes after the second dose, tumors were exposed to red light. Two PDT treatments were performed at 4 days interval.

Results: The PDT treatment hindered the tumoral progression and finally stop it. The NG acted as a synergistic factor in therapy triggering the initiation of the bystander effect. This effect was found even after the first PDT treatment.

Conclusion: In this study we reported that the PDT effect was enhanced by applying nitroglygerin (NG) ointment on the skin of tumor-bearing animals. PDT initiate the bystander effect on retinoblastomas, NG increase this bystander effect.

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MON41

Enhanced Efficacy of Photodynamic Therapy (PDT) via an Iron-Lysosome-Mitochondria Connection: Studies with Pc 4 and Dual Responsive Nanoparticles

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PDT is an alternative to surgery for treating head and neck squamous cell cancer (HNSCC). HNSCC cell lines are variably sensitive to PDT using the phthalocyanine 4 photosensitizer, Pc 4. To determine the mechanism(s) for this differential PDT sensitivity, we investigated whether mitochondrial iron uptake through mitoferrin-2 (Mfrn2) contributes to enhanced PDT cell killing. In non-erythroid cells, Mfrn2 is an iron transporter in the mitochondrial inner membrane. PDT-sensitive cells expressed ~3 fold higher Mfrn2 protein levels compared to PDT-resistant cells. High Mfrn2-expressing cells showed higher rates of mitochondrial Fe^{2+} uptake compared to low Mfrn2-expressing cells (270 and 83 nmol Fe^{2+}/min/mg protein, respectively). Bafilomycin, an inhibitor of the vacuolar proton pump of lysosomes and endosomes that releases lysosomal iron to the cytosol, enhanced PDT-induced cell killing of both resistant and sensitive cells. Iron chelators (desferrioxamine and starch-desferrioxamine) and the inhibitor of the mitochondrial Ca^{2+}, Fe^{2+} uniporter, Ru360, protected against PDT plus bafilomycin toxicity. Knockdown of Mfrn2 in high Mfrn2-expressing cells decreased rates of mitochondrial Fe^{2+} uptake and delayed PDT plus bafilomycin-induced mitochondrial depolarization and cell killing. To further assess the role of lysosomes in the PDT killing pathway, we developed Pc 4-loaded nanoparticles that selectively target sigma-2 receptors of HNSCC cells. *In vitro*, Pc 4-loaded nanoparticles self-expanded in an acidic pH and high redox potential environment. In HNSCC cells, the pH and redox dual responsive nanoparticles (DRN) were rapidly taken up by lysosomes, as assessed by confocal microscopy. Subsequently, Pc 4 co-localized with mitochondria in a time-dependent manner suggesting that Pc 4 dissociated from DRN in acidic lysosomes, allowing free Pc 4 to translocate from lysosomes to mitochondria. An *in vivo* biodistribution study showed strong Pc 4-DRN accumulation in tumors. Taken together, Pc 4-DRN is preferentially directed into tumors, where it is taken up by lysosomes. Acidic lysosomal pH releases Pc 4 from DRN. Free Pc 4 and possibly iron escape from lysosomes and translocate to mitochondria. Tumors expressing higher mitochondrial Mfrn2 protein levels may benefit more from PDT due to increased free radical formation.

MON42

Photo-activated psoralen binds the ErbB2 catalytic kinase domain, blocking ErbB2 signaling and triggering tumor cell apoptosis

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Photo-activation of psoralen with UVA irradiation, referred to as PUVA, is used in the treatment of proliferative skin disorders. The anti-proliferative effects of PUVA have been largely attributed to psoralen intercalation of DNA, which upon UV treatment, triggers the formation of interstrand DNA crosslinks (ICL) that inhibit transcription and DNA replication. Here, we show that PUVA exerts antitumor effects in models of human breast cancer that overexpress the ErbB2 receptor tyrosine kinase oncogene, through a new mechanism. Independent of ICL formation, the antitumor effects of PUVA in ErbB2+ breast cancer models can instead be mediated through inhibition of ErbB2 activation and signaling. Using a mass spectroscopy-based approach, we show for the first time that photo-activated 8MOP (8-methoxypsoralen) interacts with the ErbB2 catalytic autokinase domain. Furthermore, PUVA can reverse therapeutic resistance to lapatinib and other ErbB2 targeted therapies, including resistance mediated via expression of a phosphorylated, truncated form of ErbB2 ($p85^{ErbB2}$) that is preferentially expressed in tumor cell nuclei. Current ErbB2 targeted therapies, small molecule kinase inhibitors or antibodies, do not block the phosphorylated, activated state of $p85^{ErbB2}$. Here we show that PUVA reduced $p85^{ErbB2}$ phosphorylation leading to tumor cell apoptosis. Thus, in addition to its effects on DNA and the formation of ICL, PUVA represents a novel ErbB2 targeted therapy for the treatment of ErbB2+ breast cancers, including those that have developed resistance to other ErbB2 targeted therapies.

MON43

Integrin-Targeted, PEG-Enhanced Photosensitizer Constructs for Lysosome-Mediated Cell Death

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The uptake of photosensitizers by tumors is limited by delivery barriers, such as a lack of perfusion or the adherence to the extracellular matrix or fibrous capsules, leading to subcurative therapy in many instances. Approaches to improve photosensitizer accumulation in tumors, such as the use of highly cationic agents, can cause different problems such as dark toxicity (light independent toxicity). Furthermore, even when photosensitizers reach their intended destination, effects of the local environment can reduce cytotoxic potential. To overcome these problems, we synthesized photodynamic agents exploiting “PEG-photosensitizer shielding,” where a photosensitizer is covalently linked to a modular peptide backbone, which in turn is coupled to a single polyethylene glycol (PEG) polymer (>2 kDa). PEG shields the photosensitizer, blocking self-association (stacking), non-specific interactions with cells or proteins, and allows unhindered diffusion through tissues. We have coupled the photosensitizer EtNBS to this modular construct, along with a cyclic cell-targeting peptide, and obtained a construct with high cellular uptake and good photodynamic activity. Importantly, when delivered to large 3D in vitro tumor models, this construct had remarkably high diffusivity and readily penetrated tumor spheroids many hundreds of microns in diameter. Current studies are focused on characterizing the cytotoxicity of this construct, in both monolayer and 3D tissue cultures, investigating photodynamic environmental screening mechanisms, and exploring its application in animal studies.

MON44

Improving Tumor Responses to Photodynamic Therapy by Pretreatment with Small Molecule Enhancers of Cellular Differentiation

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Photodynamic therapy (PDT) is a cancer-treatment technique that uses either 5-aminolevulinate (ALA) or its methyl ester (MAL) as a topical pro-drug to induce intracellular synthesis of protoporphyrin IX (PpIX) within tumor cells. This therapeutic approach is becoming increasingly popular to treat cancers of the skin and mucosa (squamous cell carcinoma, SCC; basal cell carcinoma, BCC), with most clinical trials performed in Europe to date. While MAL-PDT has been approved as a licensed modality for skin cancers in most European countries, limitations to current PDT approaches have stalled its adoption for SCC and BCC treatment in this country. (Thus in the United States, PDT with ALA is only FDA approved for thin squamous pre-cancers of the skin, called actinic keratoses.) Limitations that need to be overcome include an inadequate penetration of pro-drug into deep tumor nests, and non-homogeneous distribution of PpIX in various parts of the tumor. We have pioneered a new approach toward solving this problem by manipulating tumor biology in a manner that induces cancer cells to synthesize more PpIX from the same amount of pro-drug. In work that spans laboratory studies of cultured cells and animal models, and more recently pilot clinical trials, we have pursued our initial discovery that pretreatment of SCC or BCC cancers with low amounts of methotrexate, 5-fluorouracil, or vitamin D, given daily for 3 days prior to PDT, can lead to a significant increase in PpIX

production within tumor cells. The effects are specific to neoplastic cells, since increases in PpIX do not occur (or occur to a much lower extent) in corresponding normal tissues. This presentation will review data on these new combination PDT approaches, reveal what is known about the mechanisms of enhanced tumor response, and discuss prospects for achieving improved PDT responses in epithelial cancers of several origins. We will also present data from our ongoing clinical trial that investigates the efficacy of a combination 5-fluorouracil/ALA-PDT treatment approach for squamous precancers of the skin in human patients.

MON45

Using coordination chemistry to develop light-activated anticancer agents

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A focus on targeted therapies has dominated recent efforts in drug design and development. However, not all patients within a cancer subtype express the target required for these drugs, limiting the impact of molecular targeting. As a result, the design of compounds with a general cytotoxic mechanism, such as cisplatin, is desirable to treat the greatest number of patients. One approach to reduce off-target effects while maintaining broad efficacy is to combine the reactivity of metal-based drugs with the spatial selectivity of phototherapy to increase the targeting of malignant tissues. We are utilizing structural distortion to promote photochemical reactions in a series of ruthenium polypyridyl compounds to create reactive metal centers that are strongly electrophilic and highly photo-toxic. The pro-drug compounds have a readily modifiable modular design that facilitates incorporation of different molecular components to efficiently develop systems exhibiting selectivity and light-controlled reactivity. Using this approach, we have achieved up to 1,000-fold increases in potency upon light-activation of specific compounds. However, as changes in the structure of the metal complex can also affect interactions with biological targets, we are developing screening assays to rapidly probe the biological mechanism of action of these light-activated compounds. These assays also provide direct measurements of compound efficacy, and are currently being developed for high-throughput screening. This will provide a more clear and complete picture of compound activity, facilitating rapid assessment of our compound libraries as individual chemical features are modified and optimized.

MON46

UV and vitamin D: What are we aiming for and what are we achieving?

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Vitamin D is important for bone health, is linked to many other health benefits including protection against a range of malignancies and autoimmune disorders, and is known to modulate many biological responses including cell differentiation. It is therefore important to evaluate the vitamin D status of the general population in the context of recommended target levels and public health advice on vitamin D acquisition. The accepted best indicator of vitamin D status is the circulating level of 25-hydroxyvitamin D (25OHD). The Institute of Medicine for the USA and Canada advises a target 25OHD level of ≥20 ng/ml (50 nmol/L) based on parameters of bone health, and recommends oral intake of ≥600 IU daily. The target is currently under review in the UK, where most adults are not advised of any oral vitamin D requirement as adequate intake has been assumed to occur from cutaneous synthesis following regular brief UV exposures. Through a series of intervention studies, we demonstrated that a 6 week course of low dose (1.3 SED) UV radiation exposures (3 times a week to 35% skin surface area), simulating a summer's casual sunlight exposures, could produce 25OHD ≥20 ng/ml in 90% of the white Caucasian adult (phototype I-IV, aged 20-60 years) population. However, no adults of South Asian ethnicity (phototype V) reached sufficiency following an identical course of exposures. Increased UV exposure, up to 3 times that given to white Caucasians, raised 25OHD levels in South Asians enough to avoid deficiency i.e.25OHD ≥10 ng/ml but the majority could not reach 20 ng/ml.. Our observation studies have shown that 77% of white Caucasian adults aged 20-60 years actually achieve 20 ng/ml at summer end (September) in the UK, falling to 40% at the winter trough in February. In contrast, 25OHD levels in a cohort of South Asian adults in the same age range were startlingly low, with only 7% ever achieving 20 ng/ml. Due to the diverse roles of vitamin D, suboptimal status may impact on responses to therapy, as well as on various health outcomes. Considering our observation and intervention studies in tandem, more effectively targeted guidance on sunlight exposure and oral vitamin D acquisition could assist those at risk of low levels.

MON47

Vitamin D Pretreatment Enhances the Therapeutic Efficacy of Aminolevulinate Based Photodynamic Therapy in Basal Cell Carcinoma Model

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Basal cell carcinoma (BCC) of the skin is the most common form of human cancer worldwide, and its incidence has been increasing in recent years. Photodynamic therapy, mediated by topically applied aminolevulinic acid (ALA), followed by exposure to light (either a laser or a noncoherent source) is being increasingly used for the treatment of dermatological disorders, including BCC skin cancers (particularly in Europe). However, therapeutic responses of BCC tumors after ALA-PDT treatment are currently similar, or even inferior, to some other standard medical and surgical therapies. In this study, we report that the treatment outcome of BCC can be improved by conditioning BCC tumors with vitamin D, prior to administering ALA-PDT. Our new approach has been tested on a BCC mouse model that has properties similar to the native BCC skin tumors in humans. PTCH1+/- K14-Cre-ER *p53* flox/flox mice, which were treated with tamoxifen and ionizing radiation (IR), developed multiple BCC tumors at 5-6 months of age. Histologically, these murine BCC tumors resembled nodular human BCCs and had high levels of proliferation. Using a Maestro EX *in vivo* fluorescence imaging system and *ex vivo* confocal microscopy, we confirmed that the topical application of vitamin D on BCC tumors increased the intratumoral levels of the ALA induced photosensitizer, protoporphyrin IX (PpIX), by up to 4-fold. In addition, enhanced differentiation and proliferation were identified in vitamin D pretreated tumors, enhancing the tumor specific cell death due to ALA-PDT relative to tumors treated with vehicle alone. We conclude that our new approach of combining vitamin D and ALA-PDT has great potential in achieving complete remission of BCC tumors with excellent clinical and cosmetic results and could have a beneficial impact upon patient care.

MON48

ALA-mediated PDT induces vascular response and photobleaching in superficial oral cavity lesions

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We have measured spectrally-resolved diffuse reflectance and fluorescence as part of a clinical trial of ALA-mediated PDT for superficial oral cavity lesions. Patients enrolled in the study were treated with 630-nm light administered via a microlens or balloon diffuser, depending on the treatment site, 4 to 6 hours after sensitization with orally administered 5-ALA (Levulan). Fluorescence spectra excited by 403-nm light and white light diffuse reflectance were captured sequentially with custom-built contact probe, allowing correction of fluorescence spectra for absorption and scattering artefacts. Reflectance spectra were analyzed to determine changes in hemoglobin oxygenation (StO_2) and total hemoglobin content ($[Hb]_t$)

Here, we present results from the fractionated arm of the study, in which treatment was interrupted for a 90 to 180 second break at 20% of the prescribed total fluence. In these patients, we see a response in StO_2 in all cases. In all but one case, the net change at the fractionation point is positive, however the change over the total treatment is negative for the lowest-fluence cohort, indicating a fluence-dependent evolution of StO_2 over the course of treatment. Total hemoglobin content also changes in response to treatment, but less systematically. Fluorescence photobleaching of ALA-induced protoporphyrin IX was observed in all cases. The majority of the superficial photobleaching in each case had already occurred at the fractionation point. Ongoing hemodynamic changes beyond this point likely indicate the ongoing action of deeper-lying, unbleached sensitizer to which our probe is less sensitive.

These data demonstrate reproducible trends in hemodynamic changes induced by ALA-mediated PDT and significant patient-to-patient variability, highlighting the need for patient-specific dosimetry.

MON49

Role of Nutritional Lipids and Antioxidants in UV-Carcinogenesis

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Two of the dietary tenets of the free-radical theory of cancer, put forth by Denham Harman in 1962, require, in deference to newly accrued knowledge, refinement. The first recommendation was to reduce vulnerable free-radical targets such as polyunsaturated fatty acids (PUFA). The second was to supplement the diet with one or more antioxidants. Indeed, increasing levels of omega-6 PUFA exacerbate UV-carcinogenesis in a near linear fashion, with regard to decreased tumour latent period and increased tumour multiplicity. However, dietary omega-3 PUFA dramatically inhibits UV-carcinogenesis, increasing tumour latent period and reducing tumour multiplicity. Yet the degree of unsaturation in both types of fats is almost equal. It is almost certain that the action of these two types of PUFA rests, not with degree of saturation, but with the

intermediates that each generates through the lipoxygenase and cyclooxygenase pathways. The general recommendation to reduce dietary PUFA as a mean to free-radical reduction and reduced cancer risk is oversimplified and points to the complexity faced when accurately refining this recommendation. The second recommendation requiring refinement, i.e., reducing cancer risk by addition of one or more antioxidants to the diet also represents a formidable task. Supplementation of an antioxidant into the complex milieu of the cell with its own intricate and complex defence system may result in untoward effects. In addition, each antioxidant exerts its own specific mechanism(s) of radical scavenging and may exert its own specific physiological responses. As example, butylated hydroxytoluene (BHT) was shown to markedly reduce the occurrence of UV-induced squamous cell carcinomas in mice. BHT's mode of action in inhibiting UV-carcinogenesis involves the chemical differentiation of stratum corneum, resulting in UV-dose diminution to target cells. Beta-carotene supplementation dramatically exacerbates UV-carcinogenesis under certain dietary conditions. BHT and Beta-carotene potentiate hepatic phase I and/or II detoxification enzymes that may further predispose the host to chemically-induced carcinogenesis. It may be necessary to develop an algorithm for each antioxidant based upon the risks and benefits to be derived. Experimental results underscore the oversimplification of these dietary tenets to reduce cancer risk.

MON50

Photoreactivity of human retinal lipid extracts from different age groups.

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Polyunsaturated fatty acids (PUFA), especially the most unsaturated fatty acid - docosahexaenoic acid (22:6) (DHA), are very abundant in the neural tissues including retina. Retinal PUFA are highly susceptible to oxidation being constantly exposed to strongly oxidizing environment. It has been shown that carboxyethylpyrrole (CEP), one of the products of DHA oxidation, known to be generated in situ, may modify retinal proteins and contribute to inflammation responses of the outer retina. It has been postulated that chronic inflamatory reactions could play a role in the pathogenesis of age-related macular degeneration (AMD). It is also believed that some of the lipid oxidation products are photoreactive and, upon irradiation with blue light, may generate reactive oxygen species. In this work we studied photoreactivity of oxidized retinal lipids extracted from human donors of different age groups as well as synthetic lipids with composition similar to that of naturally present in the human retina. Lipid composition of human retinal extracts has been determined by GC and LC/MS analysis. Lipid extracts (LEx) and synthetic lipids were oxidized in the dark in homogenous solutions equilibrated with air at 37°C. The photoreactivity of the studied samples was analyzed by time-resolved phosphorescence of singlet oxygen at 1270nm, EPR-oximetry, EPR-spin trapping and electrochemical detection of cholesterol hydroperoxides, employing cholesterol as a mechanistic reporter molecule. Human retinal LEx were very sensitive to oxidation despite the presence of endogenous hydrophobic antioxidants, such as carotenoids and tocopherol. Upon irradiation with blue light, oxidised LEx generated singlet oxygen and superoxide anion with moderate yields. The observed photoreactivity of the studied extracts gradually increased during their *in vitro* oxidation and was found to be higher in extracts from older donors. Our data suggest that such photoreactive products of retinal lipid oxidation may be formed in situ and contribute to photodamage of the outer retina.

MON51

Blue-light (420–453 nm) induced non-enzymatic nitric oxide generation from

photolabile nitric oxide derivates in human skin in vitro and in vivo

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It has been shown that UVA can generate nitric oxide (NO) in the human skin from No storages like nitrite and nitrosothiols. This NO is bioactive and facilitates beneficial physiological responses by increasing local blood flow and reducing blood pressure systemically when applied to the whole body. However, UVA is considered cancerogenic so we investigated UV-free wavelengths in the blue range of the spectrum (420-453nm) for their potential to generate bioactive NO from photolabile NO storages in vitro and in vivo. UV-free blue light induced a significant release of NO from nitrosothiols and from nitrite in a Cu^{1+} dependent way at neutral pH values as measured by chemiluminescence detection (CLD). Irradiation of forearms of volunteers induced an emanation of NO

from the skin. Additionally, in human skin specimen we could detect increased amounts of NO by EPR spectroscopy up to 4.5mm deep into the skin, far beyond the reach of the blue light which is absorbed in the upper epidermal layers. Blue light induced an immediate and significant increase in local cutaneous blood flow measured by micro-light-guide spectrophotometry at 6mm depth. Blue light irradiation of human skin specimen did not induce DNA damage up to 200J/cm² as shown by TUNEL analysis. Taken together our findings show that UV-free blue light is capable of releasing NO in an enzyme independent way from storage molecules (nitrite and nitrosothiols) found in high concentrations in the human skin. This NO is biologically active e.g. increasing local blood flow in a temperature independent way in human volunteers and increasing cGMP formation. This feature of UV-free blue light can be of therapeutically relevance to treat systemic and local hemodynamic disorders which are the result of an impaired availability of NO.

MON52

Measurement of intracellular pH in cancer cells *in vivo* using new genetically encoded indicator

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Intracellular pH is known to be an important regulator of many cell functions. Cancer cells generally have higher intracellular pHi and lower extracellular pHe. The reversed pH gradient is considered as hallmark of neoplastic tissue enabling cancer progression. There is a considerable interest in measuring pHi with high accuracy, precision, and spatiotemporal resolution. Green fluorescent protein (GFP) based pH indicators offers excellent opportunities for noninvasive continuous pH monitoring in cells and tissues with high sensitivity, in real time, by means of relatively low-cost optical technique.

Very recently, an improved pH-sensitive indicator generated from specific H_2O_2 indicator HyPer-2 has been introduced by Belousov et. al. New dual-excitation ratiometric pH-indicator has wider dynamic range and higher brightness than previously reported by Poburko et al. pH-sensitive YFP SypHer. pH registration with these sensors is based on detecting emission around 516 nm under excitation at two wavelengths 420 nm (I_{420}) and 500 (I_{500}) nm and calculating the ratio I_{500}/I_{420}. In more alkaline conditions excitation maximum at 420 nm decreases and at 500 nm increases, resulting in increase of the I_{500}/I_{420} ratio.

We applied newly developed genetically encoded pH indicator for pHi mapping in tumors. Ratiometric imaging of the indicator in 3D tumor spheroids and tumors in living mice was performed. HeLa Kyoto cell line stably expressing pH indicator in the cytosol was used. Fluorescence was analyzed using microscopic and whole-body imaging techniques.

To convert calculated I_{500}/I_{420} ratios to pH levels calibration curve was acquired for cells *in vitro* in buffer solutions in combination with ionophore.

The results of pHi ratiometric imaging in 3D tumor spheroids showed more basic pHi in the nodule core. We found that pHi within HeLa tumor *in vivo* was highly heterogeneous and the ratio images hardly changed during tumor growth. Additionally, histopathology and hypoxia in the tumors were characterized to interpret the data of pHi imaging.

The present work demonstrates the potential of new genetically encoded pH indicator for noninvasive pHi monitoring in tumor models *in vitro* and *in vivo*.

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MON53

Environmental UV-mediated photomodification and DNA damage induced apoptosis by Benz(a)anthracene via mitochondrial mediated pathway

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Benz(a)anthracene (BA) is an ubiquitous environmental pollutant of polycyclic aromatic hydrocarbon's (PAHs) family. The aim of the present study was to examine the pattern of UV radiation in sunlight and its impact on BA for photomodification and phototoxicity. We showed a variation in solar UVR on earth surface, during different seasons of the year. This is the first report which showed BA photo modification under sunlight exposure. In this study, we demonstrated that BA induced apoptosis in HaCaT cells through cytoplasmic and mitochondrial mediated generation of reactive oxygen species (ROS). BA induced photocytotoxicity were investigated through MTT and NRU assay. We proposed DNA insults such as single and double strand breakage and CPDs formation are the cause for cell cycle arrest and apoptotic cell death by photosensitized BA. BA induced apoptosis was caspase dependent and occurred through a mitochondrial pathway. Reduction of mitochondrial membrane potential, translocation of Bax to mitochondria and cytochrome c release favours

involvement of mitochondria in BA phototoxicity. AO/EtBr staining and TEM analysis also support apoptotic cell death. We propose a p21 regulated apoptosis via expression of Bax and cleaved PARP under sunlight exposure. Concomitantly, investigation is urgently required for the photosafety of BA photoproducts reaching in the environment through photomodification.

Key-words: Benz(a)anthracene; Photomodification; ROS; DNA damage; Apoptosis

TUES1

Photochemistry in Nanotechnology:

Bridging the Gap between Nanomaterials and Nanomedicine

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Photochemistry provides an excellent tool for the synthesis of aqueous solutions of biocompatible nanomaterials derived from gold, silver, copper, niobium, etc. Morphology, size and surface coverage can be readily 'tuned' to ensure compatibility within biocomposites while retaining long-term stability and performance.

Our research at uOttawa has emphasized the applications of photochemically generated silver nanoparticles (AgNP) in hybrid nanostructures where the AgNP are stabilized in collagen structures, both in solution and as part of hydrogels. These materials have excellent antibacterial properties, while remaining biocompatible, e.g., in the presence of fibroblasts and keratinocytes.

In other work AgNP have been incorporated into medical-grade polyurethanes in the hope of producing new polymeric materials suitable for catheter fabrication but benefiting from the anti-infective properties of silver nanostructures.

While much of the work mentioned above involves small spherical AgNP, photochemistry enables the production of other morphologies, bimetallic particles, and the control of particle size through a combination of seeding and ablative techniques. The potential of these materials in nanomedicine, and the risks associated with the potential toxicity of novel nanostructures remains to be explored in detail.

TUES2

Challenges for PDT dosimetry in small animal models.

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The use of small and larger animal models is a requirement throughout the Photosensitizer and PDT indication development. While the biology can be well approximated the physical dimensions for the target tissues and the surrounding organs at risk, particularly for orthotropic grown tumours, is often orders or magnitude smaller causing either excessive exposure to the normal tissue or oversimplified target volumes for the tumour experiencing close to homogenous fluence. The former case will limit the ability to use sufficiently high irradiances to destroy tumours whereas the latter will oversimplify the PDT dose gradient inside the target. While it is possible to scale the effective attenuation with the reduced target dimension size via an appropriate PDT activation wavelength it poses other problems in particular for treatment monitoring. For one the active volume or surfaces of photo detector need to scale with those developed for human use to measure over the same fluence-rate gradient, impacting on their responsivity. More importantly the positional accuracy in deriving the relative distance between the sources and detector need to be much better than the effective attenuation coefficient. Hence, high resolution positioning or position verification (0.5mm) is required to enable determination of the tissue optical properties (absorption and reduced scattering coefficient). This resolution is not available on standard CT or MRI imaging platform. .

TUES3

Towards PDT treatment planning.

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It has been recognized by Industry and academic units aiming to developed novel photosensitizers and treatment approached for PDT, that treatment optimization strategies are essential to maximize outcome. Treatment planning to optimize the source placement and spatial monitoring of a PDT dose parameter are the most promising parameters. As the photosensitizer concentration are somewhat under the control but the local fluence rate under a better control of the physician and physicist respectively, they are the principle parameters monitored during clinical PDT delivery and utilized for PDT treatment planning.

During the planning process only population average data for photonsensitizer accumulation and tissue optical properties are available. In the proposed approach the goals are to minimize the number of indwelling catheters needed, while reaching the

desired fluence or photon density throughout the clinical target volume. In a second step the tissue optical properties and other PDT dose determining factors need to be perturbed to identify the spatial locations at which dosimetry sensors are most responsive to the actual tissue optical and other dose determining parameters. The third step will require on line dose measurements and readjusting the delivered optical power to approximate the target dose within the confines of a now fixed source distribution.

The determination of the tissue optical properties will place very strict requirements on the known distance between the sensors and the implanted sources. For most clinical situations these distances need to be known to better than 0.5 mm thus requiring sophisticated clinical imaging or other position monitoring systems.

Treatment planning systems should complete initial planning within a couple of hours when the full planning parameter space is available (source number, position, strength, emission profile and surgical placement access) whereby the on-line treatment monitoring and plan adjustment calculations need to be completed in close to real time with only seconds lack time. The recently developed Full Monty Monte Carlo code to be executed on FPGAs appears to be capable of delivering these requirements.

TUES4

Optical measurements prior to PDT treatments of actinic keratosis are predictive of patient-specific response: our pilot clinical experience

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Photodynamic therapy (PDT) using aminolevulinic acid (ALA) as a precursor for protoporphyrin IX (PpIX) is an effective FDA-approved treatment for precancerous skin lesions known as actinic keratosis. Recent studies have shown that effectiveness persists for treatments that shorten the ALA-incubation time from the standard 14 hrs. to as short as 1 hour. However, it is an ongoing issue that some patients do not respond to the treatments, and the move to shortened incubation times increases the need to identify non-responders. This study incorporates optical measurements into a pilot clinical study of patients undergoing ALA/PpIX PDT treatments to quantitate PpIX fluorescence. Patients were treated with topical administration of 20% ALA with a 1 hour incubation and a treatment illumination with BLU-U. Patients (n=71) provided an assessment of pain on the visual analog scale during treatment, and a subpopulation of patients (n=12) provided assessments of pain and skin redness 2-3 days after treatment. Optical measurements show high inter-patient variability in PpIX fluorescence, with 50% of patients presenting extremely low values. PpIX fluorescence was higher in lesions measured (1) on the face compared with the body, and (2) in the lesions of patients reporting high levels of pain. A follow-up on a subpopulation of patients showed that PpIX fluorescence on the day of treatment was strongly correlated with pain and redness estimated 48-76 hours after treatment. This talk will discuss the potential causes of variations in PpIX production between different patients, and will make the case that measurements of PpIX fluorescence may be a more reliable metric of PDT dose than pain reported during treatment, and more cost-effective than onsite follow-up.

TUES5

LUZ11: a fluorinated sulfonamide bacteriochlorin in clinical trials for head and neck cancers

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Halogenated sulfonamide bacteriochlorins have strong light absorptions in the near infrared, long triplet lifetimes, high yields of singlet oxygen and hydroxyl radicals, photostability, low dark toxicity and amphiphilicity. We found that the dynamics of the interaction between the photosensitizer triplet state and molecular oxygen determine both the nature of the ROS generated and the stability towards such ROS. A fluorinated bacteriochlorin (LUZ11) attains a delicate balance between high yields of hydroxyl radical generation and resistance towards oxidation [1]. LUZ11 was used in cellular (drug-to-light interval DLI=72 h) and in vascular (DLI=15 min) PDT of BALB/c mice with subcutaneously implanted CT26 colon adenocarcinoma tumours. Cures (i.e., absence of palpable tumor >60 days after PDT) were observed in both cases and attained 83% of the cases in vascular PDT [2]. However, vascular PDT of nude mice with the same tumor model did not lead to any cures. Rechallenging the cured mice with the same tumor cell line 4-6 month after PDT, together with a control group of the same age and cured at the same time by surgical removal of the tumor, led to tumor regrowths in all the animals of the surgery group, but

11 of the 32 mice cured with PDT resisted tumor rechallenge. A significant increase in CD4+ T helper cells was observed in the blood 2-6 h after PDT and interleukin 6 (IL-6) was significantly increased 24h after PDT. Skin photosensitivity in rats was small 3 days after i.v. administration, consistent with the fast plasma pharmacokinetics of LUZ11 in mice and in minipigs. Encouraged by these promising results, a clinical trial is in progress.

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[3] http://clinicaltrials.gov/show/NCT02070432.

TUES6

Photodynamic therapy with long duration, ultra low level (nanowatt range) light can kill mesothelioma cancer cells in vitro

Joseph Friedberg, Michael Tenuto, Warren Naselsky, Theresa Busch, Arash Darafsheh, Christopher Murray, Ann-Marie Chacko, Taejong Paik, Daniel Pryma, Jarod Finlay

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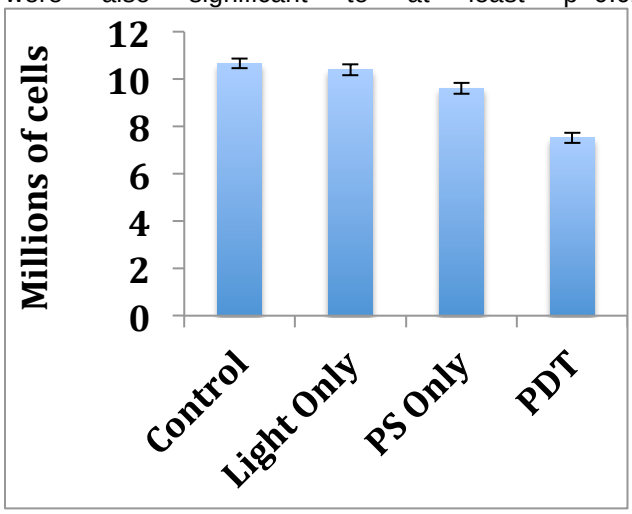
Purpose: The purpose of this study was to see if light, at an unprecedentedly low power, could be used for photodynamic therapy (PDT)-mediated killing of mesothelioma cancer cells *in vitro*.

Methods: $1.3x10^6$ AB12 murine mesothelioma cells were plated onto 60mm culture dishes. A self-illuminating 22.5x3mm tritium-phosphor tube was taped to the bottom of the dish as a light source. The light intensity from the source was estimated ~0.1 nW/cm^2 based on optical power measurement in an integrating sphere at 730nW, with a peak wavelength at 532nm. Porfimer sodium was added to medium at a concentration of 10μg/ml prior to incubation. Dishes were then protected from light and incubated, unperturbed, for 96 hours. Control groups included: medium alone, light alone and photosensitizer alone. Viability was assessed by both trypan blue exclusion cell counting and MTT assay. Experiments were performed in triplicate.

Results: Compared to untreated controls, by the trypan blue assay, the PDT-treated dishes demonstrated 29.5% cell kill, while the photosensitizer only and light only groups demonstrated 9.9% and 2.5% kill, respectively (see figure below). The cell kill for the PDT group was statistically significant to at least p=0.0001 compared to all controls. The MTT assay revealed 64.9% growth inhibition for the PDT group and 29.5% and 8.6% for the photosensitizer only and light only groups, respectively. These results were also significant to at least p=0.02.



Conclusions: To the best of our knowledge this is the first demonstration of *in vitro* PDT killing of tumor cells with light at the extraordinarily low intensity of less than a nanowatt per cm^2. This is at least several orders of magnitude less than we could find in any other report. These results are significant in that they potentially represent the first step in developing a novel intracavitary PDT treatment for surface coating cancers such as mesothelioma or ovarian cancer.

TUES7

Porphysome nanotechnology: explore new frontiers of cancer imaging and therapy

Gang Zheng

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Porphyrins are well known photosensitizers for photodynamic therapy. In the course of examining porphyrin self-quenching in liposomes to explore their potential use as activatable photosensitizers, we discovered ‘porphysomes’, the liposome-like porphyrin nanoparticles self-assembled from porphyrin-lipid building blocks. The very high porphyrin packing density results in both ‘super’-absorption and structure-dependent ‘super’-quenching, which, in turn, converts light energy to heat with extremely high efficiency, giving them ideal photothermal and photoacoustic properties that are unprecedented for organic nanoparticles. Upon porphysome dissociation, free porphyrins are released to enable low background fluorescence imaging. In addition, metal ions (e.g.,

radioactive copper-64) can be directly incorporated into the porphyrin building blocks of the preformed porphysomes thus unlocking their potential for PET, MRI and radiation therapy. As a result of their organic nature, porphysomes were biodegradable in vivo and induced no acute toxicity in mice. In a similar manner to liposomes, porphysomes can be easily scaled up via commercial extrusion techniques and the large aqueous core of porphysomes could be passively or actively loaded with drugs, opening up a new avenue for image-guided drug delivery. By changing the way porphyrin-lipid assembles, we developed ultra small porphyrin nanodiscs (<20nm), porphyrin shell microbubbles (~2um), and porphyrin microreactors (~100um), expanding the purview of porphyrin nanophotonics. Compared with classical "all-in-one" nanoparticles containing many functional modules, the simple yet "one-for-all" nature of porphysomes represents a novel approach to the design of multifunctional nanoparticle and confers high potential for clinical translation.

TUES8

Receptor Concentration Imaging (RCI) can quantify available epidermal growth factor status after photodynamic therapy in pancreatic cancer

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Verteporfin photodynamic therapy (PDT) for the treatment of pancreas cancer (PCa) is showing promise as a therapy for patients with unresectable tumors. Measured changes in tumor volume after PDT are the conventional indicator for tumor status post-therapy; however, if the tumor is imaged too close to the time of therapy these changes can be more reflective of hemodynamics, edema and inflammation. A previous magnetic resonance (MR) imaging study performed by our group indicated that PDT effect can be observed 48 hrs post-PDT but large amounts of inflammation and edema may confound results. Alternative approaches to monitoring tumor status exploit these changes by monitoring the overexpression of tumor-specific cell-signaling receptors, such as fluorescently labeled epidermal growth factor receptor (EGFR), and can be performed at much earlier time points post-therapy. Unfortunately, these techniques can be skewed by the enhanced permeability and retention (EPR) effect as well as hemodynamics and inflammation as in MR imaging. We have recently demonstrated that Receptor Concentration Imaging (RCI), a method of quantifying cell-signaling receptors, is independent of these confounding factors by monitoring the kinetics of a targeted and untargeted pair of imaging agents instead of monitoring total agent accumulation. Here, we will MR imaging, fluorescence contrast imaging and RCI using a murine xenograft orthotopic PaC to measure tumor response to interstitial verteporfin PDT (1mg/kg, 20J/cm) at 1, 3 and 7 days post-therapy. RCI has the capability to provide immediate quantification of the tumor molecular response to therapy and the potential of guiding early subsequent molecular-based adjuvant therapies.

TUES9

Her2/Neu oncogene transformation enhances 5-aminolevulinic acid mediated protoporphyrin IX production and mitochondrial accumulation

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Human epidermal growth receptor 2 (Her2, Neu, ErbB2) is a tumor oncogene often overexpressed in human breast cancers. Her2/Neu transformation has been shown to stimulate cell proliferation and alter glucose metabolism. Here we provided evidence showing that Her2/Neu transformation significantly changed porphyrin/heme metabolism as well. Overexpression of NeuT (a mutated Her2/Neu) in non-transformed MCF10A human breast epithelial cells resulted in increased biosynthesis of heme precursor molecule protoporphyrin IX (PPIX), especially after 5-aminolevulinic acid (ALA) stimulation. Western blot analysis indicated that NeuT transformation altered the expression of five out of eight enzymes involved in heme biosynthesis. Importantly, we found that PPIX was primarily accumulated in mitochondria in NeuT-transformed cells whereas in vector control cells PPIX was rapidly transported to the cell membrane and associated with cell junction molecules. The findings that inhibition of mitochondrial function by 2-deoxy glucose caused more PPIX accumulation in mitochondria in vector cells and NeuT transformation upregulated glycolysis marker pyruvate dehydrogenase kinase suggest the involvement of mitochondrial dysfunction in PPIX mitochondrial accumulation. Degradation of mitochondria by autophagy (mitophagy) induced by kinase inhibitor lapatinib reduced ALA-mediated PPIX production in NeuT-transformed cells. Collectively, these results demonstrate that abnormal porphyrin/heme metabolism is part of global metabolic alterations induced by oncogene transformation and should be further exploited for tumor imaging and targeting.

TUES10

Combination of TSPO targeted PDT and differentiation-inducing agent: image (PET and fluorescence) – guided therapy for breast cancers, especially for TNBC (triple negative breast cancers)

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Breast cancer is the second most commonly diagnosed cancer in USA (skin cancers #1) and second leading cause of cancer death among women (lung #1). Under current treatment, even for women with early stage breast cancer, more than 20% of the patients die of this disease, so that other treatment methods are urgently needed to eradicate breast cancers, especially triple negative breast cancers (TNBC), which do not provide any of so far well-defined therapeutic targets. Translocator Protein (TSPO) (also called PBR) is an 18 kDa protein primarily localized in the outer mitochondrial membrane; TSPO overexpression has been observed in a large variety of human cancers, especially highly aggressive breast cancers. Based on our published and unpublished data, here for the first time, we propose that TSPO is a suitable target for the treatment and imaging of TNBC. We also hypothesize that it is a marker of tumor aggressiveness for TNBC patients. The agent we are developing is TSPO targeted and can be used for multiple purposes: PET imaging (I-124), fluorescence imaging and photodynamic therapy (PDT). In order to image TNBC with high specificity, we use a combination of PET imaging and fluorescence imaging. PET imaging provides superior detection over CT and MRI for tracking metastasis and detecting differences between malignant and benign processes; fluorescence imaging is currently the most sensitive “Optical Molecular Imaging” technique that can detect the distribution of “Molecular Probes” in thick medium such as breast tissue. Our experimental data demonstrate that with our agent, fluorescence imaging is complementary to PET imaging in detecting metastases. As a TSPO targeted photosensitizer, our agent shows enhanced PDT efficacy compared to its counterpart without TSPO-targeting moiety. Photodynamic therapy (PDT) is a way under-explored modality for the treatment of breast cancer. Recently by collaborating with Professor Hasan of Harvard University, the University College London group has been investigating the use of PDT to treat primary breast cancers without surgery. Here we propose to use PDT with our TSPO targeted photosensitizer as an intraoperative method for eradicating residual breast cancer stem cells so as to increase the chances of cure. Another agent in our study is the steroid **1**. Due to patent-related issues, some detailed results of the study for this compound are not ready for disclosure at this meeting. We believe that the invention of compound **1** and its analogs is a major breakthrough. Compound **1** is structurally different from any other reported anti-cancer agent, and it is highly effective for lymphoma and a broad spectrum of solid tumors including breast cancer. Its main mechanism of action is promotion of cell differentiation. Differentiation-promoting anti-cancer agents produce fewer side effects than other cytotoxic agents. Compared to the best known differentiation therapy agent for cancer treatment ATRA (all-trans retinoic acid), the greatest merit of steroid **1** is that it is also highly effective for the treatment of solid tumors; in contrast, ATRA is only effective for blood cancers such as leukemia. We propose to use steroid **1** as both a neoadjuvant and adjuvant agent for the treatment of TNBC to eradicate both local and metastatic lesions. Furthermore, mutual enhancement between differentiation therapy with steroid **1** and PDT with our photosensitizer, demonstrated in our preliminary data, will combine these two therapy approaches to totally cure TNBC.

In summary, here we propose an image-guided therapy for TNBC; the therapy combines TSPO targeted PDT and differentiation-inducing therapy with steroid **1**.

TUES11

Time-dependent intracellular association of photosensitizers with organelles modulates the efficacy of photodynamic therapy

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Despite tremendous efforts to develop effective therapy, cancer remains a preeminent cause of death worldwide. Photodynamic therapy (PDT) is a promising treatment modality that can be targeted and activatable, mitigating the destruction of healthy tissues that commonly occurs in other cancer therapies. In addition, to targeting the photosensitizer (PS) to diseased tissue, the association of the photosensitizer with different intracellular organelles can alter PDT efficacy. We have developed a novel targeted PDT agent that incorporates a PS, chlorin e6 (Ce6), and a small peptide for targeting cancer, cGRD. The resulting compound, Ce6-cGRD, traffics through different intracellular organelles in a time-dependent manner, including the lysosomes and mitochondria. We compared the efficacy of Ce6-cGRD PDT in different organelles by monitoring ROS production in real-time, followed by monitoring cell death longitudinally post irradiation. The results suggest that

the intracellular distribution of PS modulates PDT efficacy.

TUES12

Discovering Ru(II) Complexes as potent tool in Photodynamic Therapy

Cristina Mari[1], Vanessa Pierroz[2], Riccardo Rubbiani[1], Malay Patra[1], Stefano Ferrari[2], Gilles Gasser[1]

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Photodynamic Therapy (PDT) offers the opportunity to kill tumor cells with a spatial and temporal control with the synergistic action of molecular oxygen, light and a photosensitizer (PS). However, the currently approved photosensitizers suffer from several drawbacks, which include tedious synthesis and purification as well as prolonged light sensitivity. Our research is focused in finding new PS with optimized characteristics. In this perspective, six novel Ru(II) complexes (Fig. 1) with strong DNA binding affinity were synthesized and characterized in-depth. Their biological behaviour was studied in the dark and upon light irradiation. Further biological analysis such as cellular localization and uptake as well as DNA damage evaluation were performed. Two complexes (**1** and **2**) present an impressive phototoxicity when irradiated at 420 nm with very low light doses (9.27 $J \cdot cm^2$), showing a dark/light toxicity ratio of 150 and 40 times, respectively.[1] Further studies are ongoing to assess the mechanism of DNA damage, to achieve higher wavelength activation and to modulate the localization of the compounds.

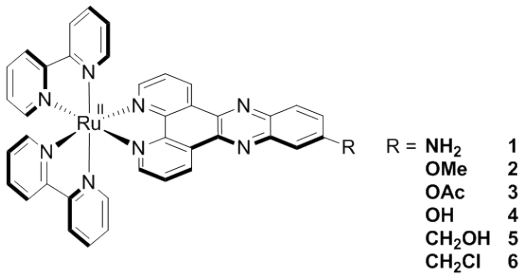

Fig. 1. Structures of Ru complexes.

[1] Mari, C. et al., 2014, *submitted*.

TUES13

Development of Porphyrin-Phospholipid Liposomes Permeabilized by Near Infrared Light

Kevin Carter[1], Shuai Shao[1], Matthew Hoopes[2], Dandan Luo[1], Bilal Ahsan[3], Vladimir Grigoryants[4], Wentao Song[1], Haoyuan Huang[1], Guojian Zhang[1], Ravindra Pandey[5], Jumin Geng[1], Blaine Pfeifer[1], Charles Scholes[4], Joaquin Ortega[3], Mikko Karttunen[2], Jonathan Lovell[1]

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Delivery of therapeutic compounds to target tissues is a central challenge in treating disease. Externally-controlled drug release systems hold potential to selectively enhance localized delivery. Here, we describe liposomes doped with porphyrin-phospholipid which are permeabilized directly by near infrared light. Molecular dynamics simulations identified a novel light-absorbing monomer esterified from clinically approved components predicted and experimentally demonstrated to give rise to a more stable porphyrin bilayer. Light-induced membrane permeabilization is enabled with liposomal inclusion of 10 molar % porphyrin-phospholipid and occurs in the absence of bulk or nanoscale heating. Liposomes re-seal following laser exposure and permeability is modulated by varying porphyrin-phospholipid doping, irradiation intensity or irradiation duration. Porphyrin-phospholipid liposomes demonstrate spatial control of release of entrapped gentamicin and temporal control of release of entrapped fluorophores following intratumoral injection. Following systemic administration, laser irradiation enhances deposition of actively-loaded doxorubicin in mouse xenografts, enabling an effective single-treatment anti-tumor therapy.

TUES14

BODIPY as Fluorescent Photosensitizers in Near IR Region

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Efficient fluorescence emission from photosensitizers can be very useful for tracking the photosensitizers both in vitro and in vivo. While weak fluorescence of most of photosensitizers is not a problem in vitro, it is not sufficient for in vivo optical imaging that provides critical information for the distribution of photosensitizer in real time. Our goal is to develop fluorescent photosensitizers having not only effective singlet oxygen generation but also sufficient fluorescence emission for in vivo optical imaging. However, it may not be easy to achieve because fluorescence emission and intersystem crossing are

competing processes. Thus, our strategy is to maximize the molecular absorptivity and balance the two processes. We chose BODIPY dye for this goal since it provides a number of unique advantages including high molecular absorptivity. We first established a synthetic route for near IR (> 700 nm) fluorescent BODIPYs and then brominated these to take advantage of heavy atom effect for enhancing the intersystem crossing. Among the brominated BODIPYs, dibrominated thieno-pyrrole fused BODIPY showed promising photophysical properties as the fluorescent photosensitizer. More recently, we observed very interesting phenomena of this class. Some BODIPY dyes showed effective singlet oxygen generation without heavy atom effects as well as sufficient fluorescence emission in near IR range for in vivo optical imaging. We will present our recent progress in fluorescent BODIPY-based photosensitizers.

TUES15

Shining Light on the Dark Side of Imaging: Exploring Photoacoustic and Non-Linear Optical Properties of Molecular Contrast Agents Based on Curcumin and BODIPY Chromophores.

Mathieu Frenette[1], Maryam Hatamimoslehabadi[2], Stephanie Bellinger-Buckley[1], Samir Laoui[2], Seema Bag[1], Olivier Dantiste[2], Jonathan Rochford[1], Chandra Yelleswarapu[2]

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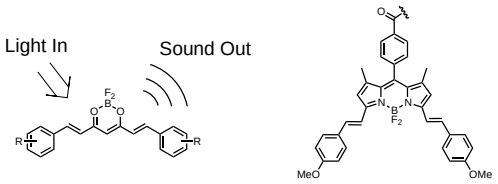
Chromophores that convert light into sound are being developed as potential probes for photoacoustic imaging—an emerging technique that combines the visualization depth of ultrasound imaging with the high resolution of optical imaging. These properties make photoacoustic imaging a promising tool for early cancer diagnosis, and in general, for high-resolution imaging in vivo.

Photoacoustic characterization, including non-linear optical effects, is here presented on a series of chromophores that are variations on BODIPY dyes and the curcumin pigment, i.e. the bright yellow spice from the natural turmeric root. By adding a BF_2 unit to rigidify the 1,3-dione portion of curcumin, the curcumin-BF_2 absorption cross-section is enhanced and shifted towards the biological optical window (~650-950 nm). Modifying the end groups of curcumin-BF_2 with various aromatic and electron-donating groups, we are able to tune their absorbance profiles and photophysical properties along with their photoacoustic efficiency.

BODIPY dyes with extended π-systems also red-shifts the absorbance spectrum towards the biological window while retaining their high molar absorption coefficient. Dimethoxystyryl-BODIPY was found to exhibit strong fluorescence, high 2-photon absorption cross-section and an enhanced photoacoustic yield.



TUES16

Biophotonics: a novel approach to the treatment and regeneration of wounds.

Emmanuelle Devemy, David Burroughes, David Ohayon, Eric DesRosiers

KLOX Technologies Inc., Laval, Quebec, Canada

Patients with chronic wounds are a challenge to treat. Depending on the type and cause of the wound, management can involve various combinations of different treatment modalities such as dressings, compression, topical antimicrobials, debridement, partial skin grafts, bioengineered skin products, growth factors, off-loading and nutritional support. However, the current standard of care is frequently insufficient to promote healing of chronic wounds.

One key new technology is the proprietary Biophotonic System designed to promote and accelerate wound healing in a safe, simple and cost efficient manner. The Biophotonic system is comprised of a Multi-LED light and a Photo Converter Wound Gel containing a fluorescent chromophore which uses its biophotonic characteristics to efficiently induce phototherapy. The chromophore physically absorbs the photons of the single wavelength Multi-LED light and converts it to multiple longer wavelength photons, resulting in the emission of blue, green, yellow and orange wavelengths in the wound. Data reported in the literature demonstrate that different wavelengths of light, particularly blue, green, yellow and orange have beneficial effects on promoting wound healing. The presentation highlights the emerging role of Biophotonic Therapy in wound healing and includes pre-clinical data.

WED1

Optical Spectroscopy and Tomography of Oxygen Delivery: From Macro to Micro and Back

David Boas

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Advances in PET and fMRI technology have enabled significant progress towards mapping cerebral O2 delivery and consumption – parameters of critical importance for understanding brain function, pathophysiology, and treatment of diseases. However, available techniques lack sufficient spatial and temporal resolution to ascertain in vivo depth-resolved imaging of the partial pressure of O2 (pO2) and its dynamic changes. Moreover, current techniques for measuring the cerebral metabolic rate of O2 (CMRO2) are inherently complex (i.e., multimodal and reliant upon advanced modeling), costly, and incompatible with the high-resolution imaging tools used to investigate of cellular and molecular processes. Therefore, a critical gap remains in our understanding of O2 metabolism, delivery, and reserve at a microscopic level, both at rest and during metabolic activation states. I will present our multi-modal microscopy platform for imaging oxygen delivery in the cerebral microvasculature of small rodents. We combined two-photon microscopy imaging of pO2 based on measuring oxygen-dependent phosphorescence lifetime with Doppler Optical Coherence Tomography based imaging of cortical blood flow. We have obtained the high-resolution and high-density pO2 maps and detailed pO2 distributions in microvascular segments down to a 450 um depth below the mouse cortical surface. We have quantified that up to 50% of the oxygen delivered to the tissue comes from the arterioles, contrary to textbook description of oxygen delivery primarily from capillaries. We have also observed that average capillary oxygenation is significantly less than venous oxygenation and that this arises from capillary transit time heterogeneity. Further, we are able to estimate CMRO2 directly from radial profiles of pO2 away from descending arterioles without any need for a measurement of flow. These new tools are enabling new exploration of brain oxygen metabolism with unprecedented spatial and temporal resolution. I will conclude by showing how these microscopic measurements are now guiding our design of novel methods to quantify cerebral oxygenation non-invasively in humans.

WED2

Quantitative Functional Assessment of Tumor Microenvironment using Contrast enhanced ultrasound and Photoacoustic Imaging

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Cancer cells are differentiated from normal cells by a number of genetically altered characteristics, one being the ability to induce abnormal angiogenesis. However, these specific traits are also regulated by tumor microenvironmental factors such as hypoxia. Alterations in the tumor microenvironment generally confer with aggressive cancer phenotypes, raising the need for *in vivo* and non-invasive methods of tracking these changes. Contrast enhanced ultrasound (CEUS) in combination with photoacoustic (PA) imaging serve as promising candidates – one has the ability to measure tissue perfusion, where as the other can be used to monitor oxygen saturation. In this study we investigate drug induced alterations to the tumor microenvironment, and validate the sensitivity of CEUS and PA imaging with histology.

Primary orthotopic tumors were surgically implanted in nude SCID mice using the 231/LM2-4 breast cancer cell line. Mice with tumors of an approximate volume of 200mm^3 were given either a single dose of 50mg/kg of Oxi-4503 or 0.9% saline (N=6/group). US imaging was performed using the VevoLAZR system with integrated PA probe at 21MHz; pre- and 4 hours post-vascular shut down. Relative tissue oxygen saturation was measured with PA imaging, and indices of relative blood volume and flow rate were assessed with CEUS. Post-sacrifice, tumour tissue was excised and fixed for histology.

Functional changes in the tumor vasculature were evident in the drug treated mice at 4 hours post-treatment as shown by the substantial decrease in average blood volume (-82.1%), flow rate (-80.5%), and oxygen saturation (-37.2%). Results for all parameters were statistically significant compared to pre-treatment. Similar degree of changes was not observed in the control group. Histological confirmation of subsequent molecular changes included CD31 staining for vessels distribution and CA9 staining for hypoxia. A higher level of hypoxia expression was found in the Oxi-4503 treated tumors, with increased areas of positive CA9 staining observed at the tumor core, confirming imaging data. Taken together, CEUS and PA imaging are potentially a sensitive tool for quantitative functional assessments of breast tumor models.

WED3

Nanoprobes for Photoacoustic Imaging and Phototherapy

Ghayathri Balasundaram[1], Chris Jun Hui Ho[1], Kai Li[3], Amalina Attia[1], Kienvoon Kong[1], Bin Liu[3], Malini Olivo[1]

[1]Biooptical Imaging Group, Singapore Bioimaging Consortium, Singapore, Singapore, [2]School of Physics, National University of Ireland, Galway, Galway, Ireland, [3]Institute of Materials Research and

Engineering, Singapore, Singapore, [4]Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore, Singapore, Singapore, [5]nstitute for Biological and Medical Imaging, Helmholtz Center, Munich, Germany

Photoacoustic (PA) imaging is becoming increasingly popular in bioimaging as it combines the high absorption contrast and specificity of optical imaging with the high spatial resolution of ultrasound imaging. Phototherapy is used to treat many medical conditions and is better than many conventional therapies. In our group, we are developing theranostic agents that can image the disease models using PA imaging and treat them using phototherapy. One such agent is a conjugated polymer (CP), an emerging class of optical contrast agents due to their unique optical properties, versatile synthetic chemistry and ease of biofunctionalization. Phantom studies were conducted to examine the PA activity as well as the targeting efficacy of the CP functionalized with folate ligand in cell suspension. Results showed that the compound exhibited strong PA signals *ex vivo* and high targeting affinity *in vitro*. Following that, we studied over time, the biodistribution and pharmacokinetic profile of the agent *in vivo* in a folate expressing breast cancer tumor model against that of a conjugated polymer with no folate ligand. Though both polymers showed strong *in vivo* PA signals, with localization at the tumor site and in various organs, such as the liver, spleen, kidneys and intestines at different time points, as well as almost total clearance from the body within a week, the *in vivo* targeting efficacy of the folate functionalized CP was faster with localization at the tumor in 1 hour postinjection, as compared to gradual passive accumulation of the non-targeted polymer in tumor over 3 days. These strong photoacoustic polymers are also shown to have photothermal therapeutic effect (preliminary studies), which is yet to be tested *in vivo*. Another class of agents we are developing is nanosensitizers comprising of photoacoustically strong gold nanoparticles of different shapes and clinically approved near infra-red photosensitizers. Our nanosensitizers are designed to have matching localized surface plasmon resonance (LSPR) for both the nanoparticle and photosensitizer in order to be excited with one wavelength for the therapy. We will be presenting our results using the promising photodynamic agent Chlorin e6, Ce6 and gold nanorods. Our preliminary work has shown that these nanosensitizers have enhanced photoacoustic signals than the nanorods themselves, which can be attributed to the photoacoustic signals from the Ce6. In our studies, the nanosensitizers will be characterized for their biodistribution in various organs of the study model, retention at the tumor site, clearance from the body and therapeutic efficacy through real-time longitudinal photoacoustic imaging.

WED4

Identifying photodynamic therapy non-responders using Photoacoustic Imaging

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[1]Massachusetts General Hospital, Boston, MA, USA, [2]Canon USA Inc, Boston, MA, USA

Early identification of treatment non-responders is critical for personalized design of impactful therapies to obtain meaningful therapeutic outcome. In this study we utilize photodynamic therapy (PDT), a clinically promising treatment used for various oncologic and dermatologic malignancies, as an example to identify non-responders using a non-ionizing, non-invasive imaging modality namely photoacoustic imaging (PAI). Photoacoustic imaging (PAI) can provide 3D information on both photosensitizer (PS) uptake and oxygen saturation (StO_2) levels and hence can provide insights into the complex interaction between the three important PDT dosimetry parameters – PS, oxygen and light dose. We present a study on identifying non-responders amongst a group of subcutaneous U87 glioma tumors treated with PDT (0.5 mg/kg Benzoporphyrin derivative, 100 J/cm^2 fluence; 100 mW/cm^2 irradiance). A significant change in StO_2 was observed in treatment responders. The non-responding tumors did not have significant change in StO_2 either immediately, or 24 hours post-PDT. In light of the fact that PAI is quickly moving towards being a clinical imaging modality, the results presented in this study showcase the applicability of PAI to PDT for cancer and other malignancies.

WED5

Silent Probes for Optical Imaging: an Overview

Norbert Lange

University of Geneva, Geneva, Switzerland

Ever since upregulated proteolytic activity was reported in diseases such as cancer or rheumatoid arthritis, research have tried to exploit this phenomenon for the selective delivery of drugs. This can be achieved mainly by creating an inactive prodrug of a drug through coupling of a protease-sensitive peptide fragment. Upon digestion by the target protease, the drugs should be released in its active form thus exerting the desired effect. However, despite an early hype, none of these approaches has reached the market until today, presumably due to three main reasons: i) the prodrug is still active, ii) the released drug peptidyl fragment is less active than the parent compound, and iii) unsufficient amounts of drug are released at the target site.

Only recently this prodrug approach was translated into functional fluorescence imaging. The underlying strategies can be roughly divided into horizontal, i.e. peptide-based and vertical, i.e. macromolecular approaches. In the latter, the protease sensitive sequence serves as linker between a polymeric carrier or a nanoparticle forming carrier and the photoactive payload. Such macromolecular approach may further benefit from passive targeting through the enhanced penetration and retention effect.

Here we will discuss the benefits and drawbacks of these pertinent strategies for the improved fluorescence imaging.

WED6

Image Guided Surgery using Near Infrared Fluorescent Light. **From Bench to Bedside**.

Alexander Vahrmeijer

Leiden University Medical Center, Leiden, The Netherlands

Despite many improvements in pre- and postoperative imaging modalities (e.g. US, CT, MRI, PET, SPECT) for oncologic surgery, the surgeon still has to rely on visual inspection and palpation to determine what structures should be resected. As a consequence, it is not uncommon that the resection margins are not free of tumor (so-called R1 resection). Due to its relatively high tissue penetration, near-infrared (NIR; 700-900 nm) fluorescent light has the potential to visualize structures that need to be resected (e.g. tumors, lymph nodes) and structures that need to be spared (e.g. nerves, ureters, bile ducts). Our group has utilized various imaging systems including the Mini-FLARE™ NIR fluorescence (NIRF) imaging system to perform first-in-human clinical trials in a variety of human surgeries, with clinically available NIR fluorescent contrast agents. NIR fluorescence contrast agents included methylene blue (MB), which emits at ≈ 700 nm and indocyanine green (ICG), which emits at ≈ 800 nm. To date, we have performed NIRF guided surgery in over 500 patients in more than 20 approved clinical trials. Many trials were focused on NIR fluorescent sentinel lymph node mapping, where a tracer is injected around the tumor to detect the first draining lymph node for evaluation of lymph node metastases. Other trials were focused on tumor identification, including rare pancreatic tumors, breast tumors and colorectal liver metastases that were invisible by conventional imaging. Moreover, trials to identify structures that need to be spared during surgery were performed and visualization of the ureter during surgery in the lower pelvis was shown to be feasible using NIRF imaging. Also the biliary tree can be identified using NIRF imaging during complicated hepato-biliary surgical procedures and routine laparoscopic cholecystectomies. We will review the key results from these studies and from recent pre-clinical and clinical studies using tumor targeted contrast agents (antibody or peptide based), discuss the advantages and limitations of the technology, and suggest various imaging system and contrast agent parameters that could be optimized in future trials for both tumor imaging as well as for the identification of normal structures. Moreover, a clear roadmap for clinical translation of targeted probes will be presented. Hopefully with NIRF imaging the number of radical tumor resections can be increased and the damage to normal tissue being prevented.

WED7

Improving Therapeutic Response to PDT through Targeting Tumor Blood Vessels at the Molecular Level

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The tumor vasculature is an important target of photodynamic therapy (PDT) for treatment with many photosensitizers and illumination schemes. In recent years, it has become apparent that numerous molecular and microenvironmental characteristics of tumor blood vessels will determine their sensitivity to PDT. We have investigated several means of modulating the molecular microenvironment of tumors towards augmentation of vascular response to PDT. The first approach utilizes molecular targeting drugs to abrogate the activation of epidermal growth factor receptor (EGFR). PDT activates survival signalling along the EGFR pathway in tumor cells and their associated vasculature. Inhibition of this activation using molecularly targeted drugs, such as the small molecule tyrosine kinase inhibitor Erlotinib, can promote the anti-vascular effects of PDT and is associated with large improvements in therapeutic outcome in PDT-treated murine tumors. In vitro PDT of mouse endothelial cells (SVEC) confirms that inhibiting EGFR signalling increases the sensitivity of this cell type to PDT. These studies found that the pre-illumination exposure of SVEC cells to Erlotinib significantly decreased cell proliferation after PDT compared to that of cells treated with only PDT. In a second approach of molecularly altering vessel response to PDT, we have targeted signalling by vascular endothelial growth factor (VEGF). Targeting with an anti-VEGF antibody was performed in a brief window days *before* the delivery of PDT. Resulting changes in tumor vascularization were associated with an increased sensitivity of these tumors to vascular-mediated damage and were accompanied by better long-term outcomes to PDT in mice that received the

anti-VEGF treatment. In continuing work, we are further elucidating the molecular and microenvironmental mechanisms by which the above and other approaches can be employed to increase the responsiveness of tumor blood vessels to subsequent treatment with PDT.

WED8

Photoactivation of sunitinib as anti-tumor strategy

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Sunitinib is an angiogenesis inhibitor, which functions by inhibiting the activity of various receptor tyrosine kinases, primarily those for VEGF and PDGF. Sunitinib is currently used in the treatment of patients with advanced renal cell carcinomas and has been shown to prolong progression-free and overall survival in these patients.

We have found that sunitinib is sequestered in the lysosomes of exposed tumor and endothelial cells, a phenomenon, which can be visualized by fluorescence microscopy, as sunitinib is a fluorescent compound. Interestingly, the exposure of cells, cultured in the presence of sunitinib, to light at the wavelength corresponding to the excitation wavelength of sunitinib leads to the immediate photo-destruction of the lysosomes and the consequent release of sequestered sunitinib into the cytoplasm, resulting in cell death.

We hypothesized that this activity could be used for vaso-occlusion by photodynamic approaches and, further, that it could be implemented as a form of anti-cancer therapy to potentially target and destroy tumor vasculature. This hypothesis was first tested *in vivo* using the chorioallantoic membrane (CAM) of the chicken embryo and colon carcinoma-bearing BALB/c mice. Treatment of the CAM vasculature with nanomolar doses of sunitinib and their subsequent exposure to 420 nm light resulted in specific dose-dependent angio-occlusion within the treated area. In human ovarian carcinoma transplanted onto the CAM, treatment resulted in the massive destruction of tumor vasculature. Effects of treatment were observable immediately after the exposure to light and resulted in a necrotic tumor mass 24 hours after treatment. Light-activated sunitinib also inhibited the growth of murine colon carcinoma as compared to tumors treated with sunitinib alone. Immunohistochemical analysis of treated tumors revealed that sunitinib-PDT resulted in a reduction in the number of CD31+ open-lumen blood vessels near the surface of the tumor.

Our results suggest that this strategy could be implemented in the treatment of cancer patients already receiving sunitinib.

WED9

Outshining drug resistance with light: How adding erlotinib to photodynamic therapy can improve therapeutic response in non-small cell lung cancer

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Non-small cell lung carcinoma (NSCLC) is plagued by a paucity of effective treatment options, making the development of new therapeutics critically important. Molecular targeting agents are frequently used to manage NSCLC, with drugs targeted against the epidermal growth factor receptor (EGFR) being particularly favored, as most NSCLC tumors exhibit aberrant expression of this receptor. However, not all NSCLC tumors will respond to EGFR inhibitors, and those that do respond will ultimately develop resistance to these drugs. Interestingly, results from our lab have shown that addition of the small molecule inhibitor of EGFR, erlotinib, to photodynamic therapy (PDT) can improve treatment response in erlotinib resistant NSCLC tumor xenografts. The improved response observed is accompanied by increased vascular shutdown and tumor cell apoptosis. Importantly, we have also noted that while erlotinib administration does not impact BPD uptake in tumor cells in vitro, uptake of the drug is increased following erlotinib administration in vivo. Elevated photosensitizer uptake coupled with changes to the microenvironment induced by erlotinib could work together to impact tumor and endothelial cell kill. These data have provided a clearer picture of the mechanisms underlying the enhanced therapeutic response observed in a class of tumors that are typically understood to be resistant to EGFR inhibition. By extension, the benefit offered to a resistant tumor population suggests that this type of combination therapy could be even more broadly applicable to other tumor types which are responsive to erlotinib therapy, but could be further improved by the addition of PDT.

WED10

Anti-angiogenic treatment at vascular normalizing doses enhances chemotherapy and photodynamic

therapy effects in a preclinical model of human ovarian carcinoma

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Anti-angiogenic agents were reported to modulate the tumor microenvironment and improve other treatment outcomes, but they often are used at high doses in the clinic to prune tumor vessels and paradoxically may compromise various therapies. Here, we demonstrated that targeting the tumor vasculature with a lower vascular-normalizing dose of axitinib, a small molecule tyrosine kinase inhibitor (TKI), specifically targeting the VEGF receptors 1, 2 and 3 can temporarily normalize the tumor vasculature. Axitinib-mediated treatment of human ovarian carcinoma grafted on the chorioallantoic membrane (CAM) administered at a dose of 56 µg/kg caused a transient increase in intra-tumoral oxygenation, with a peak in tumor oxygenation at 30 hours after axitinib administration.

We combined axitinib-mediated treatment with intravenous administered doxorubicin. Increased intratumoral doxorubicin fluorescence was observed after administering a dose 35.2 mg/kg of doxorubicin. The distribution of doxorubicin was monitored for 15 minutes after administration using fluorescence detection of doxorubicin excited at a wavelength of 450-490 nm. An increase in fluorescence was seen when doxorubicin was administered in the peak of the normalization widow. The potential to increase treatment efficacy based on increasing intratumoral doxorubicin delivery was further tested in the CAM model by administering 3.5 mg/kg doxorubicin either in the normalization window (30 h after axitinib administration) or before. Visudyne®-PDT was applied using a suboptimal light dose of 2.5 J/cm^2 during the peak of the normalization window, i.e. 30 hours after the administration of 56 µg/kg of axitinib, and resulted in a statistically significant tumor growth inhibition. The treated tumors were inhibited by 84% compared to the final control tumor volume, while treatment with 56 µg/kg axitinib and 2.5 J/cm^2 PDT resulted in 50% and 35% inhibition, respectively, indicating synergistic tumor growth inhibition. More importantly, tumor growth inhibition was also significantly inhibited as compared to the same treatments given in a different regimen (i.e. when PDT was performed before the induction of vascular normalization), where only 60% of tumor growth inhibition was observed.

WED11

Combination of photodynamic therapy and cancer molecular targeted agents

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Photodynamic therapy (PDT) induces cell damage and even cell death through the generation of reactive oxygen species (ROS). Depending on the type of cells, photosensitizer and light doses, PDT has been shown to induce cell apoptosis, necrosis and autophagy. PDT is also known to activate cell survival pathways. The final PDT outcome is dependent on the interplay between PDT-induced cell death and survival signals. To enhance the therapeutic outcome of PDT, it is necessary to further potentiate PDT-induced death signal and/or inhibit PDT-induced survival signal.

To achieve this goal, we combined PDT and molecular targeted anticancer agents in this study. Because PDT induces cell death by generating oxidative proteins which can be detoxified by proteasomes, it is hypothesized that PDT in combination with proteasome inhibitor bortezomib will increase cell death by inducing the accumulation of oxidative proteins. Based on the finding that sub-lethal PDT induced a significant upregulation of phosphorylated AKT, a pro-survival cell signal, we hypothesize that PDT in combination with phosphatidylinositol 3-kinase (PI3K) pathway inhibitor BEZ235 increases PDT outcome by preventing cell regrowth. In this talk, I will summarize our in vitro and in vivo studies on the interaction between PDT and cancer molecular targeted agents. Our results demonstrate that PDT in combination with proteasome inhibitor bortezomib or PI3K inhibitor BEZ235 leads to enhanced therapeutic outcome.

WED12

An Ideal Sunscreen – How to Achieve It

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Ideal sunscreens provide uniform protection across the ultraviolet B and ultraviolet A radiation (UVR) range, similar to shade and clothing. Ideal sunscreens would thus perform like a neutral density filter with the same protection at any wavelength. In such an ideal case the transmitted UVR at one minimal erythema dose (1 MED) has a value of 7.9 J/cm². We use this value to normalize the transmitted UV dose @ 1 MED (NTUVD). The closer to 1, the more ideal is the spectral profile of the respective sunscreen. Currently available sunscreens have NTUVD values up to 7. This means by the time the UV-protected skin just reaches the erythema threshold of 1 MED, the skin has in fact already received 7 times the amount of UVR compared to the ideal sunscreen. This extra

energy comes mainly from the UVA-I range (340-400nm), since the protection profiles of non-ideal sunscreens are UVB biased. There are four key requirements for good UV protection that help us estimate how far we progressed on the path towards the ideal sunscreen on a ranking scale between poor and perfect (0 and 100%). Technology is leading with an estimated score of 80%, followed by Performance Assessment at 70%, Norms and Standards around 50% and Compliance trailing at only around 30%. UV filters are the heart of the product technology. Besides UVB-filters, plenty of UV filters for UVA II and UVA I protection are now available everywhere, except in the USA. Sunscreen efficacy depends on UV filter type (organic or inorganic), photostability, and the addition of Sun Protection Factor (SPF) boosting agents. Performance assessment is still not ideal; SPF is based on human testing, whereas in vitro methods to assess UVA protection are on a good way to be harmonized globally. The high bar for achieving the highest UVA protection level of the various classification standards released in Europe (2006) and the USA (2007) already helped increasing UVA protection considerably. Unfortunately the US gave up their tough “proposed rule” and settled with a much inferior “final rule” (2011). The most essential problem however, remains poor compliance due to physical as well as psychological barriers. Providing cosmetically pleasing formulations that people like to wear and communicating what sunscreens are and how they work are the key elements in improving topical UV protection.

WED13

The Role of Botanicals and Antioxidants in Sun Protection

Mary Matsui

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Physical and chemical sunscreens are well established and regulated agents used for photoprotection world-wide. They are an important part of the American Academy of Dermatology sun protection messaging. However, sunscreens do not provide as much protection as they could, for several reasons. This talk will review the rationale for complementary modes of protection and focus on the potential benefits of botanical extracts and anti-oxidants. Both topical and ingestible sun protection will be included. Because these non-sunscreen ingredients do not absorb efficiently in the ultraviolet wavelengths, alternative efficacy endpoints will be discussed. Experimental evidence for the activity of several specific extracts, molecules and vitamins, particularly in human clinical studies, will be reviewed. In addition, a number of factors that limit the use of botanicals and antioxidants for photoprotection will be examined, including stability, bio-availability, and testing protocols.

WED14

Changes to the *Stratum Corneum* After Narrow-Band UVB (311nm) Phototherapy in Polymorphic Light Eruption Patients

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Narrow-band ultraviolet-B (NBUVB) phototherapy is a successful treatment for many patients with the common photodermatosis, polymorphic light eruption (PLE). Prophylactic doses of NBUVB administered prior to the summer months can alleviate PLE symptoms. However, the mechanisms behind successful NBUVB phototherapy in PLE patients are not understood. The most discussed hypothesis is that so-called ‘skin hardening’ occurs during the process. To examine this hypothesis we investigated the effect of NBUVB phototherapy on the viable epidermis and the *stratum corneum*, a critical structural component of skin barrier function, in PLE patients (n=6, phototypes I-III). Patients received whole-body NBUVB phototherapy (Philips TL-01, peak 311nm) for 15 sessions over 5 weeks. Exposure doses started at 70% of the individual patient's minimal erythemal dose and increased in ~20% increments over the treatment course. Biopsies were taken from PLE skin before and after the phototherapy course. Haematoxylin and eosin was used to stain gross epidermal structure and quantitative (Image J) immunofluorescence of filaggrin (FLG), involucrin (INV) and loricrin (LORI) was used to investigate epidermal differentiation markers.

The results showed that whilst the total thickness of the viable epidermis + *stratum corneum* was significantly thinner ($P<0.001$); the *stratum corneum* was significantly thicker ($P<0.05$) after NBUVB phototherapy. The intensity of expression of the epidermal differentiation markers, FLG and INV, were both significantly reduced ($P<0.05$) after NBUVB phototherapy whereas LORI expression was unaffected.

These results suggest that NBUVB PT has profound effects on epidermal differentiation and morphology. The viable epidermis and differentiation markers, FLG or INV, were reduced after NBUVB phototherapy whereas the *stratum corneum* increased in thickness, suggesting a compensatory mechanism to maintain or

reinforce, skin barrier function. The thickened *stratum corneum* would be expected to result in increased photoprotection and a strengthened barrier against exogenous 'photoallergen' ingress. Both of these effects may provide mechanistic insight into the observed 'skin hardening' effects of phototherapy in PLE.

WED15

Controversies on Photoprotection

Henry Lim

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The following topics will be covered:

- FDA Final rule on sunscreens. Released June 2011, implemented Dec 2012. Critical wavelength is now used as an assessment of protectiveness of sunscreens in the UVA range;

- Inflammatory properties of UV filters. In animal model. Clinical significance to be determined.

- Effect of sunscreen use on serum vitamin D. In laboratory setting, vit D levels were suppressed with sunscreen use. This was not shown in actual daily usage.

- Safety of oxybenzone. There was estrogenic effect in mouse model. Oxybenzone was detected in urine and milk. Clinical significance will be discussed.

- Safety of nanoparticles. Current evidence shows that they remain on top of the skin.

- Non-topical form of photoprotection. Promising, but still in early stages of development.

WED16

Mapping microscopic viscosity in cells using molecular rotors

Marina Kuimova

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Viscosity is one of the main factors which influence diffusion in condensed media. In a cell viscosity can play a role in several diffusion mediated processes, such as drug delivery, signalling and mass transport. Previously, alterations in viscosity in cells and organs have been linked to malfunction; however, mapping viscosity on a single-cell scale remains a challenge.

We have imaged viscosity first inside lipid mono- and bi-layers and in cells using fluorescent probes, called molecular rotors [1]. In molecular rotors the speed of rotation about a sterically hindered bond is viscosity-dependent [2-5]. This approach enabled us to demonstrate that viscosity distribution in a cell is highly heterogeneous and that the local microviscosity in hydrophobic cell domains and model membranes can be up to 100 times higher than that of water.

We have also demonstrated [3] that viscosity in cells increases significantly as a result of photoinduced cell death during the Photodynamic Therapy of cancer (PDT).

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WED17

Predominant photogeneration of singlet oxygen or free radicals by selected nanoparticle photosensitizers

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While the aerobic photochemistry of fullerenes is driven by high quantum yield of their triplet excited state and efficient formation of anion radical forms, the photoreactivity of nano-crystalline titanium dioxide is determined by the formation of the electron-hole pair. Using C_{60} fullerene substituted with three quaternary pyrrolidinium groups and nanocrystalline TiO_2 with surface modified with hematoxylin or bromopyrogallol red, the effect of NADH, azide, thiocyanate and sulphite on the nanoparticle ability to photo-generate singlet oxygen and free radicals was examined by time-resolved phosphorescence at 1270 nm, electron paramagnetic resonance (EPR) oximetry and EPR-spin trapping. Although irradiation of both types of nanoparticles with blue light induced oxygen consumption that was significantly accelerated by the addition of NADH and, to a lesser extent of histidine, high concentration of azide strongly inhibited photoxidation of histidine only when the photoreaction

was mediated by the fullerene. NADH dramatically increased the rate of photogeneration of superoxide anion by the fullerene and only moderately by TiO_2 nanoparticles. In the presence of azide, photoexcitation of the fullerene generated azide radicals in an oxygen independent manner. TiO_2-mediated photogeneration of azide radicals was substantially less efficient and required oxygen. The photoexcited fullerene induced efficient oxidation of NaSCN, which was clearly mediated by singlet oxygen. This pseudohalide had negligible effect on photo-consumption of oxygen mediated by TiO_2 nanoparticles. On the other hand, sulphite, a key product of the interaction of singlet oxygen with thiocyanate, was rapidly photooxidized by TiO_2 nanoparticles to sulfur trioxide radical anion. This photoreaction did not require oxygen; however, oxygen was promptly photoconsumed when present in the sample. Although fullerenes can photogenerate efficiently singlet oxygen and free radicals, TiO_2 nanoparticles operate exclusively via Type I photochemistry. We postulate that the use of modified TiO_2 as photosensitizers for PDT could be advantageous under low oxygen concentration.

WED18

Photomechanical Responses of Photoreceptors

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Fly eyes have the fastest visual responses in the animal kingdom, but how they achieve this has long been an enigma. Phototransduction in Drosophila microvillar photoreceptors is mediated by a G-protein coupled phospholipase C (PLC) cascade culminating in activation of “transient receptor potential” (TRP) and TRP-like (TRPL) channels by a still unresolved mechanism. Here we show that these light-sensitive channels are not ligand but mechanically gated. Using atomic force microscopy we found that light exposure evoked rapid contractions of the photoreceptor cells. These contractions were even faster than the cell's electrical response and appeared to be caused directly by PLC activity. Photoreceptor light responses were facilitated by membrane stretch, and modulated by amphipaths and different diets, which both alter lipid bilayer properties. When we replaced the native light-sensitive channels with mechano-sensitive channels, photoreceptors still generated electrical signals in response to light. These results indicate that splitting of the membrane lipid PIP_2 by PLC reduces the membrane area, which leads to an increase in membrane tension and change in curvature, and ultimately causes the contractions of the cells. They furthermore suggest that the resultant mechanical forces contribute to gating the light-sensitive channels, thereby introducing the concept of mechanical force as an intermediate or “second messenger” in metabotropic signal transduction.

WED19

On the unique light production from the marine worm *Chaetopterus*:

Where do we stand?

Dimitri Deheyn

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The marine worm *Chaetopterus* sp. inhabits the seafloor from shallow coastal areas to deeper canyons, covering a widespread geographical distribution worldwide. The worm builds a U-shaped tube in which it lives, with the two tips of the tube opening to the seafloor surface. The worm secretes a mucus that produces a long lasting blue bioluminescence that can be seen spewing out of the tube openings upon disturbance. Ecological functions associated with the bioluminescence remain speculative at this stage, but could involve defensive strategies such as the burglar alarm. Chemistry leading to the light production remains unknown to this day. Here I will describe the recent progress made in my laboratory about the unique mechanism of light production in *Chaetopterus*. The light production involves a photoprotein that appears controlled by an inhibitor factor, as observed by dilution experiments as well as dose-response experiments with hydrogen peroxide, inhibitory in this particular chemistry. Iron is required for the bioluminescence and the oxido-reduction of iron forms could be involved in control of the light production. The mucus contains riboflavin (vitamin B12) that could be the chromophore of the photoprotein, as suggested by the spectral shift of mucus fluorescence over time, going from blue to a green similar to the one of riboflavin. Three major proteins have been purified from light producing fractions and are currently being sequenced. Based on specific biochemical characteristics, I will address the attractive prospects the *Chaetopterus* light-producing system could offer for a variety of biotechnological applications.

WED20

Biomechanical Imaging with Brillouin Microscopy

Giuliano Scarcelli

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In the past years, we have developed a biomedical imaging technology based on Brillouin light scattering

to measure the biomechanical properties of biological tissue and biomaterials. Spontaneous Brillouin scattering arises from the interaction of light and acoustic phonons that are inherently present in a sample. Upon this interaction, part of the scattered light acquires a GHz frequency shift directly related to the longitudinal elastic modulus of the sample. Thus, by detecting this small spectral shift, the sample's elastic properties can be measured without physical contact. We will discuss the development of the high-throughput spectrometer that enabled transforming Brillouin technology from point-sample spectroscopy requiring high laser power to an imaging modality that can be safely applied in vivo. The first area of biomedical applications we have explored is in ophthalmology where Brillouin imaging can measure changes in corneal and lens elasticity by aging, by progression of disease or in response to treatment/drugs. This has resulted in an on-going clinical trial to test the potential of Brillouin technology as diagnostic and therapy-monitoring tool for corneal ectasia. We will conclude the talk by discussing our current efforts to develop high-resolution Brillouin confocal microscopy for cell biomechanics.

WED21

Melanoma, UV and melanin- clues from mouse models

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The development of mouse models has facilitated investigations into the role of UV radiation in mammalian melanoma. Hepatocyte growth factor transgenic (HGF/SF) mice have extrafollicular melanocytes in the skin, are black and hyperpigmented on the C57BL/6 genetic background and develop melanomas recapitulating human disease after neonatal UV exposure. We delivered precise spectrally defined UVA (320-400nm) or UVB (280-320nm) at biologically relevant doses to this model and identified two UV wavelength-dependent pathways for induction of CMM and a significant role for eumelanin in melanomagenesis. UVB radiation initiated melanoma associated with direct UVB DNA damage and independent of pigmentation but melanoma induction by UVA required melanin and was associated with melanin-dependent oxidative DNA damage in melanocytes. Spontaneous melanomas also required the presence of melanin. In HGF/SF transgenics, melanin was confined to melanocytes and protective epidermal melanin was sparse, enabling direct exposure of melanocytes to UV. Mice homozygous for an inactivating mutation in the melanocortin-1 receptor (Mc1r e/e) produce more phaeomelanin than eumelanin and exhibit yellow pigmentation. In yellow Mc1r e/e B-RAF mutant mice, phaeomelanin has been implicated in spontaneous melanoma. Yellow Mc1r e/e HGF mice, in contrast, produced no melanomas either in response to UV radiation or spontaneously. Further, Mc1r e/+ HGF mice, heterozygous for Mc1r deficiency, were black and indistinguishable from black Mc1r competent (Mc1r +/+) HGF transgenics but produced UV or spontaneous melanomas with much less efficiency. Thus, an interaction between the Mc1r and HGF signaling pathways, independent of melanin production, was required for HGF-dependent melanoma. Since HGF is critical to treatment failure of B-RAF inhibitors in melanoma patients this interaction may be significant in human disease. The role of melanin, particularly with UVA, in melanoma requires further investigation.

WED22

HGF/SF does not affect melanogenesis but increases the number of extra-follicular melanocytes in mouse skin.

Agnieszka Wolnicka-Glubisz

Jagiellonian University, Krakow, Poland

Melanins are an important factor determining vulnerability of the mammalian skin to UV and UV-induced cancers. However, its exact role in this pathology is still unclear. In this study we compare in a qualitative and quantitative way melanogenesis of neonatal and adult C57BL/6 mice and their transgenic variants overexpressing hepatocyte growth factor/scatter factor (HGF/SF) which have melanocytes outside the hair follicles, in contrast to their wild type controls. Pigmented HGF/SF neonatal mice are more susceptible to melanoma than are albino HGF/SF animals, in contrast to humans where dark skin is protective against melanoma, raising the question of the effect of transgenic HGF/SF on melanization. Here, we demonstrate the methodology of determination of melanogenesis in intact skin and hair shafts, and quantify the effects of HGF/SF overexpression. Our electron paramagnetic resonance studies supported with histology, transmission electron microscopy, Western blotting and zymography revealed that HGF/SF overexpression does not change the type of melanin produced in the skin from eumelanin towards pheomelanin. Transgenic HGF/SF does not affect the terminal intensity of melanin production, because it does not change the melanin content in a standard sample of hair. Therefore, the increased inducibility of melanoma in pigmented transgenic HGF/SF mice is not due to pheomelanin, nor to quantitative changes in the pathway of melanocytic melanin production. We have also shown that HGF/SF overexpression does not influence the hair-cycle/morphogenesis-related changes in skin

thickness. The only influences of HGF/SF overexpression on the skin/hair follicle melanogenesis, are an increase of the number of hair follicles and the presence of an additional population of melanocytes outside hair follicles.

WED23

The Two Faces of Melanin – Protective and Anti-protective.

Julian Menter

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"Melanin" refers to a *group* of pigments, Eumelanin is thought to comprise numerous cross-linked 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) polymers, Pheomelanin differs from eumelanin in that its oligomer structure incorporates benzothiazine and benzothiazole units that are produced instead of DHI and DHICA. Neuromelanin is formed from catecholamine oxidation pathways. All of these melanins possess broad featureless absorption bands, can act as semiconductors, can bind metals and organic material (drugs) and act as free radical scavengers. These properties confer the ability of melanin to act simultaneously as a protector and/or as a sensitizer. For example, melanin sequestration of drugs or metals can protect vulnerable cells or tissue from deleterious effects by these agents. On the other hand, bound transition metals (e.g. iron) can lead to formation of harmful reactive oxygen or nitrogen species. The particular chemistry of melanin can influence the relative importance of protective vs. anti-protective behavior to solar radiation. Eumelanin is generally thought to be photo-protective, while pheomelanin is a photosensitizer. Neuromelanin can bind large of iron and is thought to play a role in iron homeostasis. However under iron overload it could play a toxic role by promoting redox reactions. Extensive electron delocalization stabilizes melanin radicals but also allows melanin "mediate" potentially harmful redox reactions between electron donor and acceptor molecules adsorbed to the melanin backbone.

We have previously demonstrated that synthetic dopa-melanin and sepia melanin can couple the oxidation of catecholic skin depigmenters to potassium ferricyanide reduction *in vitro*. More recently, we have shown that co-adsorbed nitric oxide (NO) and molecular O_2 will react to form reactive nitrogen species (RNS), most likely $ONOO^-$ and NO_2 at rates much faster than would occur in the absence of melanin pigment. This latter observation is of significance to keloid pathology, since NO is known to up-regulate type I collagen in humans, and since keloid scarring is observed preferentially in darkly – pigmented persons. Funded in part by GRANTS: MBRS #GM08248, RCMI #8G12MD007602, and DOD # 911 NF – 10 – 1 0448. There are no conflicts of interest.

WED24

Tanning Lamps and Health.

Henry W. Lim

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In 2009, International Agency on Research on Cancer has classified solar radiation, UV-emitting tanning devices, and ultraviolet radiation as group 1 carcinogens. Since 2007, there is now strong evidence that exposure to tanning beds are associated with increased risk of non-melanoma skin cancer and melanoma. Because of generation of β-endorphin, there is a biologic basis for the additive behavior to the use of tanning beds observed in frequent users. Intentional tanning among US adolescents continues to be prevalent. Tanning bans or restrictions are now in place in several states in the US, and in several countries. In March 2010, US FDA has also held a public hearing the reclassification of the tanning lamps, which are now classified as a Class I device. Following the hearing, the consensus of the FDA Advisory Board is that tanning lamps should not be classified as a Class I device.

WED25

A 3-year follow-up of sun behavior in patients with cutaneous malignant melanoma based on ultraviolet radiation measurements and sun diary data

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We aimed to assess sun behavior in the years following diagnosis of cutaneous malignant melanoma (CMM) in a 3-year follow-up, observational case-control study.

Twenty-one patients with CMM, and 21 controls matched individually to patients by gender, age, occupation, and constitutive skin type participated. Ultraviolet radiation (UVR) exposure was assessed the first and the second summers (N=20) and the first and the third summers (N=22) after diagnosis. Data from 40 participants were analysed.

UVR exposure was assessed by personal electronic UVR dosimeters measuring time-related UVR in standard erythema dose (SED) and corresponding sun diaries (mean: 74 days per participant each participation year).

Patients' daily UVR dose and UVR dose in connection with various behaviors increased during follow-up (quantified as increase in daily UVR dose each year [95% CI]); all days (0.3 SED [0.05–0.5]); days with body exposure (0.6 SED [0.07–1.2]); holidays (1.2 SED [0.3–2.1]); days abroad (1.9 SED [0.4–3.4]); and holidays with body exposure (2.3 SED [1.1–3.4]). After the second year of follow-up patients' UVR dose was higher than that of controls who maintained a stable UVR dose. There was no difference between groups in the number of days with body exposure or the number of days using sunscreen in the second and third year of follow-up. Our findings suggest that patients with CMM do not maintain a cautious sun behavior in connection with an increase in UVR exposure, especially on days with body exposure, abroad and on holidays.

WED26

Measurements in the built environment: UV reflection in small scale systems and what it means for outdoor workers

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The behaviour of UV radiation within localised areas will be reviewed with respect to workers in outdoor occupations. Man-made structures have been shown to influence UV exposure obtained by outdoor workers by either increasing or decreasing potential UV exposure, through increasing or decreasing UV radiation reflected from surfaces surrounding the workers. In addition to the review of the current knowledge of UV reflection for different surface types, structure and the resulting relationships between the two, the definitions of albedo and reflection will be compared and contrasted. How UV reflection in the atmosphere is understood needs to be reviewed with respect to how this information can be used to contribute to UV radiance modelling, an increasingly important tool that can be used for more accurate UV radiation level predictions in the future.

WED27

Horizon Sky Radiance – The Relevance for Ocular UV Dosimetry

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There have been a number of studies of human ocular exposure to ultraviolet (UV) radiation, but most emphasize the measurement or calculation of corneal irradiance, frequently without consideration of the field-of-view, or angle of acceptance, of UV radiation. In past studies of UV radiation arriving at the cornea, lens and (the trace amounts reaching the) retina, it became apparent that the eyes' UV exposure was greatly less than that incident on the skin. Only under conditions of high UV ground reflectance (surface albedo) does the eye experience photokeratitis ("snow blindness") – generally in late winter although UV global irradiance incident on the ground is highest in mid-summer. Since ground reflectance of UV-B plays such a key role – with green grass reflecting ~ 1%, sand, asphalt, concrete and building materials reflecting of the order of 10% and surf and water about 20%, it became clear that direct exposure from the sun and overhead diffuse radiation exposure from the sky play only a minor role in the total exposure for the human eye.[1] Studies of upper-lid blocking of overhead irradiation and squinting outdoors (Deaver et al.)[2] showed that the typical field-of-view for the eye was limited to ~ 15^{o} in bright summer sunlight. This led us to conduct spatially selective measurements of the actinic UV (~ $S(\lambda)$-weighted) environmental UV with two Solar Light Co. UV monitors, side by side at Aberdeen Proving Ground, MD. One measured global UV, the other measured only the contribution from the horizon sky up to ~15^{o}. The ratio of horizon sky/global irradiance was measured, thus providing an approximate relative contribution of UV entering the eye's pupil relative to the global actinic UV irradiance typically measured for UV skin-exposure studies. Measurements of radiance with a narrow-field acceptance, portable UV meter were also made under different field conditions. Results were in agreement with our experience of photokeratitis and outdoor UV exposure conditions and compared to a mathematical model for UV sky radiance (Streicher).

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WED28

Observed and predicted levels of ultraviolet radiation at the Earth's surface

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Solar ultraviolet (UV) radiation has been measured with ground-based instruments since the late 1980s. Satellite data allow the reconstruction of the UV irradiance at the Earth's surface back to 1978 when the first NASA TOMS instrument became operational. Here we provide a review of ozone and UV changes observed during the last four decades and discus scenarios of UV levels for the 21st century. Biospherical Instruments has measured UV radiation at high latitudes with high-resolution spectroradiometers continuously since 1988, supported by the National Science Foundation. Because of the "ozone hole", the UV Index (UVI) at the South Pole during October and November is now 55-85% larger than the 1964-1980 mean. At mid-latitudes, total column ozone has declined by about 6% between 1964–1980 and the early 1990s, but has partly recovered since. At many high- and mid-latitude locations, changes in the UVI over the last decades have been driven by factors other than total ozone. For example, decreases in tropospheric aerosols caused by improvements in air quality standards have increased UV radiation at many urban centres. At high latitudes, decreases in sea ice and snow cover have decreased downwelling UV irradiance but have increased the penetration of radiation into those parts of the ocean that were formerly covered by ice. Without the Montreal Protocol to reduce ozone-depleting substances, summer-time UVIs at mid-latitudes would have increased by a factor of three by 2065. Because of the Protocol's success, the expected scenario of future UV is very different. By the middle of the 21st century, mid-latitude ozone is expected to exceed values observed in the 1960s. This "super-recovery" is partly caused by stratospheric cooling from the continued increase in green house gases. With the exception of high-Southern latitudes, future UV levels will be dominated by factors other than changes in stratospheric ozone. In eastern China and India, UV is predicted to increase substantially over the next 50 to 70 years as air quality and aerosol burden are expected to improve, but uncertainties are high. While measurements of scanning spectroradiometers are still the "gold standard" for UV radiometry, new products, such as affordable filter instruments, personal dosimeters, cell phone apps, and "big data" applications are becoming available to better estimate personal UV exposure.

WED29

Cell Killing and Transformation Induced by Polychromatic UV Light: an Integrated Theory

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The production of DNA damage by ultraviolet radiation (UV) in cells is a linear function of incident dose, but most biological end points are non-linear functions of dose. If certain conditions apply, the biological effects of polychromatic UV can be calculated by replacing the dose term in a monochromatic dose response function with the integral of the product of the cross section for damage and the spectral intensity at each wavelength, but problems were anticipated when the dose appears more than once is the dose response function, the situation for the most general expressions for both cell survival and transformation[1]. The repair-dependent theory of survival overcomes this difficulty[2]. This theory in now extended to include transformation by assuming that the probability of transformation is proportional to the number of lethal lesions repaired in surviving cells or the interactions of such lesions (abstract this meeting and reference 3). This results in an expression for transformation frequency that is the sum of modified linear, quadratic, and higher order terms, each following the corresponding power of dose for low doses (high survival). But the modification, expressed as a ratio of regularized gamma functions, results in a plateau in transformation frequency at high doses (low survival). The parameters that define the shape of each term in the transformation frequency series can be determined by parameters are obtainable from either survival or transformation data. Experimental data for the neoplastic transformation frequency of mammalian cells[4] can be fit using only the modified quadratic expression; a linear component is not required.

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WED30

Development and Applications of a Radiance Model

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Radiance is the most versatile of troposphere radiation metrics. From the three dimensional radiance field, actinic flux and surface irradiance can be computed. Photochemical reaction rates are proportional to actinic flux. Surface irradiance can be calculated for surfaces of arbitrary orientation (slope and aspect) by integration of the radiance field over the 2 pi steradian field of view. Development of a comprehensive radiance model must address the functional dependence of radiance on some 20 independent variables. These variables may be grouped into categories of spectral, geodesic, atmospheric, and physiographic. Radiance, as typically computed with comprehensive radiative transfer models, are extremely precise, but unacceptably slow for complex geometries or dynamic applications. Presented here is a synopsis of the functionality and selected applications of a radiance simulation model. Developed as a suite of process-specific sub-models, each addresses a physically separable component of the radiance field. The integration of the sub-models then seamlessly computes the radiance field with the speed of a regression model and the accuracy of a radiative transfer solver.

THUR1

Role of NO induced by repeated treatments with Pb*a*/PDT in prostate cancer cells

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Cell recurrence in cancer photodynamic therapy (PDT) is an important handicap that is poorly understood. It has become clear that nitric oxide (NO) is a modulator of PDT activity. By modifying the dysregulated NF-κB/YY1/RKIP survival/anti-apoptotic loop, NO can either stimulate or inhibit apoptosis. We have reported that PDT induces the release of NO in prostate cancer cells in a concentration-dependent manner and, hence, NO modifies the loop activity by either inducing or inhibiting tumor cell growth. In the present study, we examined if repeated treatments with a low dose of PDT (40 nM) will induce tumor cell growth in prostate cancer cell lines. Experimentally, we used (a) a metastatic (PC3) prostate cancer cell line (b) Pheophorbide *a* (Pb*a*), a chlorophyll derivative as a photosensitizer and (c) a white halogen lamp with red filter (660 nm) with a fluence of 0.82 J/cm^2 to irradiate the cells after 3h of Pb*a* incubation. We repeated the treatments 8 times (overall duration: about 6 weeks). Following the last treatment, we determined the cell growth proliferation by both FACS analysis and a clonogenic assay. We also measured in tumor cell lysates the protein levels of each member of the NF-κB/YY1/RKIP loop. Since this loop is also linked to the epithelial mesenchymal transition (EMT), we also measured E-cadherin and vimentin expression levels. To assess the presence of a more aggressive cell population (comprising of cancer stem cells, CSCs), we identified the CSCs by FACS analysis following treatment with fluorescent CD24 and CD44 antibodies. The findings demonstrated that repeated treatments with a low dose of Pb*a*/PDT in prostate carcinoma cell lines, through the continuous induction of a low NO level, resulted in the stimulation of cell growth of the more aggressive CSC tumor subpopulation that was resistant to PDT-mediated cytotoxicity.

THUR2

Pro-Survival Signaling by NOS2-Derived NO in Photodynamically-Stressed Cancer Cells

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Many cancer cells produce nitric oxide (NO) at low (sub-micromolar) levels to help avert apoptosis and stimulate proliferation. There is evidence that such NO plays an important role in tumor resistance to radiotherapy and chemotherapy. Using human breast tumor COH-BR1 cells as an *in vitro* model for 5-aminolevulinic acid (ALA)-based PDT, we discovered that these cells rapidly and persistently overexpressed inducible NO synthase (iNOS) along with NO after a moderate ALA/light challenge. This was clearly a cytoprotective response because apoptotic photokilling increased dramatically when an iNOS inhibitor, NO scavenger, or iNOS knockdown was employed. This was the first known example of cancer cells per se mounting a NO-dependent resistance to PDT eradication. We have since identified some key signaling events that underlie iNOS/NO induction and NO-mediated resistance; these will be briefly discussed. More recently, we discovered that human prostate PC-3 cells also exploit iNOS/NO for protection against ALA/light-induced apoptosis, exhibiting a more dramatic upregulation of iNOS than COH-BR1 cells at 4 h post-irradiation (10-12-fold *vs*. 2-3-fold), which persisted for at least another 20 h. In addition to this, we observed a striking increase in proliferation rate of PC-3 cells surviving a photostress and this was also iNOS/NO-dependent. Cell cycle phase analysis revealed a large NO-dependent increase in S-phase occupancy of stressed cells, consistent with accelerated proliferation. Moreover, we found that photostressed PC-3 survivors migrated more rapidly than controls and exhibited substantially greater invasiveness; both effects were negated by an iNOS inhibitor, again consistent with iNOS/NO dependency.

The observed NO-stimulated growth, migration, and invasion responses pose serious concerns if they occur in the clinical PDT setting. Rational pharmacologic intervention with a suitable iNOS inhibitor would be called for in this case. (Supported by NIH/NCI grant CA70823)

THUR3

Combination of nitric oxide therapy, anti-oxidative therapy, low level laser therapy, plasma rich platelet therapy and stem cell therapy as a novel therapeutic application to manage the pain and treat many clinical conditions.

Salaheldin Halasa

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From hypertension to diabetes, cancer to HIV, stroke to memory loss and learning disorders to septic shock, male impotence to tuberculosis, there is probably no pathological condition where nitric oxide does not play an important role. Nitric oxide is an analgesic, immune-modulator, vasodilator, anti-apoptotic, growth modulator, angiogenetic, anti-thrombotic, anti-inflammatory and neuro-modulator. Because of the above actions of nitric oxide, many clinical conditions associated with abnormal Nitric oxide (NO) production and bioavailability. Our novel therapeutic approach is to restore the homeostasis of nitric oxide and replace the lost cells by combining nitric oxide therapy, anti-oxidative therapy, low level laser therapy, plasma rich platelet therapy and stem cell therapy.

THUR4

Revisiting early studies on the impact of nitric oxide on PDT response

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In 1990s, nitric oxide (NO) has become widely recognized as a major molecule in a diverse array of physiologic and pathologic processes, which has also led to the development of various agents modulating specifically tissue levels of this transient gaseous species. Potential of NO to have a major influence on the outcome of cancer therapies, particularly those associated with the generation of reactive oxygen species was acknowledged soon thereafter. This has prompted us to propose that the response of tumors to photodynamic therapy (PDT) can be markedly influenced by the following effects of NO: i) vasodilatation, ii) modulation of platelet activity, iii) attenuation of the increase in vascular permeability and consequent vascular leakage, iv) suppression of activated neutrophil aggregation in tumors, v) dampening adhesion and extravasation of circulating leukocytes in tumor blood vessels, vi) averting mast cell degranulation, and vii) causing (if produced at high levels) cytotoxic injury to the endothelium and cancer cells. Critical NO-sensitive processes enfolding after PDT include ischemia perfusion injury, cancer cell apoptosis, and development of immune response against treated tumor. This inspired studies that demonstrated that NO participates in the events associated with PDT-mediated tumor destruction, particularly the vascular response. It was further shown that the level of endogenous NO production in tumors is an important determinant of sensitivity to PDT. Importantly, these early studies established that the critical role of NO can be exploited for therapeutic gain with PDT. Renewed efforts in this direction are certainly warranted.

THUR5

Role of nitric oxide and other soluble mediators in the acute inflammatory response to ALA-PDT in human skin

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Acute inflammation occurs in human skin following topical aminolevulinic acid photodynamic therapy (ALA-PDT). Experimental models of PDT have identified potential mediators of this response but their involvement following PDT in human skin is little explored. We previously showed histamine to mediate the immediate ALA-PDT induced inflammatory response, but not the more prolonged erythema. We have additionally, through a series of novel studies in human volunteers, examined the involvement of proinflammatory mediators prostaglandin (PG) E_2 and nitric oxide (NO) in the erythemal response. Duplicate dose-series of ALA were applied to the skin of each ventral forearm using the quantitative delivery system of iontophoresis, and exposed to 100 J/cm^2 red light. In separate studies, arms were randomised within subject to receive treatment with the cyclooxygenase inhibitor indomethacin or control, or to receive treatment with NO synthase inhibitor N_{ω}-nitro-L-arginine (L-NAME), or control. Following PDT, the erythemal response was quantified to determine the impact of treatment. Release of PGE_2 and NO following ALA-PDT was also assessed directly using the technique of dermal microdialysis. Microdialysate was collected over 30 min periods immediately pre-irradiation, during irradiation and up to 24 h post-irradiation and mediators quantified by ELISA and chemiluminescence assay, respectively. An ALA dose-

related erythema occurred by 3 h post-PDT which persisted to 48 h. Application of topical indomethacin immediately following ALA-PDT reduced the slope of the erythemal dose-response assessed at 3 h and 24 h post-PDT. Intradermal injection of L-NAME was also shown to reduce the ALA-PDT-red cell flux dose-response at 24 h post-PDT, and to reduce the red blood cell flux at sites treated with ALA-PDT from 3 to 48 h post-PDT. Analysis of dermal microdialysate confirmed NO and PGE_2 to be released by PDT, with different time courses. In conclusion, topical ALA-PDT upregulates production of PGE_2 and NO in human skin, both of which mediate the clinical inflammatory response. These mediators may play a role in PDT-induced acute adverse events and on PDT efficacy in human topical ALA-PDT, and could potentially be modulated to influence PDT outcomes.

THUR6

Estimating receptor concentration in solid tumors noninvasively using multi-tracer fluorescence tomography

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The abnormal expression of receptors on tumor cells has become a major focus of efforts to individualize cancer therapy. Receptors involved in cell proliferation and programmed death are commonly targeted with antibody therapies, and new treatment modalities seek to exploit this abnormality to preferentially deliver toxic payloads to tumor cells. As efforts to target these molecular abnormalities accelerate, the ability to noninvasively quantify receptor availability in vivo will become increasingly important. However, current molecular imaging technologies, which report the distribution of a targeted tracer, are generally incapable of quantifying receptor activity in vivo. This is largely due to the confounding effects of tumor vasculature and leakage which result in significant non-specific uptake of the targeted tracer.

To address this challenge, we have developed a noninvasive optical imaging approach that accounts for tracer pharmacokinetics and thus is capable of estimating receptor concentration in tumors. This capability is enabled by imaging the kinetics of two fluorescent tracers injected simultaneously, one targeted to the receptor of interest, and the other a non-targeted counterpart. Spectral discrimination of the tracers facilitates simultaneous imaging of both tracers, a capability unique to optics. The non-targeted tracer reports only non-binding uptake mechanisms and is therefore used to remove these effects from the targeted tracer measurements. Fitting the time course data of both tracers to a compartmental model facilitates the recovery of specific receptor-tracer binding.

Applying this dual-tracer approach to MRI-guided fluorescence tomography, a technology which enables volumetric fluorescence imaging through several centimeters of tissue, allows receptor concentration estimation in sub-surface tumors. This approach was demonstrated using mouse models implanted with glioma xenografts which over-express EGFR. Recovered values of EGFR concentration in these tumors were consistent with values estimated from independent studies, suggesting that noninvasive quantification is feasible. Dual-tracer optical imaging could have a significant impact on drug development programs; enabling longitudinal tracking of treatment response in preclinical studies, patient stratification for clinical trials, and treatment monitoring in clinical practice.

THUR7

Photodynamic Therapy May Mitigate the Risk of Surgical Tract Site Tumor Seeding for Malignant Pleural Mesothelioma

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Brackground: Malignant pleural mesothelioma (MPM) is known to recur in prior chest wall surgical sites in up to one-third of patients (pts). Prophylactic irradiation of tracts (PIT) can limit surgical tract chest wall recurrences and is commonly used after biopsies and extrapleural pneumonectomies. Limited data exist defining the benefit of PIT after a lung-sparing radical pleurectomy (RP), and there are no prior reports accessing how photodynamic therapy (PDT) influences tract site recurrences. We hypothesized that PDT can sterile microscopic disease and limit tract site recurrences when delivered with RP. **Methods:** 79 consecutive pts treated with RP and intraoperative PDT on one of two IRB-approved prospective trials were analyzed. Using porfimer sodium or 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a (HPPH) with 24 or 48 hour drug-light intervals, respectively, PDT was delivered to 60 J/cm^2 at 630nm (porfimer sodium) or 15-60 J/cm^2 at 661-665nm (HPPH) after all gross tumor was debulked to ≤5mm in thickness. Unlike other series that uniformly deliver PIT to all pts, PIT was only administered to pts with high risk features of gross residual disease, multi-station mediastinal nodal metastases, aortic or extensive chest wall invasion, or existing tumor seeding along a prior incision site excised during RP. **Results:** Eleven

pts (14%) received adjuvant PIT for residual disease (n=2), nodal metastasis (3), aortic invasion (1), extensive chest wall invasion (6), and/or prior tract seeding (1). None of these pts developed tract site recurrences at a median of 23.4 months after PDT. Among the 68 pts not receiving PIT but still receiving PDT, only 4 (6%) recurred in a tract site at a median of 10.6 months after PDT. PIT did not influence the rate of tract site control (100% vs. 94%, p=0.42), locoregional or distant control, or overall survival (all p>0.05). **Conclusions:** This study demonstrates significantly fewer than expected surgical tract chest wall recurrences following RP and intraoperative PDT, even among pts not receiving PIT and those with high risk features. The ability of PDT to sterilize microscopic residual disease may be the primary factor in achieving a low rate of tract site recurrences in this study. PIT can be safely omitted in pts without high risk features who undergo an en bloc RP and intraoperative PDT. Tract site recurrence rates after RP alone without PDT should be assessed.

THUR8

Optogenetic control of chemokine receptor signal and T cell migration

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Adoptive cell transfer (ACT) utilizing tumor-targeting cytotoxic T lymphocytes (CTLs) has been used with some success to mediate the durable complete regression of metastatic melanoma. However, effective trafficking of the CTLs to tumor sites is the main barrier to achieving successful melanoma remission in the clinic. The introduction of chemokines into the tumor environment results in the recruitment of relevant leukocyte subsets and decreases the tumorigenicity of malignant cells. However, current approaches for targeting chemokines in cancer immunotherapy have shortcomings due in part to the redundant effects of chemokines on tumor metastasis and angiogenesis. To address this issue, we developed a strategy for optically controlling chemokine-mediated T cell trafficking *in vivo*. The intracellular loops of $G\alpha_t$-coupled rhodopsin were replaced with those of the $G\alpha_i$-coupled chemokine receptor CXCR4. Photoactivatable CXCR4 (PA-CXCR4) transmitted intracellular CXCR4 signals in response to 505-nm light. Localized activation of PA-CXCR4 induced T cell polarization and directional migration ("phototaxis") both *in vitro* and *in vivo*. Directing light onto the melanoma was sufficient to recruit PA-CXCR4-expressing tumor-targeting CTLs and improved the efficacy of adoptive T cell transfer immunotherapy, with a significant reduction in tumor growth in mice. These findings suggest that the use of photoactivatable chemokine receptors allows remotely controlled leukocyte trafficking with outstanding spatial resolution in tissue and may be feasible in other cell transfer therapies.

THUR9

Targeting Physical and Stromal Determinants of Ovarian Cancer Biology in Bioengineered Models to Inform PDT-based Combination Regimens

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Metastatic progression is influenced by an array of physical and biological factors including flow-induced shear stress, signalling with stromal partners, and matrix composition, which play key roles in determining the fate of disseminated tumors. Comprehensive research platforms that integrate these determinants of tumor growth and treatment response are critically needed to identify effective mechanism-based combination therapies. Here the impact of hydrodynamic stress and stromal biology is evaluated in the context of ovarian cancer (OvCa), which disseminates predominantly via flushing of ascites along preferential fluidic pathways and communicates with the local microenvironment to initiate peritoneal implants. The motivation for this study stems from clinical observations that the most stubborn tumors are often found in regions such as the peritoneal gutter, a common site of resistance and recurrence, and also a region that is subjected to fluidic stress from ascites. A microfluidic model that supports 3D tumor growth was developed to establish the role of fluidic stress on the heterogeneity of metastatic OvCa. Tumor nodules cultured under flow showed increased epithelial-mesenchymal transition (EMT) compared to non-flow 3D cultures. Molecular and morphological changes consistent with EMT included a transcriptionally-regulated significant decrease in E-cadherin, a significant increase in vimentin, and significant decrease in fractal dimension, a metric adapted to quantify spindle-like morphology. A concomitant significant post-translational upregulation of epidermal growth factor receptor (EGFR) expression and activation was seen under flow. Our group and others have shown that

photodynamic therapy (PDT) enhances the efficacy of conventional agents. Combination treatments with PDT and the anti-EGFR antibody, Erbitux, administered as individual monotherapies show synergistic enhancement of tumor destruction and survival in a clinically-relevant mouse model for metastatic OvCa. Co-delivery of the photosensitizing agent on a single targeted construct (photoimmunotherapy, PIT) provides enhanced selectivity and reduced chemotherapy cycles in vivo. The potential value of using bioengineered models to guide customized, rationally-designed PDT-based combination regimens will be presented.

THUR10

Repair of DNA Photolesions in Chromatin

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The first order of chromatin compaction is the nucleosome, in which the intimate association of histones to DNA prevents repair enzymes from accessing UV photolesions. Posttranslational modifications to histones, including acetylation, occur in cells after exposure to UV light, and play a role in regulating repair of UV damage. Recent work in our lab is focused on determining the direct effect of UV damage on nucleosome stability, and the possible role of histone modifications in promoting repair in chromatin. As nucleosomes exist in a dynamic equilibrium in which portions of the DNA molecule spontaneously unwrap and transiently expose DNA, we used FRET and restriction enzyme accessibility to study changes in nucleosome dynamics following DNA damage by UV radiation. Our data show that the presence of UV photoproducts enhances spontaneous unwrapping of DNA from histones, and the increased unwrapping dynamics is concomitantly associated with increased restriction enzyme accessibility to histone-loaded DNA. To examine the roll of histone acetylation in DNA repair, we generated nucleosomes containing acetylated H3 at Lys-14 (H3K14ac), a modification found in the chromatin of cells after UV exposures, and investigated possible mechanisms by which H3K14 acetylation modulates repair. H3K14ac does not alter nucleosome unfolding dynamics or enhance the repair of UV-induced cyclobutane pyrimidine dimers by UV photolyase. However, nucleosomes with H3K14ac have a higher affinity for purified chromatin remodeling complex RSC (Remodels the Structure of Chromatin) and show greater cyclobutane pyrimidine dimer repair compared with unacetylated nucleosomes. Our studies indicate that nucleosome dynamics of UV-damaged DNA in chromatin may provide intrinsic lesion exposure for efficient repair of buried DNA lesions, and H3K14 acetylation plays an important role in coordinating the chromatin remodeling activity needed for efficient repair of UV damage.

THUR11

UV-Induced Psoralen Photoadducts and their Rapid Detection by Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)

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UVA-activated psoralens are used to treat hyperproliferative skin conditions due to their ability to form antiproliferative DNA photoadducts. Although UVA (320-400 nm) is more commonly used, clinically, studies have shown that UVB (280-320 nm) is also effective. Because 8-methoxypsoralen (8-MOP) has a greater extinction coefficient at 300 nm (11,800 $M^{-1}cm^{-1}$) compared to 365 nm (2,016 $M^{-1}cm^{-1}$), it was expected that photoadduct levels would be greater. MALDI-TOF, a technique combining chromatography with mass spectrometry, was used to detect 8-MOP photoadducts in a ten base alternating A-T oligonucleotide (AT-10). Additional data was obtained using HPLC analysis of enzymatic digests. In this report, we describe the extent and distribution of photoadducts. For UVB-activated 8-MOP, photoadduct formation was three times greater than UVA. AMT contains a protonated amino group which greatly facilitates its dark binding intercalation with DNA (K_d=1.8x10^5 *vs* 770 for 8-MOP) and hence a much greater extent of AMT photoadduct formation. Our results demonstrate a novel modality to assess psoralens as therapeutic agents and could be used to screen for new, more active derivatives.

THUR12

Why do solar-UV signature mutations occur preferentially at TCG context?

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Ultraviolet radiation (UVR) has a genotoxicity, producing DNA photolesions such as cyclobutane pyrimidine dimers (CPDs) and inducing specific types of mutations called UV signature, namely C ® T and CC ® TT base substitutions at dipyrimidine sites. I have studied UVR-induced mutations in the skin, using a transgenic mouse whose transgene, the bacterial *lacZ* gene, can be used as a mutational reporter. I observed that longer wavelengths of UVR (UVB~UVA)

induced a specialized type of UV-signature mutation, solar-UV signature, C ® T at dipyrimidine sites associated with methylated cytosine (mC), namely at Py-mCpG sites. Moreover, I found that the solar-UV signature mutations occurred preferentially at 5'-TmCG-3' context, not at 5'-CmCG-3'. On the other hand, reactive oxygen-mediated mutations were hardly induced in mouse skin even by UVA1. The preference of the solar-UV signature for mCpG sites can be explained partially by the observation by Drouin's and Pfeifer's groups (1997, 2009) that UVB and solar UVR produce CPDs more preferably at mCpG sites than at non-methylated ones. The preference for 5'-TmCG-3' context could be explained by the observation that cytosine deamination in CPDs was extremely enhanced in 5'-TmCG-3' context compared to 5'-CmCG-3' context (Cannistraro & Taylor, 2009), if deamination-mediated error-free bypass of CPD by DNA polymerase h were assumed as the relevant mechanism. Solar-UV signature mutations were also detected in the *p53* genes from mouse and human nonmelanoma skin cancers. However, the preference for TCG context was observed only in mouse. In human, such preference disappeared and rather shifted for CCG context. This apparent discrepancy from mouse data might suggest a specific evolutional change of human *p53* gene to adapt to the environmental UVR.

THUR13

Compared to UVC, UVB irradiation generates more cyclobutane pyrimidine dimers in dipyrimidine sites potentially more frequently mutated in skin cancer.

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Ultraviolet (UV) radiation from sunlight is the major factor responsible of the development of skin cancer. The formation of DNA damage is the first step of skin carcinogenesis and the cyclobutane pyrimidine dimers (CPD) are the most important pre-mutagenic damage involved in this process. UVC irradiation is frequently used to study the UV-induced CPD frequencies and their distribution between the four types of dipyrimidine sites, even if we are not naturally exposed to these wavelengths. In addition, the influence of the length of a dipyrimidine run on CPD frequencies is poorly understood. To study the impact of UV wavelength and DNA sequence within a dipyrimidine run on CPD frequencies and their distribution, we exposed normal human primary fibroblasts (*in cellulo*) and purified DNA (*in vitro*) to 10 KJ/m2 UVB or 0.2 KJ/m2 UVC. Using ligation-mediated PCR, we quantify the CPD formation at 952 dipyrimidine sites on the *PGK1*, *c-jun*, *H-ras*, *K-ras*, *N-ras*, and *p53* genes. *In cellulo*, we found that TT dipyrimidine sites were more damaged after UVC than after UVB irradiation while CC, TC and CT dipyrimidine sites were more damaged after UVB than after UVC irradiation. In addition, UVC-induced CPD were more frequent at TT and TC than at CC and CT, while UVB-induced CPD were more frequent at CC than at CT. Moreover, CC were more damaged after UVB than UVC irradiation, independently of the length of the dipyrimidine runs. All these results highlight that, compared to UVC, UVB generates more CPD at potentially more frequently mutated dipyrimidine sites confirming the importance to study the different steps of skin carcinogenesis using different UV wavelengths reaching the earth surface.

THUR14

New Approaches for Developing Near-Infrared Light-Controllable Drug Carriers

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I will discuss two approaches for making drug carrier whose payload can be released upon near infrared (NIR) light exposure. On the one hand, lanthanide-doped upconverting nanoparticles (UCNPs) are introduced into ultraviolet (UV)-sensitive polymer-based carriers (such as block copolymer micelle, hydrogel and polymer-coated mesoporous silica), acting as an internal UV light source. Under NIR excitation from a continuous-wave diode laser, UCNPs emit UV photons inside the carrier that, in turn, can "execute" the photochemical reaction resulting in disruption of the carrier and release of the payload. On the other hand, block copolymer hydrogel exhibiting an unusual upper critical solution temperature (UCST) is developed. In this case, by adding NIR light absorbing nanofillers (such as gold nanorods) in the hydrogel, exposure to NIR light gives rise to a photothermal effect that can bring the local temperature above the UCST and lead to the gel-sol phase transition and concomitant release of the payload.

THUR15

Biocompatible (light)responsive polymer layers to manipulate cells.

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A current challenge to address in tissue engineering and cell biology is the remote control of cell migration, proliferation, and cell fate down to the resolution of a

single cell. One way to achieve such a control is based on stimuli-responsive substrates that undergo on demand switches from a cell-repellent or inactive state, into a state imparted with high affinity for either protein receptors or for the plasma membrane. This presentation will illustrate current macromolecular "toolboxes" designed to switch interactions between polymers and cell membranes, including photolabile coatings, with particular focus on two systems that were designed in our group to be easily implemented with no need for specific instrumentation: i/ azobenzene-containing Amphipols that adsorb on lipid membranes and photo-trigger mild cytosolic penetration of soluble peptides,[1] ii/ stimuli-triggered polymer brushes that present short peptides to control cell adhesion and migration.[2] The first system is based on the photovariation of hydrophobic/hydrophilic balance of micelle-forming amphiphilic macromolecules that under their commercial form (devoid of azobenzene) are used to solubilize and stabilize membrane proteins. Depending on their (photoswitchable) polarity, these macromolecules can penetrate in lipid membranes and open pores of a few nanometers in diameter. The second system is based on spontaneous adsorption of cationic comb-like polymers with poly(ethylene oxide) side chains to form a dense, cell-repulsive brush on culture dishes. Attachment on the top of the polymer brush of soluble peptides (by *in situ* copper-free click chemistry) readily triggers cell adhesion, and enables patterning of cell deposition for co-culture.[2] Current development of temperature-responsive and light-responsive coatings will be cited.

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THUR16

Controlling cellular proteins with light

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Controlling the activity of proteins with light offers new opportunities to study biological processes with high spatiotemporal resolution. The gain in resolution compared to traditional genetic and pharmacological control methods is due to the light illumination itself, which can be tuned with submicrometric and submillisecond resolutions. To regulate the activity of a protein of interest with light, various strategies can be envisioned. First, one can rely on light-sensitive modules attached to the protein. Activation of these modules with light induces a change of the protein activity, and therefore a biological response. This approach requires however a great knowledge of the structure of the protein under study and some engineering to ensure light-dependent response. The second strategy is more versatile and consists in manipulating the cellular concentration of the protein by for instance the control of its synthesis using light-activatable transcription factors. Compared to the first strategy, the photocontrol of transcription enables to control a priori any proteins, which explains its generality. However, the timing between the photoactivation and the effect depends on several cellular mechanisms and is therefore rather slow (hour time scale), which decreases the time resolution of the approach. In this talk, I will show how controlling with light proteolysis – the reverse mechanism of protein synthesis – can be used to regulate protein levels within cells. Light-induced removal of a specific protein can inhibit (if the target is an activator) or activate (if the target is an inhibitor) specific biological functions with high spatiotemporal control.

THUR17

Efficient upconversion of 800 nm near infrared via novel core-shell lanthanide-doped nanocrystals

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Near infrared (NIR) light (800-1000 nm) as a trigger for release of bioactive molecules offers the advantages of higher tissue penetration and lower scattering in living tissue than shorter wavelengths of light, as well as the high spatiotemporal resolution possible with lasers. However, few chemical moieties efficiently translate the absorption of NIR into chemical rearrangement. To overcome this limitation, we have recently shown that upconverting nanoparticles (UCNPs) composed of $NaYF_4$:Yb^{3+},Tm^{3+} allow triggered release from UV-responsive polymeric nanoparticles upon irradiation with 980 nm light, as they convert this wavelength to UV light (Viger et al., Adv. Mater. 2013). Nonetheless, the very low quantum efficiency of these UCNPs limits the potential for translation of this strategy for NIR-triggered release to biological settings. Moreover, the strong water absorption at 980 nm may cause local heating, so UCNPs that can upconvert non-water-resonant wavelengths would be preferable. To this end, we have designed and synthesized nanostructures that can upconvert biologically benign NIR excitation wavelengths (800 nm) under low excitation flux to visible emission that can be potentially used to degrade photoresponsive polymers. A core-shell platform, wherein a shell of uniform thickness thoroughly shields the core from surface quenching, allows upconversion of 800 nm NIR excitation to visible emission. Excitingly, these UCNPs are at least an order of magnitude brighter upon 800 nm excitation than Nd^{3+}-doped UCNPs, the only material yet shown

to upconvert this wavelength. We are currently exploring the utility of this new UCNP platform in triggered release from polymeric nanoparticles that degrade upon UV irradiation; it also holds promise for other applications, such as photodynamic therapy.

THUR18

Newly Cloned GFP from Rhacostoma Jellyfish and a novel spot test for BPA

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Recently, Brighter Ideas, Inc. has cloned a novel GFP from a New Jersey shore jellyfish, Rhacostoma atlantica. A patent application for Rhacostoma GFP was submitted in March, 2014. This new GFP is red-shifted relative to native Aequorea GFP, having absorption and emission spectra similar to the mutant of wild-type Aequorea GFP known as E-GFP. The spectral shifts observed in E-GFP (both excitation and emission) are caused by a single amino acid substitution in the chromophore region—from Serine 65 to Threonine. In Rhacostoma there is an Alanine in position 65. The hexapeptide encompassing the chromophore in wild-type Aequorea GFP has the amino acid sequence FSYGVQ while the corresponding region in Rhacostoma GFP is LAYGVT.

Rhacostoma GFP was expressed in E. coli and purified directly from spun down cells by three-phase partitioning (using aqueous ammonium sulphate and tert-butanol) followed by column chromatography on a Phenyl Sepharose FF gel filtration column. This preparative column step was followed by a polishing step on a Sepharose 6-B gel filtration column. These three steps produced a very clean sample as judged by size exclusion HPLC. The absorption (excitation) spectrum of Rhacostoma GFP peaks at 466 nm with a molar extinction coefficient of 58,000. Fluorescence emission peaks in the region 495 nm to 500 nm. The fluorescence quantum yield is 0.73, slightly greater than that of E-GFP (0.6). Most notably, the amino acid sequence of Rhacostoma GFP is only 45% identical to the sequence of GFP from its closest phylogenetic neighbor, suggesting that this novel GFP may provide a useful scaffold for mutagenesis.

In addition, we will display an educational kit for measuring BPA (bis-phenol-A), an endocrine disruptor found in plastic bottles and epoxy-lined food cans. Our spot test reveals extraordinarily high levels of BPA in 40% of the 1000 store receipts we tested. Many of the receipts have 200,000 times as much water-leachable BPA as these other well-known sources of BPA contamination. BPA is detected by a color change from teal to lavender. The teal color is produced by a free-radical form of ABTS generated by H2O2 in the presence of peroxidise. Further reaction with BPA changes the teal color to an unmistakable lavender spot.

THUR19

CLIPT for Progression of Breast Cancer

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Chest wall recurrences of breast cancer following mastectomy are initially treated with a combination of radiation, chemotherapy, and/or surgery. In many cases, secondary recurrent and/or progressive disease can be seen within prior radiation fields. These patients, particularly those with large surface area involvement, have limited therapeutic options. Based on promising pre-clinical data, a novel form of Photodynamic Therapy using Continuous Low-Irradiance Photodynamic Therapy (CLIPT) may provide an alternative therapy for patients who have exhausted all other treatment modalities.

The following discussion of CLIPT will examine the outcomes of Phase I pilot studies, the expansion of the therapy in a pivotal clinical study and the technology development of a mobile light delivery device that is used to deliver the therapy.

THUR20

Biostatistical Considerations in Designing Clinical Trials

Mary Putt

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Introduction: Investigators sometimes view interactions with statisticians as necessary evils, in the same category as a trip to the dentist. This session will interactively model the interaction between statistician and investigator through discussion of a case study involving the design of a clinical trial. We will build on positive experiences working as a multi-disciplinary team on the UPenn PDT program, inviting active audience participation along the way.

Background: Patients with locally advanced (97% Stage III/IV) epithelial-based malignant pleural mesothelioma (MPM) who receive lung-sparing radical pleurectomy (RP) followed by photofrin-based (60 J/cm2, 24h drug-light interval) photodynamic therapy (pPDT) have median overall survival (OS) rates that exceed 31 months post-surgery. These are exciting findings in light of historical survival rates on the order

of 13 -23 months post-diagnosis for patients treated with RP alone or extra-pleural pneumonectomy (EPP) alone.

Proposed Study: A randomized clinical trial to quantitatively answer the question of whether RP/pPDT enhances OS compared to RP or EPP alone.

Questions of Interest:

1. Why consider a randomized trial at all?
2. Is it ethical to randomize patients given the observed success of the RP/pPDT treatment to date?
3. What options are available for implementing the randomization process
4. Can we blind a surgical trial?
5. What are the advantages/disadvantages of early stopping in the event of futility or superiority?
6. How do we decide on sample size?
7. What if the trial fails to conclude that RP/pPDT is the superior treatment? What then?

THUR21

A Spreadsheet for Detection of Possible Data Fabrication in Numerical Data Sets of the Type Frequently Encountered in Cell and Radiation Biology Survival Studies

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The growing awareness of the frequency of falsified and fabricated data in scientific and medical reports highlights the need for tools to identify problematic data prior to publication. Programs to detect plagiarism are routinely used by a number of publishers and have led to a decrease in this type of misconduct. Image manipulation can be detected by using programs such as one available on the Office of Research Integrity website. Routine use of such programs will help to curtail this type of fraud, as well. We have constructed a spreadsheet that can process numerical data sets of a type frequently encountered in cell and radiation biology survival studies to detect possible data fabrication. Such detection is based on the fact that fabrication may be indicated by the presence of unusual, unexpected patterns in data. For example, rightmost terminal digits of counts taken from particle and scintillation counters should be relatively uniform, whereas numbers invented by individuals do not generally have uniform terminal digits. Hence, significant deviation from uniformity of the terminal digits of count data could be cause for concern. Another suspect pattern, more specific to survival studies in radiation biology and pharmacology, where samples are generally tested in triplicate, is an unusually high frequency of triples that include values close to their mean as one of their elements. Since in this such research it is the mean value of these triples that is of specific interest, an investigator wishing to guide the results of his investigations, might well be inclined to invent triples by choosing a near mean value as one of the elements, and numbers equidistant on either side as the two others. When simple links to spreadsheets containing data triples in adjacent columns are set up, our Excel spreadsheet: 1) tabulates frequencies of terminal digit data values; 2) counts the number of data values that have duplicated terminal digits; and 3) counts the number of triples that contain their mean or a near mean value, and applies appropriate statistical tests to identify data anomalies. Screening using a spreadsheet of this type could help to further minimize data fabrication and falsification and elevate statistics to its rightful place in the armamentarium of fraud-detecting software.

THUR22

A climate model to predict population exposure to UVR in coming decades based on personal UV measurements

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As the climate and the environment changes this will influence personal UVR exposure.

Personal UVR exposure was measured in Standard Erythema Doses (SED) using a wrist worn electronic dosimeter. Behaviour like work/off-work, sunbathing and sunburn was recorded in a dairy on a daily basis.

The population comprised 44 Danish farmer families with 152 participants in total, who participated in the period from May through September 2009. The families had from 1 to 3 participating children. The mean ages were 44 years (range 32-67 years) for the adults and 11 years (range 5-19 years) for the children. All the farmers were male and all the spouses were female. A total of 148 people completed the study and data from 17303 days of the 19995 collected days was analysed.

Meteorological data was collected on a daily bases for the whole period: ambient UVR, daily maximum temperature, rain, sunshine-hours, and cloud cover. From these meteorological data categories was constructed. All the grouped metrological parameters was used as “model input variables” to predict the personal UVR exposure for farmers, spouses and

children separately. The average measured UV dose (SED) was 206.1, 143.7 and 187.6 for the farmers, spouses and children respectively. The modelled doses were 203.6, 143.3 and 184.0.

Assuming uniformity in behaviour under the same conditions, risks and/or benefits we estimated future population UVR exposures for a given scenarios of environments using output of a regional climate model (RCM- HIRHAM from the Danish Meteorological Institute (DMI, www.dmi.dk), as part of the EU project ENSEMBLES (ensembles-eu.metoffice.com)) and future ozone data (SPARC (Stratospheric Processes And their Role in Climate) report. no. 5, chapter 9 (sparc-climate.org)).

We found that the model predicts an increase in personal UV dose (~10%) in the period 2050 to 2060 compared to that measured in 2009.

(Work was done in collaboration within EC-project ICEPURE(227020) www.icepure.eu)

THUR23

Body modelling of UVR exposure under different solar environments

Alois Schmalwieser[1], Antony Young & team[2], Hans Christian Wulf & team[3], Paul Eriksen & team[3]

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A variety of UV exposure measurements can be found in the literature. These measurements were made at certain body sites and under certain solar environments. The UV exposure depends strongly on the individual behavioral pattern and is defined by the duration of irradiation, the geometry of the receiving surface (body site) in relation to the sun, the protection by clothes, the use of sunscreens, the amount of hair and shadowing. Behavioral patterns are influenced by occupational activities, spare time activities and holiday destinations.

Body modelling of UVR exposure allows generalizing such measurements to any other condition and to any other location. Body modelling enables the calculation of UV exposure at any date of the past or in the future. We model the UVR exposure by using 3-d (wire frame) models of the human body. There are models for each gender and for different ages (gender and age modify the body shape) available. Each body model consists of tens of thousands of polygons, which give a much higher resolution and are more realistic than models used in a number of recent publications. Another advantage of our type of model is that the posture can be changed, as well as the clothing and hairstyle. Additionally our models can perform movements such as walking, running or cycling.

Input parameter is the solar UV radiation coming from all sky directions. This can be measured or modeled. The solar UV radiation can be weighted with any action spectrum to get the UV exposure e.g. for erythema, vitamin D photosynthesis or pigmentation. The exposure pattern differs for different action spectra. The evaluation of the modelled exposure distribution was done by equipping volunteers with a dozen of personal UV dosimeters all over the body. The mesh models copied posture respectively activity. The agreement of modelled and measured values is best for clear sky situations. The agreement for cloudy sky depends strongly on the time scale: as longer the period the better is the agreement.

The modeled UV exposure patterns allow the identification of body parts, situations and activities of high risk for overexposure. With that, body modelling may become a very helpful tool for sun protection and health care.

THUR24

Sun and ski holidays improve vitamin D status, but are associated with high levels of DNA damage

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Skin cancer is caused by solar ultraviolet radiation (UVR), which is also essential for vitamin D production. DNA damage (cyclobutane thymine dimers: T-T dimers) and vitamin D (25(OH)D) synthesis are both initiated by ultraviolet B radiation (UVB). We aimed to investigate the adverse and beneficial effects of solar UVB exposure simultaneously in holidaymakers. Sun-seekers and skiers (n = 71) were observed over 6 days with on-site monitoring, personal diaries, and recording of personal UVB exposure doses with electronic dosimeters. Urine and blood samples were analysed for T-T dimers and 25(OH)D, respectively. The volunteers had a statistically significantly increase in vitamin D and T-T dimers. There were strong associations between UVB exposure and post-levels of T-T dimers and vitamin D, as well as between post-level T-T dimers and vitamin D. We conclude that beneficial UVB induced vitamin D

synthesis is associated with considerable DNA damage in the skin. These data, on two major health predictors, provide a basis for further studies that may result in better risk/benefit analysis in the future.

THUR25

Skin colour has no effect on vitamin D photosynthesis

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Vitamin D is important for skeletal health and is likely to play an important role in many other health outcomes. Global data show a high level of vitamin D deficiency/insufficiency, which is more prominent in people with heavily pigmented skins. It is widely stated that this is because melanin inhibits cutaneous vitamin D synthesis but there are few, and contradictory, laboratory data to support this hypothesis. We tested the ability of skin melanin to inhibit vitamin D synthesis by exposing skin types I-VI (n=34) to fluorescent solar simulation radiation (SSR). Studies were done in young healthy adults in winter/early spring in London, UK. Participants were exposed to 5 whole body exposures, each of 2 standard erythema doses (SED) SSR with intervals of 3-4 days between exposures. Blood samples were taken prior to exposures and 3 days after the final exposure. Vitamin D status was assessed measuring serum 25(OH)D with LC/MS. Linear regression analysis within given skin types showed linear responses (25(OH)D vs. SSR dose) with slopes that were independent of skin type. Intercepts varied with skin type to show that baseline 25(OH)D decreased with increasing skin type. All skin types showed an increase of about 30-40 $nmol.L^{-1}$ 25(OH)D after 10 SED SSR. We conclude that skin colour (melanin) has no effect on vitamin D photosynthesis and that other factors explain differences in vitamin D status in different skin types.

P O S T E R S

POS1

Towards prevention of infectious diseases: microbial control of wastewater by photoactivated ZnO nanoparticles

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Worldwide the need for clean water is increasing because of population increase and contamination of conventional water sources. WHO reported that 1 billion people are at risk because they do not have access to potable water, another 2.6 billion people lack access to clean water. Thus, innovative approaches to increase availability of clean water are highly needed.

The aim of this study is to evaluate antimicrobial activity of photoactivated (400 nm) ZnO nanoparticles against food-borne pathogens found in wastewater from agricultural and food industries: *Escherichia coli* O157:H7, *Listeria monocytogenes and Enterococcus faecalis*.

Results indicate that 200 nm size ZnO nanoparticles in suspension as well as surface coated with 200 nm size nanorods, synthesized in our laboratory, and photoactivated with visible light, exhibit strong antimicrobial properties and can inactivate previously described waterborne pathogens reducing bacterial counts by 6 log. Moreover clear degradation of bacterial biofilms on the surface of polyolefine was found after treatment with photoactivated ZnO nanoparticles or nanosurfaces. Scanning electron microscopy images allow conclude that cell wall disintegration and cell lysis take place in treated bacteria.

The inactivation of waterborne pathogens by ZnO nanoparticles or surfaces coated with ZnO nanorods in the presence of visible light implies potential ex situ application under sunlight for water decontamination at ambient conditions.

In conclusion, it is expected that exploitation of nanosize ZnO properties will help to prevent waterborne infections as has potential to decontaminate water in efficient and cost-effective way with highly reduced energy consumption.

POS2

Dendrimeric-like Hexadecahydroxylated Zinc Phthalocyanine. Synthesis And *in Vitro* Evaluation of Photodynamic Efficiency.

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Phthalocyanines are promising photosensitizers for photodynamic therapy thanks to their stability and maximum absorption in the near-infrared region of the electromagnetic spectrum. Even though there are few phthalocyanines[1][2][3] clinically used or on advanced trial stages, most phthalocyanines face a challenge of water-solubility in order to be used as a drug. Water-solubility of phthalocyanines can be tailored by adjusting the substitution pattern[4]. Since the use of glycerol as a photosensitizing substituent was promising[5], a new diglycerol-based substituent was developed and lead to a dendrimeric-like water-soluble phthalocyanine with appropriate photophysical, photochemical and biological properties.[6]

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Keywords: Photodynamic therapy, phthalocyanine, water-soluble.

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POS3

UVB radiation increases MCPIP-1 expression in HaCaT cells.

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MCPIP1 (Monocyte Chemotactic Protein-1 Induced Protein) is a recently identified protein which downregulates the inflammatory response due to its RNase activity to mRNA IL-1β, IL-6 and negative regulation of NF-κB activity upon stimulation with IL-1β and LPS.

NF-κB, which can be acivated by UV radiation triggers the production of interleukins and TNF-α.

Since UVB (280-320 nm) radiation induces apoptosis and inflammatory response in the skin, we studied the role of MCPIP1 gene in the regulation of this process on HaCaT cells, an immortal human keratinocyte line.

HaCaT cells were irradiated with UVB (0.5mW/cm^2) for 30-120 sec, which induced apoptosis and the death in about 50% of cells. The induction of apoptosis was verified by the measurement of caspase 3/7 activity and analysis by flow cytometry of Annexin V-stained cells, and the overall phototoxicity of UVB was estimated by MTT assay performed 24h after the treatment.

UVB radiation (0.3-0.6 kJ/m^2) of HaCaT cells resulted in a significant increase of MCPIP1 expression, both at mRNA and protein level which was blocked by actinomycin D. This suggests the involvement of *de novo* mRNA synthesis in the increase of MCPIP transcript and protein following UVB radiation.

To study if cells with silenced MCPIP1 in contrast to wild type cells or non-specific control are more resistant to UVB induced apoptosis we diminished MCPIP1 expression in HaCaT cells using retrovirus vectors containing shRNA specific for MCPIP1. Two of five tested vectors (sh2 and sh4) gave 70-100% of gene silencing vs. control cells (treated with nonspecific siRNA). However silencing of MCPIP1 results decreased the viability and proliferation rate of HaCaT cells. Interestingly, MCPIP1 inhibition influences activity of signaling pathways important for cell growth and metabolism. Work supported by the National Science Centre Grant number 2012/05/B/NZ1/00004 and 2011/03/B/NZ1/00023.

POS4

UV-Stressed *Daphnia pulex* and Freshwater Algal Species Increase Fitness Through Uptake of Vitamin D

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With the high variability of ultraviolet radiation in aquatic ecosystems, it has been suggested that UV-exposed organisms may demonstrate enough phenotypic plasticity to maintain the relative fitness of natural populations. Our long-term objective is to determine the potential photoprotective effect of vitamin D on *Daphnia pulex* exposed to acute or chronic UV radiation. The questions posed in this specific study are three-fold: 1. big picture effects of vitamin D on fitness of the organisms; 2. organismal quantification of vitamin D; and, 3. route of vitamin D sequestration and the biological mechanisms by which it may act as a photoprotectant. Significantly higher fitness was observed in the *D*. *pulex* with vitamin D than those without (most extreme effects observed were 0% survival in the absence of vitamin D and 100% with 10 ppm D in acute UV-B, 3.18 kJ/m2/nm, exposures). Vitamin D was isolated from the culture media, the algal food (*Pseudokirchneriella*), and the *D*. *pulex* and quantified using high performance liquid chromatography (HPLC). Vitamin D was fluorescently labeled using a phenothiazinium dye and added to cultures of *D*. *pulex*. Images demonstrating the uptake of vitamin D into the tissues and carapace of the *D*. *pulex* were acquired. In this endeavor, initial findings

suggest a strong bioaccumulation mechanism on *D. pulex* through various freshwater algal species. Changes in various freshwater algal populations and their concomitant effects on the *Daphnia* are being investigated and early evidence suggests that the algal interaction with the vitamin D may be the most important in this photoprotection puzzle.

POS5

Hair dye induced DNA damage and Differential Protein expression in Human keratinocyte under environmental UV radiation.

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Hair dyes are widely used as cosmetic agents to change the color of hair, and/or to color the gray hair around the world in recent years which increases the risk of skin cancer and other skin disease. 2-Amino-3-hydroxypyridine and Paraphenylenediamine are extensively used in hair dye for coloring.Thus the safety of hair dye products, is a matter of concern for human health consequences. Photosensitizing potential of 2-Amino-3-hydroxypyridine (A132) was studied by using human keratinocyte (HaCaT cell line) under UVA (1.6 mW/cm²) UVB (0.6mW/cm²) and sunlight irradiation at different concentrations (5-100 µg/ml). Several studies have demonstrated that UVR-induced cell death occurs through the generation of reactive oxygen species. The consequent oxidative stress includes the impairment of cellular antioxidants, the induction of DNA damage and the occurrence of apoptosis.The photostability of A132 was assessed by photodegradation study under the exposure of UVR for different time period. Mechanism of phototoxicity was evaluated by photochemical generation of singlet oxygen ($_1O^2$), superoxide anion radical ($O^{\cdot.2}$) and hydroxyl radical ($^{\cdot}OH$). Photochemical generation of reactive oxygen species (ROS) was confirmed by the specific quenchers like DABCO, NaN3 and SOD. Photosensitizing capacity of A132 leads to lipid peroxidation. The photocytotoxicity of A132 was assessed by MTT & NRU (neutral red uptake) assays. Single cell gel electrophoresis (SCGE) shows the induction of DNA damage under UVB exposure. Cell cycle study by flow cytometer showed G0 phase arrest in HaCaT cell line. Photosensitized A132 also induced apoptosis which was confirmed by staining with acridine orange and ethidium bromide. Effect of A132 on the expression of Bcl2, Bax, genes was assessed by real time PCR, which was further confirmed by western blot analysis at protein level. Simultaneously Differentially expressed proteins in HaCaT cell line treated with A132 were screened by 2D gel electrophoresis that also confirms the oxidative stress on A132 treated HaCaT cell line. Thus, our study reveals that phototoxic potential of A132 under environmental intensities of UVA and UVB. Therefore the effectiveness and safety measurement of hair dye ingredients should be primary concern for human health.

POS6

Oxidative stress mediated apoptosis and identification of marker proteins by benzophenone under environmental UV radiation

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The use of sunscreens has increased many fold now a days due to excessive sun exposure which increases the risk of skin cancer, skin aging and other skin diseases. Benzophenone and its derivatives are extensively used in sunscreens as UV blocker. Apart from sunscreen Benzophenone is used in other cosmetics like shampoos, soap, perfumes, body lotions, paints and as packaging material of food stuff. Thus the safety of UV filters, and sunscreen products, is a matter of concern for human health consequences. Photosensitizing mechanism of benzophenone was studied by using human keratinocytes (HaCaT) cell lines under environmental ambient intensities of UVA (1.6mW/cm²) and UVB (0.6mW/cm²) at different concentrations (5-50µg/ml). Photostability test showed that benzophenone is not stable in UV radiation and forms two photoproducts which were identified by LC/MS-MS. Mechanism of phototoxicity was evaluated by photochemical generation of singlet oxygen ($^1O_2$), superoxide anion radical ($O^{\cdot}{}_2-$) and hydroxyl radical ($^{\cdot}OH$). Which were further confirmed by specific quenchers. The photo oxidative degradation of DNA base 2'**-**deoxyguanosine (2'-dGuo) by the benzophenone was studied. The photocytotoxicity of benzophenone was assessed by MTT and NRU (neutral red uptake) assays, which showed 60% reduction in cell viability. The intracellular ROS generation was done by H_2DCF-DA assay which confirmed H_2O_2 generation. Single cell gel electrophoresis (SCGE) showed the induction of DNA damage under UVA exposure. Benzophenone induced leakage of LDH in culture media and formation of melonaldehyde as end product of lipid peroxidation was measured in concentration dependent manner. Cell cycle study by flow cytometer showed sub G1 fractions which represent apoptotic cells. Benzophenone induced apoptosis in HaCaT cell lines was measured by staining with Annexin V FITC conjugate and Propidium iodide. which was further confirmed by the expression of Bcl2, Bax, and p21 genes by real time PCR, there was no significant change in the expression of Bax was noticed but the

expression of bcl_2 was highly down regulated which favors the bcl-2:bax rheostat towards apoptosis, a significant up regulation of p21 was observed . The western blot result of bax, bcl-2 and p21 favors the result at gene level. The differentially expressed proteins in UVA treated HaCaT cells by benzophenone were screened by two dimensional gel electrophoresis. 13 protein spots which were found significantly differentially expressed were identified by MALDI/TOF-TOF against MASCOT search using NCBInr database. Most of these proteins were found first time to be associated with damage of HaCaT cell line induced by benzophenone at ambient UVA radiation. Thus, our study reveals that phototoxic potential of benzophenone which induced DNA damage and apoptosis under the exposure of environmental intensities of UVA and UVB. Therefore use of benzophenone in sunscreens as well as in others cosmetics may be deleterious to human health.

POS7

DRPDT2: a new compound to improve photodynamic therapy

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Prostate cancer (PCa) is the third leading cause of cancer-related deaths among men. Individuals who succumb to advanced stages of PCa inevitably become refractory to conventional therapy. Therefore, there is an urgent need to develop new drugs for these patients. While not currently used in the treatment of PCa, Photodynamic Therapy (PDT) has been successfully applied clinically in the management of both neoplastic and non-neoplastic diseases. We have reported the important role of Nitric Oxide (NO) in enhancing the PDT-mediated anti-tumor activity, both *in vitro* and *in vivo* as the result of the inhibition of the resistant NF-κB/YY1/RKIP loop in tumor cell lines. Hence, we hypothesized that chemical conjugates between photosensitizer (in this case pheophorbide *a*) and an NO donor may represent novel and potent cytotoxic agents against refractory PCa; in fact in this way it is possible to obtain simultaneously high production of reactive oxygen species and NO induction, increasing the oxidative damage due to PDT. Thus, we have evaluated *in vitro* the effectiveness of a new photosensitizer-NO derivative, namely DRPDT2, on cell growth and viability on human carcinoma prostate PC3 cell lines. Preliminary data show that DRPDT2 inhibits proliferation and induces cell death. Experiments on molecular signaling mediated by DRPDT2 are underway.

POS8

Differences in expression of genes controlling metabolic equipment of co-cultured human melanocytes and keratinocytes. **Modulation by solar UV or H_2O_2 exposure**.

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Skin is particularly exposed to oxidative stress and environmental insults such as sunlight or pollution which impact metabolic homeostasis (e.g. UVB and AhR or UVA and Nrf2). In this study, metabolic status of normal human keratinocytes and melanocytes from same donors was assessed in coculture at the transcriptional level. Basal expression of about 200 genes encoding phase I or phase II metabolism was compared in both cell types. Half of genes were equally expressed, however some significant differences were noticed. For instance, melanocytes displayed a higher expression of NQO1, HO1, Ferritin, whereas GPX genes family was mainly expressed in keratinocytes. When cells were exposed to simulated solar UV (UVB+UVA, cell number over 50% of control 24h post exposure) HO1 was induced in both cell types, but clear inductions (over 2 fold) were mostly observed in keratinocytes (e.g. GPX2, PSTG2, CYP1A1, CYP1B1…). Similar experiments were performed using H_2O_2 (100 or 200 µM): HO1 and Thioredoxin Reductase were induced in both cell types, but here again most of overexpressions were noticed in keratinocytes (GCLC, NQO1, Ferritin, CYP1B1…). The return kinetics to basal expression level was higher in melanocytes than in keratinocytes. These results show that epidermal cells sharing the same genetic background and growing in the same culture medium display differences in basal and stress-induced expression of genes controlling metabolism. Melanocytes seem to be less sensitive to stress than keratinocytes. Even if pathways like Nrf2-ARE, AhR or inflammation are involved in response to H_2O_2 or solar UV, there are also stress-specific gene modulations both in melanocytes and in keratinocytes.

POS9

Comparative study of in-vitro photodynamic effect of free and liposome-encapsulated chlorophyll derivative in de-pigmented melanoma

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Cutaneous melanoma that develops in melanin-producing melanocytes is among the most unresponsive cancers to known therapies. It has the highest potential to metastasize in all body organs and represents a life threatening disease. Photodynamic therapy (PDT) has proved to be a potential treatment modality in various types of cancerous and non-cancerous diseases. However, melanin, the light-absorbing pigment in melanoma, reduces PDT efficacy by acting as a free radical and reactive oxygen species (ROS) scavenger and competing with the capability of the photosensitizer to absorb light energy.

The objective of this study is to assess the PDT-mediated cell killing in de-pigmented melanoma using free and liposomes-encapsulated Chlorophyll derivative (CHL) in B16.F10 melanoma cell line.

Phenylthiourea is used as a reversible tyrosinase enzyme inhibitor which is a rate-limiting enzyme in melanin synthesis. Conditions were optimized for phenylthiourea to 200 uM, as a non-cytotoxic concentration, and 2-day incubation before application of CHL. These conditions showed 49.8% melanin inhibition.

Light and dark control experiments were performed and proved safety of all employed light doses from a 650 nm optic fiber red laser and concentrations of free and liposomes-encapsulated CHL, respectively.

Several liposomes formulae were prepared and assessed for PDT effect. There was a positive correlation between the cholesterol content in different formulae and PDT efficacy, thus, the formula with the highest cholesterol content, particle size less than 200 nm and % encapsulation efficiency of CHL (82.15%) was chosen for further investigation.

The *in-vitro* phototoxicity at 24-hour incubation period and a light dose of 56.25 J/cm ^{2} showed LC_{50} of 2 uM and 30 uM, in free and liposome-encapsulated CHL respectively.

POS10

Evaluation of growth, biomarker expression and matrix remodeling in 3D cultures of drug-resistant pancreatic cancer cells reveals elevated invasiveness and increased sensitivity to PDT

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Pancreatic adenocarcinoma is one of the most lethal cancers, partly because of its likelihood to metastasize before diagnosis. The highly invasive potential of pancreatic cancer cells is associated with a mesenchymal phenotype and resistance to chemotherapy, so improvements in treatment options for patients will be essential for enhancing their life expectancy and quality. In photodynamic therapy (PDT), photosensitized cells are exposed to light, initiating photochemistry that results in targeted tumor destruction primarily by generation of cytotoxic singlet oxygen. We have generated an oxaliplatin-resistant PANC-1 cell line (PANC-1-OR) and sought to determine its phenotype, metastatic potential, and sensitivity to photodynamic therapy. Based on immunofluorescence and western blotting, PANC-1-OR cells express markers of a mesenchymal and invasive phenotype. PANC-1-OR cells also extensively invade into the surrounding matrix when grown in 3D matrigel cultures, as shown by particle tracking microrheology that quantitatively monitors matrix degradation as a measure of invasiveness. In imaging-based treatment assessment studies in a 3D cell culture model, PANC-1-OR cells exhibit better response to benzoporphyrin derivative monoacid ring A (BPD)-based photodynamic therapy than PANC-1 cells.

POS11

Sequential [4+2] Diels Alder Reaction of 3,4',5 Trimethoxy-Trans-Stilbene with Singlet Oxygen

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Resveratrol (trans-5-(para-hydroxystyryl)-resorcinol) is a polyphenol commonly found in the skin of grapes, mulberries, and wine. The trihydroxylated stilbene has been reported to exhibit preventive effects towards photo-oxidative damage in epithelial cells; however, the mechanism as to how this occurs is not well explored. We have established that resveratrol has two known pathways of reaction with singlet oxygen ($^1O_2$): [4+2] cycloaddition with the central double bond and the adjacent double bond from the phenol ring, and [2+2] cycloaddition to form an unstable dioxethane. However, in vivo, the 4' hydroxyl group, is glycosylated and 4'-methylated derivatives have also been reported. We suggest that the reaction of $^1O_2$ with trimethoxylated stilbene (3,4',5 trimethoxy-trans-stilbene) produces the usual [2+2] cycloaddition product and a [4+2] reaction pathway followed by another sequential [4+2] Diels Alder reaction to produce a bisendoperoxide product. Kinetic experiments using time-resolved luminescence

spectroscopy has shown that resveratrol and its methylated derivative are poor $^1O_2$ scavengers with k_t values of $1.6x10^6$ $M^{-1}s^{-1}$ in CD_3CN and $8.03x10^5$ $M^{-1}s^{-1}$ in CD_3CN, respectively.

POS12

Degradation of bio-based oligomer/polymers from sustainable materials

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Synthetic polymers derived from fossil fuels play an indispensible role in everyday life. However with the problem of limited availability of fossil fuels and increased demand for polymers we are forced to look into viable alternatives such as renewable biomass. One of the major challenges associated with many of the synthetic polymers is their non-toxic degradability that makes them harmless landfills. We have been interested in utilizing biomass-derived compounds as oligomers/polymers that can be degraded with light. The poster will highlight our strategy of synthesizing photodegradable bio-based polymers from these sustainable sources.

POS13

Anticancer effect of blebbistatin under blue light

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Cancer is one of the main challenging and societal problem in modern world. It is estimated that number of deaths from cancer in 2012 was more than 8.2 millions. New strategies and approaches in treatment of cancer diseases are being developed continuously. Numerous scientific investigations are devoted to the search for potential anticancer agents effectively inhibiting activity of cancer cells. Blebbistatin derived its name from ability to block cell blebbing could be such a novel therapeutic agent. It was discovered in a screen of inhibitors of myosins, particularly myosin II, playing important role in cellular architecture, division and migration.

Here we show that blebbistatin can be used as a potential anticancer agent. Cytotoxic effects of blebbistatin were investigated in four different human cancer cell lines, prostate adenocarcinoma androgen independent Du145 and androgen dependent LNCaP, glioma U87 and melanoma FEMX-I. Immortalized human fibroblasts F11-hTERT served as a control. Phototoxic effects were also investigated in mentioned cell lines upon exposure to blue light lamp (370 – 470 nm, peak 420 nm). Blebbistatin at a studied range of concentrations induces comparable cytotoxicity in cancer and normal human cell lines. However, in a combination with blue light, blebbistatin exhibits selective phototoxic effect towards cancer cells with a cytotoxicity enhancement ratio to be greatest for FEMX-I cells followed by LNCaP, Du145, U87 and F11-hTERT. It is proposed that main mechanism of phototoxic action of blebbistatin is via formation of reactive oxygen species (ROS) but not singlet oxygen.

In conclusion, blebbistatin is a new promising inhibitor of cell activity. In addition, blebbistatin is a photosensitizer with selective photodynamic effect on human cancer cells.

POS14

Longitudinal monitoring of cancer micrometastases using activatable immunoconjugates and fluorescence microendoscopy

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We are developing fluorescence microendoscopy and quantitative image analyses to guide and monitor treatment of disseminated cancer micrometastases. Traditional imaging modalities often fail to detect residual, microscopic tumour deposits that frequently cause disease recurrence. To help address this problem, we present an approach that utilizes a tumor-targeted, activatable immunoconjugate and fluorescence microendoscopy to track cancer micrometastatic burden in an orthotopic xenograft mouse model of peritoneal carcinomatosis. Histopathology, chromophore tissue extraction and a quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay—that counts the number of viable human cancer cells within the entire peritoneal cavity—were applied to validate *in vivo* imaging of immunoconjugate pharmacokinetics and micrometastatic burden. The findings demonstrate that this approach enables *in vivo* imaging for: (i) recognition of tumours as small as 30 μm with a sensitivity of 90% and a specificity of 90%; (ii) quantitative monitoring of immunoconjugate pharmacokinetics and tumour-selective activation;

and, (iii) quantitative monitoring of micrometastatic burden in select sites known to frequently harbour residual disease.

POS15

Long term stability of isotropic detectors calibration using an LED-coupled integrating sphere

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Light dosimetry is a critical clinical parameter for photodynamic therapy (PDT). Routine measurements of light fluence use isotropic detectors composed of an optical fiber with a scattering tip. For an accurate *in vivo* light dosimetry, it is necessary to individually calibrate each detector. This study examines the long term calibration stability of isotropic detectors calibrated using a light-emitting diode (LED) light source (Philips LumiLED) built into an internally baffled 4" integrating sphere. The LED emits over a broad range of wavelengths centered at approximately 630 nm. The sphere is fabricated from a plastic sphere coated with barium sulfate coating (Spectraflect, Labsphere), and the LED and its driver circuitry are built into the housing which holds the sphere.

The isotropic detectors used in this study were made by Medlight, with a tip of 0.5mm. Calibration was performed for wavelengths of 630-730nm. The calibration factor is defined as fluence rate per voltage read by a detector calibrated for the wavelength in question. The illumination of the detector is independent of wavelength in this case; the variation in calibration factor with wavelength reflects the wavelength-dependence of the photodiode detector's response and the optical fiber's transmission. This can be quantified by a wavelength correction factor, equal to the ratio of calibration factors at two wavelengths. Stability of LED light source over time is examined by leaving the LED on over xx hrs and measure light fluence rate repeatedly over several times.

The LED-coupled integrating sphere described here allows accurate calibration of isotropic detectors in a portable, simpler to use, and less sensitive to the details of the experimental setup. The study was made for over 4 years and the relative maximum (standard) deviation of the calibration factor was ± 11% (5%) for 630nm wavelength, compared to 25% (11%) based on an alternative collimated direct laser beam calibration method.

POS16

Repair-dependent Cell Radiation Survival and Transformation: an Integrated Theory

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The repair-dependent model of cell radiation survival is extended to include radiation-induced transformations. The probability of transformation is presumed to scale with the number of potentially lethal damages that are repaired in a surviving cells or the interactions of such damages. The theory predicts that at doses corresponding to high survival, the transformation frequency are the sum of simple polynomial functions of dose; linear, quadratic, etc., essentially as described in widely used linear quadratic expressions. At high doses, corresponding to low survival, the ratio of transformed to surviving cells asymptotically approaches an upper limit or plateau. The low- and high dose domains are separated by a transition region. Published transformation data for mammalian cells show the high-dose plateaus predicted by the repair-dependent model for both ultraviolet and ionizing radiation. For the ultraviolet- and ionizing radiation induced neoplastic transformation experiments that were analyzed, the transformation frequency data can be fit with only the repair-dependent quadratic function, which approaches being strictly quadratic in the low-dose limit, but has a sigmoidal shape over a wider range of doses. Inclusion of data from the transition region in a traditional linear-quadratic analysis of neoplastic transformation data can exaggerate the magnitude of, or create the appearance of, a linear component. Quantitative analysis of survival and transformation data shows good agreement for ultraviolet radiation; the shapes of the transformation components can be determined from survival data. For ionizing radiations, both neutrons and X-rays, survival data overestimate the transforming ability for low to moderate doses. The presumed cause of this difference is that, unlike UV photons, a single x-ray or neutron may generate more than one lethal damage in a cell, so the distribution of such damages in not accurately described by Poisson statistics. However, the complete sigmodial dose-response data for neoplastic transformations can be fit using the repair-dependent functions with all parameters determined only from transformation frequency data.

POS17

***In vitro* photodynamic inactivation of *Candida* species with chloroaluminium phthalocyanine nanoemulsion**

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Candida species are the main cause of opportunistic mycoses in humans. The selection of fungicide-resistant strains and the high toxicity of the currently used fungicides make the development of alternative fungus-control techniques highly desirable. Photodynamic antimicrobial chemotherapy (PACT) is a promising method which combines a nontoxic photosensitizer (PS) with visible light to cause selective killing of microbial cells. The development of PACT to treat mycoses or kill fungi in the environment depends on identifying effective PS for the different pathogenic species and delivery systems able to expand and optimize their use. In the present study, the *in vitro* susceptibilities of *Candida albicans* and *Candida tropicalis* to PACT with chloroaluminium phthalocyanine in nanoemulsion (ClAlPc/NE) in combination with red light were investigated. PS concentration- and fluence-dependent cell survival after illumination was compared, before and after unbound extracellular PS had been washed out. The PS uptake and its subcellular localization were also investigated. Exposure to light in the absence of the PS and treatment with the PS in the absence of light did not kill the fungi. Cells were killed in a fluence-dependent manner. PACT with ClAlPc/NE (0.045 μM and 50 J cm^{-2}) resulted in reductions up to 4 logs in the survival of *C*. *albicans* and *C*. *tropicalis*. Washing the cells to remove unbound PS before light exposure did not avoid fungal photodynamic inactivation, suggesting that cell photosensitization was mainly carried out by cell bound ClAlPc. Internalization of ClAlPc by *C*. *albicans* and *C*. *tropicalis* was confirmed by confocal fluorescence microscopy, and the degree of uptake was dependent on PS concentration.

POS18

Evaluating the efficacy of photodynamic therapy with glioblastoma neurospheres enriched in cancer stem-like cells

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Recent studies have shown that the cancer stem cells identified in glioblastoma tissues (glioblastoma stem cells; GSC) are potent tumor initiators and a source of tumour recurrence. Studies have also shown that GSCs identified by CD133 immunostaining, a biomarker associated with GSCs, exist in various glioblastoma cell lines and CD133+ GSCs are resistant to the conventional treatments. In order to achieve improved treatment for glioblastoma, successful elimination of GSCs, which show multi-potency and self-renewal, is essential. It has been reported that differentiating agents can modulate the GSC subpopulation to become sensitive to conventional treatments. Photodynamic therapy (PDT) is a promising cancer therapeutic strategy that uses a photosensitizer and light. In clinical trials of glioblastoma, fluorescence-guided surgery followed by PDT using a non-fluorescent pro-drug (5-aminolevulinic acid, ALA), which is converted into fluorescent and photodynamic porphyrins (protoporphyrin IX, PpIX), has shown promising clinical results. Prior photodynamic studies have shown that cancer stem cells may resist PDT via transporter efflux of certain photosensitizers. The studies using epithelial cancer cell lines have shown that differentiated cells convert more ALA to PpIX resulting in better PDT efficacy. Given these facts, we hypothesized that the accumulation of photosensitizer and the efficacy of PDT may be differentiation-dependent. In this study, we carried out preliminary experiments with glioblastoma spheroid cultures to evaluate the accumulation of photosensitizer and the treatment response of subsequent PDT in CD133+ and CD133- cells using flow cytometry. We also evaluated agents that affect the population of CD133+ in combination with PDT.

POS19

Autocatalytic-Assisted Photorelease of a Sensitizer Drug Bound to a Silica Support

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The photorelease of a sensitizer from a fluorinated silica surface occurs by a reaction of singlet oxygen with the vinyl ether bond linker with scission of a dioxetane intermediate. Irradiation of the released sensitizer generates singlet oxygen, which accelerates the release of more sensitizer via an autocatalytic reaction. Sigmoidal behavior of sensitizer release in n-butanol and n-octanol occurs at an optimal temperature of 20 °C. The photorelease efficiency was reduced at low temperatures, where the sensitizer was retained on the surface due to a long-lived dioxetane with inefficient scission, and also reduced at high temperatures, due to a slower reaction of $^1O_2$ with the vinyl ether bond. Immediate acceleration is a result of released sensitizer being used as a dopant to eliminate the induction step, further implicating an

autocatalytic mechanism. However, the sigmoidal sensitizer release was not correlated to solvent viscosity, heat, or light from the dioxetane decomposition or to minor O_2 solubility enhancements caused by the fluorinated silica. The mechanistic information collected here can be used to help control the pace of drug release; however, it remains to be seen whether an autocatalytic-based drug delivery system has an advantage to those with non-sigmoidal kinetics.

POS20

Synergism Between Airborne Singlet Oxygen and a Trisubstituted Olefin Sulfonate for the Inactivation of Bacteria

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The reactivity of a trisubstituted alkene surfactant (8-methylnon-7-ene-1 sulfonate, **1**) to airborne singlet oxygen in a solution containing *E. coli* was examined. Surfactant **1** was prepared by a Strecker type reaction 9-bromo-2-methylnon-2-ene with sodium sulfite. Sub-micellar concentrations of **1** were used which reacted with singlet oxygen by an 'ene' reaction to yield two hydroperoxides (7-hydroperoxy-8-methylnon-8-ene-1 sulfonate and (*E*)-8-hydroperoxy-8-methylnon-6-ene-1 sulfonate) in a 4:1 ratio. Exchanging the H_2O solution for D_2O where the lifetime of solution-phase singlet oxygen increases by 20-fold, led to but a ~2-fold increase in yield of the hydroperoxides pointing to surface activity of singlet oxygen with the surfactant in a partially solvated state. In this airborne singlet oxygen reaction, *E. coli* inactivation was monitored in the presence and absence of **1** and by a LIVE/DEAD cell permeabilization assay. It was shown the surfactant has low dark toxicity to the bacteria, but in the presence of airborne singlet oxygen produces a synergistic enhancement of the bacterial inactivation. How the 'ene' derived surfactant hydroperoxides can provoke $^1O_2$ toxicity and be of general utility is discussed.

POS21

Incorporation of an ^{18}O-Label in the Photooxidation of Aromatic Nitrosoamines with Singlet Oxygen ($^{18}[^1O_2]$)

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Despite the intense interest in nitrosamine chemistry and biology, no attempt to investigate O-atom exchange from singlet oxygen has been reported. Using GC/MS and tandem mass spectrometry (HPLC MS/MS), we find evidence that ^{18}O isotopically labeled singlet oxygen ($^{18}[^1O_2]$) reacts with nitrosamines (*N*-methyl-*N*-(*p*-tolyl)nitrous amide and *N*-nitrosodiphenylaniline) and exchanges an ^{16}O for an ^{18}O atom. The oxygen exchange results are consistent with a [2 + 2] of singlet oxygen to the nitrosamine N=O bond with formation of a 4-membered ring trioxazetidine followed by its decay in a retro [2 + 2] process. Density functional theory evidence supports the notion of O-atom exchange via a trioxazetidine intermediate.

POS22

Singlet Oxygen Generation on Porous Superhydrophobic Surfaces: Effect of Gas Flow and Sensitizer Wetting on Trapping Efficiency

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We describe physical-organic studies of singlet oxygen generation and transport into an aqueous solution supported on superhydrophobic surfaces on which silicon-phthalocyanine (Pc) particles are immobilized. Singlet oxygen ($^1O_2$) was trapped by a water-soluble anthracene compound and monitored in-situ using a UV-VIS spectrometer. By flowing oxygen through the porous superhydrophobic surface, singlet oxygen generated in the plastron (i.e. the air layer beneath the liquid) is transported into the solution within gas bubbles, thereby increasing the liquid-gas surface area over which singlet oxygen can be trapped. Significantly higher photooxidation rates were achieved in flowing gas, as compared to when the gas in the plastron was static. Superhydrophobic surfaces were also synthesized so that the Pc particles were located in contact with, or isolated from, the aqueous solution to evaluate the relative effectiveness of singlet oxygen generated in solution and the gas phase respectively; singlet oxygen generated on particles wetted by the solution was trapped more efficiently than singlet oxygen generated in the plastron, even in the presence of flowing oxygen gas. A mechanism is proposed that explains how Pc particle wetting, plastron gas composition and flow rate as well as gas saturation of the aqueous solution affect singlet oxygen trapping efficiency. These stable superhydrophobic surfaces which can physically isolate the photosensitizer particles from the solution

may be of practical importance for delivering singlet oxygen for water purification and medical devices.

POS23

Superhydrophobic Photosensitizers. Mechanistic Studies of $^1O_2$ Generation in the Plastron and Solid/Liquid Droplet Interface

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We describe here a physical-organic study of the first triphasic superhydrophobic sensitizer for photooxidations in water droplets. Control of synthetic parameters enables the mechanistic study of "borderline" two- and three-phase superhydrophobic sensitizer surfaces where $^1O_2$ is generated in compartments that are wetted, partially wetted, or remain dry in the plastron (i.e., air layer beneath the droplet). The superhydrophobic surface is synthesized by partially embedding silicon phthalocyanine (Pc) sensitizing particles to specific locations on polydimethylsiloxane (PDMS) posts printed in a square array (1 mm tall posts on 0.5 mm pitch). In the presence of red light and oxygen, singlet oxygen is formed on the superhydrophobic surface and reacts with 9,10-anthracene dipropionate dianion (**1**) within a freestanding water droplet to produce an endoperoxide in 54-72% yields. Control of the $^1O_2$ chemistry was achieved by the synthesis of superhydrophobic surfaces enriched with Pc particles either at the PDMS end-tips or at PDMS post bases. Much of the $^1O_2$ that reacts with anthracene **1** in the droplets was generated by the sensitizer "wetted" at the Pc particle/water droplet interface and gave the highest endoperoxide yields. About 20% of the $^1O_2$ can be introduced into the droplet from the plastron. The results indicate that the superhydrophobic sensitizer surface offers a unique system to study $^1O_2$ transfer routes where a balance of gas and liquid contributions of $^1O_2$ is tunable within the same superhydrophobic surface.

POS24

Bacterial Inactivation by a Singlet Oxygen Bubbler: Identifying Factors Controlling the Toxicity of $^1O_2$ Bubbles

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A microphotoreactor device was developed to generate bubbles (1.4 mm diam., 90 µL) containing singlet oxygen at levels toxic to bacteria and fungus. As singlet oxygen decays rapidly to triplet oxygen, the bubbles leave behind no waste or byproducts other than O_2. From a comparative study in deaerated, air saturated, and oxygenated solutions, it was reasoned that the singlet oxygen bubbles inactivate *E. coli* and *Aspergillus fumigatus*, mainly by an oxygen gradient inside and outside of the bubble such that singlet oxygen is solvated and diffuses through the aq. soln. until it reacts with the target organism. Thus, singlet oxygen bubble toxicity was inversely proportional to the amt. of dissolved oxygen in solution. In a 2nd mechanism, singlet oxygen interacts directly with E. coli that accumulate at the gas-liquid interface although this mechanism operates at a rate 10 times slower. Due to encapsulation in the gaseous core of the bubble and a 0.98-ms lifetime, the bubbles can traverse relatively long 0.39 mm distances carrying $^1O_2$ far into the solution; by comparison the diffusion distance of $^1O_2$ fully solvated in H_2O is much shorter (150 nm). Bubbles that reached the outer air-water interface contained no $^1O_2$. The mechanism by which $^1O_2$ deactivated organisms was explored through the addn. of detergent mols. and Ca^{2+}. Results indicate that the preferential accumulation of *E. coli* at the air-water interface of the bubble leads to enhanced toxicity of bubbles containing $^1O_2$. The singlet oxygen device offers intriguing possibilities for creating new types of disinfection strategies based on photodynamic ($^1O_2$) bubble carriers.

POS25

Efficacy of Extracorporeal Photopheresis in Systemic Sclerosis is not associated with an Increase in Lung Cancer

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Systemic sclerosis (SSc) is a chronic disease of the connective tissue associated with fibrosis of the skin and internal organs (e.g.: Lung). Extracorporeal photopheresis (ECP) in many cases can improve skin sclerosis in a significant manner. Evidence suggests that SSc is associated with an increased risk of lung cancer. The aim of the present study was to determine the risk of lung carcinoma in ECP-treated SSc-patients. A cohort study with an anonymous retrospective analysis of 71 patients with SSc treated

with ECP between 1991 and 2014 at the Photopheresis Unit of the Department of Dermatology at the Medical University of Vienna, Austria, was performed.

ECP treated patients had a standardized incidence rate (SIR) of 2.34 (95% CI 0.84 to 4.58) for developing lung cancer. Compared to the Austrian general population SSc patients of our cohort had a risk of 10% and thus an enhanced risk to develop lung cancer. All of the lung carcinoma patients had been diagnosed with a non-specific interstitial pneumonia (NSIP) prior to the development of lung carcinomas. Comparison of our data (SIR 2.34) with a recent meta-analysis (SIR 3.18, Onishi et al., 2013) showed that our cohort of ECP-treated SSc patients was not at increased risk for lung cancer. Conclusions: 1) In accordance with previously published studies, patients with SSc have an increased risk of developing lung cancer. 2) NSIP could be a risk factor for the development of lung cancer, since lung cancers appeared only in SSc patients previously diagnosed with a NSIP. 3) ECP does not increase the risk of lung cancer in SSc patients

POS26

Phosphorescence of bilirubin and efficiency of bilirubin-sensitized generation of singlet oxygen

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Unsuccessful attempts to detect phosphorescence of bilirubin and register bilirubin-sensitized generation of singlet oxygen have lasted for over period 40 years. The problem is caused by a very low value of quantum yield to triplet state for bilirubin molecules (φ_{isc}~0.01-0.05). In this work we for the first time, using phosphoroscope, managed to detect phosphorescence of bilirubin in rigid glass media ($\varphi_{ph} \sim 10^{-5}$, $\tau \sim 50$ μs, λ_{max} = 760-775 nm) on the background of intense bilirubin fluorescence (quantum yield of fluorescence $\varphi_{fl} \sim 0{,}40$, τ = 3-4 ns, λ_{max} = 500-520 nm) at liquid nitrogen temperature (77 K) and at laser excitation (semiconductor laser λ = 405 nm or 445 nm) as well as to register position of triplet level of pigment. Phosphorescence spectra of bilirubin were registered in Triton X-100, 2-Methyltetrahydrofuran, Dimethyl sulfoxide, *N,N*-Dimethylformamide; we were unable to register phosphorescence of bilirubin in a complex with human serum albumin (HSA) and bovine serum albumin (BSA) in aqueous solutions.

Also, for the first time we registered (in organic solution and aqueous solution in a complex with HSA and BSA) bilirubin-sensitized luminescence of singlet oxygen in the region of 1270 nm and calculated quantum yield (φ_{Δ}) of its generation at room temperature. The samples were excited with laser pulses with duration of 15 ns and energy ~ 10 μJ, pulse repetition rate of 15 Hz at a wavelength of 355 nm. The efficiency of singlet oxygen generation is characterized by following values: for chloroform - φ_{Δ} = 0.02, bilirubin-HSA complex - φ_{Δ} = 0.01, bilirubin-BSA complex - φ_{Δ} = 0.008. It is shown that bilirubin-sensitized generation of singlet oxygen is realized by mean of transfer of energy of electronic excitation from triplet state of tetrapyrrole.

In conclusion, the processes of photoisomerization of pigment (φ_{is}~0.13) play a determining role in mechanism of photoconversion of bilirubin upon exposure to radiation at its long-wave absorption band whereas the reactions of self-sensitized oxidation are ineffective.

POS27

Effect of Laser Radiation of Red and Near Infrared Spectral Regions on the Zooplankton *Artemia Salina L*.

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At analyzing the mechanisms of photophysical processes determining the regulatory effect of low-intensity laser radiation of the visible spectrum, the presence of photobiological effect is generally ascribed to absorption of radiation by protein macromolecules containing prosthetic groups (hemoglobin, cytochrome c oxidase, superoxide dismutase, catalase, etc.) or to the activation of endogenous photosensitizers by light (especially, photosensitizers of porphyrin nature). It is believed that in the first case exposure to radiation leads to the change of oxygen transport function of hemoglobin and increase of local concentration of oxygen due to its photodissociation from oxyhemoglobin as well as change of activity of enzymatic systems (cytochrome c oxidase, superoxide dismutase, catalase). In the case of determining role of sensitized reactions in mechanism of biological activity of laser radiation the priority is assigned to the processes of change in the permeability of cellular membranes due to reactions of lipid peroxidation. As a test, to check the action of laser radiation, percentage of nauplii hatched from cysts (protective shells) after activation of eggs in salt water in a stable thermal

regime was chosen. The following types of continuous-wave lasers were used for exposure: HeNe laser, λ = 632.8 nm; diode lasers with λ = 808 and 976 nm; diode pumped Nd:YVO_4 lasers (λ = 1064 and 1342 nm) and Nd:YVO_4 laser generating 1064 nm wavelength with Raman conversion (λ = 1176 nm). It was found for the first time the ability of radiation of red spectral region (632,8 nm) as well as near infrared spectral region (800-1340 nm) that is located outside electronic absorption band of main chromophores to have a regulatory effect on biochemical processes, which control the hatching of nauplii of brine shrimp *Artemia salina L*. upon irradiation of its cysts. Among possible acceptors of optical radiation of near infrared spectral region (at least on some of mentioned wavelengths) can be molecular oxygen. Biological activity of laser radiation can be explained by direct triplet-singlet excitation of molecular oxygen dissolved in biological tissues and its subsequent influence, as a signal (trigger) molecule, on physiological processes. Besides, water can be acceptor of radiation because absorption of aqueous solutions of biological molecules is entirely explained by absorption of solvent in region of λ = 1200 – 2500 nm.

POS28

Growth under visible light increases mucilage and conidia production and tolerance to UV-B radiation in the plant-pathogenic fungus *Colletotrichum acutatum*

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Colletotrichum acutatum is an important plant-pathogenic fungus that causes anthracnose in several commercially important fruit crops and postbloom fruit drop of citrus. During the asexual stage of its life cycle on the host plant, *Colletotrichum* produces abundant conidia embedded in a water-soluble extracellular mucilage. Conidia are responsible for fungal dispersion and host infection. Light conditions (i.e. spectral distribution and irradiance) during the fungal growth can influence the development in different ways. Some spectral intervals can induce important and positive physiological and morphological changes, such as conidial production, while others, being deleterious, can kill the conidia reducing the population size and limiting fungal dispersion. We investigated the effects of exposures to visible light during the growth of *C. acutatum* colonies on the mucilage and conidia production and on the UV-B tolerance of the produced conidia. Conidial tolerance to an environmentally realistic UV-B irradiance was determined both in conidia surrounded by mucilage on sporulating colonies and in washed conidia. Exposures to visible light during fungal growth increased mucilage and conidia production and also increased tolerance to UV-B of the conidia. Colonies exposed to light produced approximately 1.7 times more conidia than colonies that grew in the dark and the UV-B tolerances of conidia produced under light were at least two times higher than the tolerance of conidia produced in the dark. Conidia embedded in the mucilage on sporulating colonies were more tolerant to UV-B than the washed conidia. Conidial tolerance to UV-B radiation varied among 5 selected strains. Exposures of 2 h were enough to kill from 50% (strains FDC 52 and FDC 82) to 80% (FDC 03) of the conidia. Exposures of 4 h killed approximately 95% of the conidia of the more tolerant strain (FDC 52) and exposure of 6 h killed 100% of the conidia of all the five strains.

POS29

Electron transfer processes in cytochrome-cytocrome oxidase system studied by laser induced optoacoustic spectroscopy.

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The long-range (10 to 25 Å) charge transfer process in protein complexes is a fundamental process in respiratory and photosynthetic machinery. There are several open questions about the contributions of the environment in these long-range interactions as well as about the influence of the separation between the donor (D) and acceptor (A) partners.

In this work the structural movements in proteins due to charge transfer are studied by laser-induced photoacoustic measurements (LIOAS). The proteins studied are the Cu_A centres of cytochrome C oxidase subunit II [1] and the cytochrome c_{552}.

Deconvolution methods for signal analysis, in combination with an appropriate model were used for the determination of structural volume changes. Three different systems were analyzed: each protein separately and the mixing of the two proteins with their absorbances matched, in phosphate buffer solution. In each case, three well-separated processes were identified: a fast one (prompt) with a decay time $t_1 \leq 10$ ns; a slower process with a lifetime t_2 ca. 200-400 ns; and a longest-lived component with a lifetime longer than 1 μs.

The pre-exponential factors φi of the three components at several temperatures were plotted as El φi vs (cpρ/β). In all cases, good linear correlations were obtained. The slopes of the lines represent the structural volume change associated with each of the processes, in each case multiplied by the respective quantum yield of the process.

POS30

Modeling Heterotypic Communication in Tumor Growth and Treatment Response: The Role of Tumor Endothelial Cells and Stromal Fibroblasts

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The tumor microenvironment plays a critical role in the biological characteristics and response to therapy of metastatic disease. Understanding the therapeutic implications of heterogeneities that result from crosstalk between tumor cells and stromal partners is critical for designing more effective therapy regimens. Stromal partners such as tumor endothelial cells (TEC) and tumor-associated fibroblasts (TAF) are emerging as important biological modulators of many cancers, including ovarian cancer (OvCa) and pancreatic cancer (PanCa). Heterocellular 3D tumor arrays that restore communication with stromal partners may be increasingly important complements to existing systems. However, these models lack the ability to differentiate between cell populations, which may vary in their susceptibility to therapy. Here we present a fluorescent dye-labeling method for extended longitudinal monitoring, and for the first time the quantitative evaluation of differential responsiveness to therapy between tumor and stromal cells in heterocellular 3D tumor arrays.

Fluorescence was monitored using confocal microscopy 24 hours and 7 days post-plating, and toxicity was assessed by MTT Proliferation Assay. OVCAR-5 and HUVEC-C, or MiaPaCa-2 and MRC5 cells were labeled at a concentration of 25μM, the concentration that conferred little to no toxicity while allowing imaging over two weeks, and grown in 3D co-culture arrays. To assess the effect of Vybrant dyes on susceptibility to treatment, OVCAR-5 and HUVEC-C cells were labeled at a concentration of 25μM (5X recommended concentration) in monolayer. The cultures were treated with photodynamic therapy, an emerging light-based modality, at doses ranging from 1 J/cm^2 – 10 J/cm^2 (0.25μM BPD, 150mW/cm^2), or a clinically-relevant chemotherapy cocktail at a fixed ratio of 1:2500 based on published IC_{50} values for each agent (1–10nM Paclitaxel + 2.5–25μM Carboplatin).

These findings are anticipated to be valuable to answer a broad array of questions in drug discovery, including the identification of mechanism-informed targeted and combination therapies. The use of biologically-relevant heterocellular 3D tumor models, such as those presented here, will improve the efficient allocation of valuable time and resources to the most promising candidate agents and modalities.

POS31

Cytometric approach for a rapid evaluation of *Candida albicans* susceptibility to photodynamic antimicrobial chemotherapy with phenotiazinium photosensitizers

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Photodynamic antimicrobial chemotherapy (PACT) is a promising method which combines a nontoxic photosensitizer (PS) and visible light to cause selective killing of microbial cells. We investigated the susceptibilities of *Candida albicans* to PACT with two phenothiazinium derivatives, new methylene blue N (NMBN) and the novel pentacyclic phenothiazinium photosensitizer S137 in combination with red light. The effectiveness of each PACT was determined based on cell survival. Additionally, we evaluated a cytometric protocol using propidium iodide (PI) to determine the susceptibility of *Candida* to PACT. PI is a probe often used to stain non-viable cells. Dead or dying cells with injured membranes can incorporate PI. Light exposures alone (5 to 25 J cm^{-2}) and treatment with the PSs (2.5 μM) in the absence of light did not kill *C. albicans*. PACT both with NMBN and S137 killed the cells in a fluence-dependent manner. PACT with NMBN and S137 resulted in a reduction in the survival of the cells from 0.97 log (5 J cm^{-2}) to 5.12 logs (25 J cm^{-2}) and from 3.65 logs (5 J cm^{-2}) to 3.87 logs (25 J cm^{-2}), respectively. Treatment only with NMBN and S137 resulted in PI staining of 8 and 21% of the cells, respectively. PACT with NMBN stained the cells in a fluence-dependent manner from 30% (5 J cm^{-2}) to 90 % (25 J cm^{-2}). PACT with S137 resulted in PI staining of the cells from 90% (5 J cm^{-2}) to 99% (15 and 25 J cm^{-2}). The use of PI and flow cytometry appears to be a good, fast and reliable alternative to the classical survival-based method for determining the susceptibility of *Candida* to PACT with phenotiazinium photosensitizers.

POS32

"Pointsource" Delivery of a Photosensitizer Drug and Singlet Oxygen: Eradication of Glioma Cells in Vitro

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We describe a pointsource sensitizer-tipped micro-optic device for the eradication of glioma U87 cells. The device has a mesoporous fluorinated silica tip which emits singlet oxygen molecules and small quantities of pheophorbide sensitizer for additional production of singlet oxygen in the immediate vicinity. The results show that the device surges in sensitizer release and photokilling with higher rates about midway through the reaction. This was attributed to a self-amplified autocatalytic reaction where released sensitizer in the extracellular matrix provides positive feedback to assist in the release of additional sensitizer. The photokilling of the glioma cells was analysed by global toxicity and live/dead assays, where a killing radius around the tip with ~0.3 mm precision was achieved. The implication of these results for a new PDT tool of hard-to-resect tumors, e.g. in the brain, is discussed.

POS33

Rapid Optical Determination of Beta-Lactamase based Antibiotic Susceptibility

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The absence of rapid tests evaluating antibiotic susceptibility results in the empirical prescription of antibiotics for infections. This can lead to treatment failures due to escalating antibiotic resistance, and also furthers the emergence of drug-resistant bacteria. There is thus a critical need for rapid methodologies that can provide information on antibiotic susceptibility and/or bacterial resistance.

This study reports a rapid optical method to detect beta-lactamase and thereby assess activity of beta-lactam antibiotics, which could provide an approach for targeted prescription of antibiotics. Beta-lactamase enzymes produced by bacteria are a major antibiotic resistance mechanism against the widely used beta-lactam antibiotics. The methodology is centred on a fluorescence quenching based probe (Beta-LEAF – Beta-Lactamase Enzyme Activated Fluorophore) that mimics the structure of beta-lactam antibiotics. Beta-LEAF is designed such that fluorescence remains quenched until the probe is cleaved enzymatically by beta-lactamase, which allows for lactamase detection. Antibiotic susceptibility is analysed by virtue of competition between the probe and tested beta-lactam antibiotic for the lactamase.

The beta-LEAF assay was performed for rapid determination of beta-lactamase production and activity of tested β-lactam antibiotics in this context (beta-lactamase based antibiotic activity) on a panel of *Staphylococcus aureus* ATCC strains and clinical isolates, with cefazolin as a test antibiotic. Four of the clinical isolates were determined to be lactamase producers, with the capacity to inactivate cefazolin, out of the twenty-five isolates tested. These results were compared against gold standard methods, nitrocefin disk test for beta-lactamase detection and disk diffusion for antibiotic susceptibility, showing results to be largely consistent. Furthermore, in the sub-set of beta-lactamase producers, it was demonstrated and validated that multiple antibiotics) could be assessed simultaneously to predict the antibiotic that would be most active for a given bacterial isolate.

The study establishes a rapid assay for beta-lactamase detection and prediction of antibiotic activity using *S. aureus* clinical isolates. Although the focus in the current study is beta-lactamase-based resistance, the overall approach represents a broad diagnostic platform. In the long-term, these studies form the basis for the development of assays utilizing a broader variety of targets, pathogens and drugs.

POS35

50th Anniversary of the Foote/Wexler Discovery: A Milestone for Singlet Oxygen Research

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This year (2014) marks the 50th anniversary of the discovery of the role of singlet oxygen in photosensitized oxidation reactions by Christopher Foote and Sol Wexler at UCLA. Foote and Wexler reported that the oxidation of organic molecules by sodium hypochlorite and hydrogen peroxide yielded near identical product distributions from those generated independently in dye-sensitized photooxidations. This led to their suggestion for the intermediacy of singlet oxygen in photosensitized oxidations, which is now widely accepted and

constitutes an important milestone in the history of photooxidation chemistry. Up to that point, a sensitizer-oxygen complex (moloxide) was generally assumed. This talk will provide an overview of the Foote/Wexler work, how it set the stage for rapid developments in photosensitized processes, and describe how the field continues to flourish, including singlet oxygen as a critical species in photodynamic therapy (PDT).

POS36

The Effects of Modified Fibronectin on ARPE-19 Cells as Model Systems for Ageing and Inflammation in Human Bruch's Membrane

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Age-related macular degeneration (AMD) is the leading cause of blindness in the Western world. This ocular disorder is characterized by the degeneration of photoreceptors as a result of damage to retinal pigment epithelium (RPE) and Bruch's membrane (BM). The underlying cause of AMD is not completely understood as the disorder is multifactorial. The risk factors for AMD include age and genetics as well as environmental components such as smoking. Therefore, in this study, we have investigated the effects of modified fibronectin on RPE cells in order to gain insight into the development of AMD. Fibronectin (FN) has the RGD amino acid sequence that binds to the α5β1 integrin region of the RPE cells. This protein was modified by blue light mediated A2E damage, non-enzymatic glycation, and non-enzymatic nitration which served as model systems for ageing and inflammation. In order to determine the effects of modified FN on ARPE-19 cell attachment, the cells were seeded onto the modified FN and the MTT assay was used to assess the cell viability. Furthermore, the cell death mechanism of ARPE-19 cells was determined 24 hours after the cells were seeded onto modified fibronectin and exposed to UV irradiation with flow cytometry. In this study, we determined that modified FN, especially glycated and nitrated FN, had the greatest effect on ARPE-19 cell attachment. Twenty-four hours after the ARPE-19 cells were exposed to modified FN and UV irradiation, necrosis was the major cause of cell death. Altogether, these data suggest that the alteration of BM structure leads to RPE damage. This may provide an explanation for the loss of photoreceptors and development of AMD.

POS37

Transfersomal Chlorophyllin Derivatives: A Novel Model in the Photodynamic Treatment of Malignant Brain Tumors

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Glioblastomas are the third most common cause of cancer deaths in patients of different ages. It is well documented that the application of photodynamic therapy on malignant gliomas offers more selectivity compared to chemotherapy, radiotherapy and surgery. Chlorophyllin (CHL) derivatives are natural products extracted from green plants and exhibit several advantages: they are endorsed by the Food and Drug Administration (FDA) as food additives, highly effective with low cost and show the highest levels of human safety.

In this work the efficacy of the photosensitizer (CHL) is evaluated against human glioblastoma cell lines in a free form as well as in transfersomal nanoformulations. Transfersomes are lipid nanovesicles reported to show high elasticity and flexibility so that they can penetrate through pores even smaller than their sizes. CHL was loaded in transfersomes composed of phosphatidylcholine added to span or sodium deoxycholate surfactants (with lipid: surfactant ratios of 5:1, 10:1, and 20:1 w/w). Transfersomes containing surfactants sodium deoxycholate in ratio 20:1 and span in ratio 5:1 were chosen due to their high encapsulation efficiencies of 70.16 and 92.6% respectively, with average particle sizes ranging between 70-90nm. Different CHL concentrations (from 13 to 138μM) were applied to U373 glioblastoma cell lines, which were then irradiated with light doses ranging from 4.6 to 168 J/cm^2.Significant photocytotoxicity was observed with both free and transfersomal CHL, however more pronounced in the transfersomal system. Annexin/propodium iodide fluorescence staining indicated the predominance of apoptotic cell death.

In conclusion, CHL proved to be effective in the photodynamic treatment of glioblastoma, and its incorporation into transfersomal nanovesicles enhances its penetration and efficacy towards the malignant cells.

POS38

Compositional Studies of Human Retinal Lipofuscin: Wet versus Dry Age Related Macular Degeneration

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Age related macular degeneration (AMD) is a common retinal disorder found in the elderly and is the leading cause of blindness in the Western world. The excess

buildup of lipofuscin in the retinal pigment epithelium (RPE) is considered to be a major risk factor for AMD. This study aims to further elucidate the chemical composition of human retinal lipofuscin including the investigation of fluorophores and photooxidative byproducts in order to better understand AMD. Human retinal lipofuscin is isolated from human donor eyes diagnosed with either wet or dry AMD according to the method previously described by Feeney-Burns. The organic soluble fraction of lipofuscin is collected, dried, and reconstituted using methanol for use in high performance liquid chromatography tandem mass spectrometry (LC/MS) coupled with a photo diode array and fluorescence detectors. Total ion current chromatograms observed from LC/MS analysis suggests unique chemical composition for tissue diagnosed as wet versus dry AMD. These data support the hypothesis that wet and dry AMD are two distinct diseases. The extensively studied fluorophore A2E and its photooxidation products were observed in lipofuscin extracts diagnosed as wet AMD but not from tissue diagnosed as dry AMD further postulating the two are different diseases. Understanding the chemical composition and fluorophores found in these samples can aid in furthering the treatment, diagnosis and prevention of wet and dry AMD.

POS39

Photoacoustic monitoring of photosensitizer photobleaching rate to predict photodynamic therapy response

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In photodynamic therapy (PDT), the photosensitizer (PS) concentration at the treatment site plays a major role in determining PDT outcome. More commonly the PS photobleached due to PDT is assessed using fluorescence imaging and is used as a treatment predictor. Fluorescence images do not provide the complete 3D image of the heterogeneous PS accumulation. In this study we utilize photoacoustic imaging (PAI), a non-invasive and non-ionizing technique to provide 3-D tumoral PS biodistribution and monitor PS photobleaching rate during PDT. Specifically U87 glioblastoma cells were implanted subcutaneously on the flank of 4-6 weeks old nude mice. 10 days post implantation, Benzoporphyrin derivative (BPD) in a liposomal formulation is injected via tail vein at a concentration of 0.5 mg/kg of mouse body weight. The mouse was placed in a custom-built laboratory setup to simultaneously perform PDT at 690 nm laser illumination and PAI (using VisualSonics Vevo LAZR imaging system). Our results from this pilot study demonstrated PAI has the ability to monitor the heterogeneous PS uptake and PS photobleaching rate. This information can then be utilized to personalize PDT parameters, such as adjustment of light dose or administration of additional PS for robust and predictable therapeutic outcome.

POS40

Kinetics of photosynthetic response to ultraviolet and visible light in *Synechococcus* WH8102 (CYANOBACTERIA)

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The picoplanktonic cyanobacteria, *Synechococcus* spp., (Nägeli) are important contributors to global ocean primary production that can be stressed by solar radiation, both in the photosynthetically active (PAR) and ultraviolet (UV) range. To better understand how this affects marine primary productivity, we studied the responses of PSII quantum yield (active fluorescence), carbon fixation (^{14}C assimilation) and oxygen evolution (membrane inlet mass spectrometry) in *Synechococcus* WH8102 under moderate UV and PAR. PSII quantum yield decreased during exposure to moderate UV and UV+PAR, with response to the latter being faster (6.4 *versus* 2.8 min, respectively). Repair processes were also faster when UV+PAR exposure was followed by moderate PAR (1.68 min response time) than when UV was followed by very low PAR (10.5 min response time). For the UV+PAR treatment, the initial decrease in quantum yield was followed by a 50% increase (“rebound”) after 7 min exposure, showing an apparent photoprotection induction. When exposed to increasing levels of PAR, PSII activity continued to increase even when CO_2 fixation was light-saturated (saturation parameter of CO_2 fixation was 123 µmol photons m^2 s^{-1} compared to 147 and 205 µmol photons m^2 s^{-1} for net and gross oxygen evolution). Oxygen uptake increased as a function of PAR as well, suggesting that this oxygen-dependent mechanism may be acting as a photoprotective electron sink. However, oxygen uptake did not change under UV, suggesting that this mechanism is not an important strategy of photoprotection for *Synechococcus* WH8102 against UV. We used propyl gallate, an antioxidant, to test for plastid terminal oxidase (ptox) or ptox-like enzymes activity, but it caused nonspecific and toxic effects on *Synechococcus* WH8102.

POS41

Treating pancreatic cancer with Nano-PDT and liposomal irinotecan

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Late diagnosis and significant systemic toxicities from standard chemotherapies have maintained the grim statistics for pancreatic cancer (PanCa). Several innovative combinations (*i.e*. hedgehog inhibitors plus gemcitabine) have been evaluated in clinic, but most have failed due to their ineffectiveness. Photodynamic therapy (PDT) and liposomal irinotecan chemotherapy are already promising PanCa treatment modalities in Phase I and III clinical trails, respectively. Recognizing the clinical challenges, the genetic complexity, and the crosstalk between various survival signalling pathways in PanCa, this study leverages nanotechnology to combine PDT and irinotecan (referred to as photochemotherapy), allowing for dose reduction, non-overlapping side effects and enhanced treatment outcome. The mechanistic interactions of the two fundamentally different treatment modalities were investigated from macroscopic to microscopic scale. Specifically, our results suggested that irinotecan aids in reducing tumour hypoxia to a PDT-favourable condition, PDT destroys efflux transporters increasing the intracellular irinotecan concentration, and PDT blocks the expression of irinotecan-induced survivin. In this study, using orthotopic PanCa xenograft models, we performed photochemotherapy using both lab-made and clinical liposomal formulations of irinotecan to dramatically control tumour growth without systemic toxicity. We are currently facilitating the combinations with single-agent NCs for rapid clinical translation, and on the other hand, advancing multi-agents NCs for a forward-looking, targeted combination strategy.

POS42

Ultraviolet B Sensitivity of BALB/c 3T3 Cells. Increased UVB Exposure Can Be Used in the 3T3 Neutral Red Uptake Phototoxicity Test for the Evaluation of UVB-absorbing Test Materials

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The OECD guideline for the testing of chemicals, In vitro 3T3 NRU phototoxicity test, No. 432 defines the UVA (320-400 nm) dose for evaluation of phototoxic potential of test materials as 5 J/cm^2. The dose of UVB (290-320 nm) (if any) to be used in the assay is not defined in this guidance. The guidance and the literature indicates that UVB is cytotoxic to 3T3 cells and thus the conventional wisdom has grown that UVB is to be minimized in or excluded to ensure a valid assay. This minimization of the UVB component is a concern when a test material absorbs either entirely or primarily in the UVB portion of the spectrum, as the ICH S10 Guidance on Photosafety recommends the 3T3 Assay as the first assay to be used for evaluation of phototoxic potential of a test material, and a negative result in this assay indicates that further photosafety testing is not warranted. Should the assay not be relevant for evaluation of UVB absorbing test materials, an *in vivo* preclinical model or clinical evaluation would be necessary for this evaluation. In this laboratory, the spectrum of the xenon arc solar simulator equipped with a 1 mm WG 320 filter used for the 3T3 assay delivers ~20 mJ/cm^2 of UVB through the plate lid along with the 5 J/cm^2 of UVA, with percent cell survival (~91%) and OD_{540} (~0.675) values well above the minimum criteria set by the OECD 432 Guidance (80% and 0.400, respectively). To address the actual sensitivity of the cells to increased UVB exposure while delivering the same UVA dose, the lid was removed from the tissue culture plate during irradiation, increasing the UVB dose to ~30 mJ/cm^2. This increased UVB dose resulted in the reduction of the % cell sensitivity and OD_{540} values to ~84% and ~0.655, well above the Guidance's minimal recommendations. Thus, the assay can be modified to increase the UVB dose without loss in cell survival that would cause the assay to fall outside of these criteria and jeopardizing the assay's validity. The positive control articles chlorpromazine and promethazine also perform as expected, allowing for a valid assay to be performed using an increased UVB dose. These results indicate that the increased UVB dose is acceptable in the 3T3 NRU phototoxicity test for evaluation of the phototoxic potential of those test materials that absorb primarily or entirely in the UVB portion of the spectrum.

POS43

Detection of singlet oxygen using photomultiplier-tube to evaluate photodynamic therapy

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This study was to investigate the feasibility for the newly developed Photomultiplier-tube (PMT)-based singlet oxygen detection (SOD) system, which uses a monolithic fiber optic cable to direct the diode laser to the target tissues or cells and collects the $^1O_2$ emission. We measured the production of $^1O_2$ with PS in the four cancer cell lines. Produced $^1O_2$ level was

measured with the PMT-based SOD system and the NaN_3 quenching experiments. $^1O_2$ photon counts were compared with the fluorescence intensity at a variety of PS concentrations. The association between the production of $^1O_2$ and the tumor cell killing, cell viability was determined by MTT assay. The standard curve was drawn using $^1O_2$ photon count and FL-meter values. Lifetime and photon count of $^1O_2$ was decreased as NaN_3 concentration was increased. MTT assay, performed in a 96 well plate, showed a relationship between the two results, indicating the PMT-SOD system could accurately measure $^1O_2$ production even in a small number of cells. The PMT-SOD system detected the $^1O_2$ production directly and could detect the $^1O_2$ production in small cell numbers, which enabled simultaneous analysis of various cell lines or diverse PSs.

POS44

ATP-binding cassette sub family G member 2 inhibition effect on photodynamic therapy efficacy in colon cancer

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The ATP-binding cassette sub family G member 2 (ABCG2) is an ATP-binding cassette transporter protein which has a role in the regulation of endogenous protoporphyrin levels. It is not well known whether the efficacy of photodynamic therapy (PDT) is dependent on the expression level of ABCG2 protein in colon cancers. The aim of our study was to examine the role of endogenous ABCG2 expression in porphyrin-based PDT in the colon cancer cells. We checked the ABCG2 expression level in five colorectal cancer cell lines. In the tested cells, SW480 and HT29 cells were selected for further experiments, as they showed the lowest and highest ABCG2 expression levels, respectively. Cells were incubated with PPa in the presence or absence of the ABCG2 activity inhibitor,Ko-143. They were exposed to a diod laser emitting at 670 nm wave length with total radiation dose of 4 J/cm2. SW480 cells, which expressed lower level of ABCG2, showed the higher uptake of PPa than HT-29 cells. The uptake level of PPa was significantly correlated with the decreased cell viability after PDT. Pretreatment with Ko-143 significantly enhanced the PDT efficacy in HT29 cells. To confirm the ABCG2 effect on PDT, we established ABCG2 over-expressing stable cells in SW480 cells (SW480/ABCG2), which showed the lower uptake level of photosensitizer than the control cells. Furthermore, SW480/ABCG2 cells showed significantly decreased PDT effect compared to the control cells The increased or decreased cell survival was significantly correlated with the production level of singlet oxygen after PDT. These results indicate that ABCG2 expression can be an important protein determining the PDT efficacy deriving from photosensitizer efflux in colon cancers.

CONCLUSION: These results indicate that ABCG2 expression can be an important protein determining the PDT efficacy deriving from photosensitizer efflux in colon cancers.

POS45

Photoluminescent Metal Complex Probes: A Tale of Metals, Light and Time

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Metal complexes present unusual properties such as red emission, large Stokes shifts, and long lifetimes. These properties are highly desired in the design and synthesis of probes for different applications. Our group uses time-resolved techniques in combination with photoluminescent metal complexes to overcome challenges commonly found for conventional fluorophores. First, the use of time-gating will be demonstrated as an effective method to improve the performance of photoluminescent probes to detect specific copies of DNA. Time-resolved spectroscopy will then be used in combination with long-lived iridium complexes to illustrate how amino acids such as cysteine and histidine can be detected in complex autofluorescent environments. Similar principles will be presented in the detection of amyloid-β aggregation (a peptide associated with the onset of Alzheimer's disease) by ruthenium dipyridophenazine complexes. Finally, a combination of time-resolved and steady-state techniques will be used to identify solvent vapors employing a rhenium metal complex entrapped within the cavities of a faujasite zeolite.

POS46

Dose Construction Parameters for Photodynamic Targeting of Multifocal Nodules in a 3D Tumor Model

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Photodynamic targeting of complex disease sites requires optimization of dosimetric parameters to maximize efficacy. A range of preclinical tools are required to identify the dose construction variables that provide the most significant and durable response to photodynamic therapy (PDT). Here a three-dimensional (3D) platform for ovarian cancer (OvCa) is used to assess the impact of modulating key PDT dose parameters (photosensitzer x fluence) on tumoricidal durability. This study builds on efforts by us and others to improve the predictability of outcomes from PDT through a better understanding of the biological and dosimetric factors that contribute to variability in treatment response. The viability and size of residual disease is longitudinally evaluated following PDT with three concentrations of benzoporphryin-derivative monoacid-A (BPD-MA) (0.25μM, 1 μM, and 10 μM) to construct three fixed PDT doses (1.25 μM x J/cm^2, 5 μM x J/cm^2, and 10 μM *J/cm^2). The data demonstrate that for all PDT doses, 0.25 μM and 1 μM BPD-PDT produce the most significant and durable cytotoxic response, and smallest residual disease. For all doses, 10 μM BPD-PDT is the least effective at reducing viability of 3D OvCa nodules. Significantly more photobleaching is observed in nodules treated with 0.25 μM BPD-PDT compared to higher BPD concentrations. There is no correlation between activated caspase-3 levels and PDT dose for 0.25 μM and 1 μM BPD-PDT, suggesting complex death and recovery pathways. The present findings demonstrate that the kinetics of tumor destruction and regrowth are significantly impacted by the parameters used to construct a given PDT dose. The optimal parameters for biomedical applications are dependent on the photosensitizer and target disease and should be evaluated as a complement to traditional dose escalation studies. This approach will inform rational combinations and focused in vivo validation studies with the goal of establishing more effective photochemistry-based targeting strategies.

POS47

Impact of Physical Forces on 3D Ovarian Cancer Biology: Targeting **Epithelial-Mesenchymal Transition, Cellular Heterogeneity and Biomarker Modulation Induced by Flow**

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The objective of this study is to determine if hydrodynamic physical forces alter the genetic, molecular and morphological characteristics of ovarian cancer (OvCa) metastases to develop targeted photodynamic therapy (PDT)-based combinations informed by understudied physical and stromal cues. It is becoming increasingly evident that metastatic OvCa is not a monolithic disease. OvCa disseminates as single cells and tumor aggregates along ascitic currents and communicate with the local microenvironment to initiate metastatic peritoneal implants. It is not clear what impact ascitic currents have on the biology of OvCa metastases. The motivation for this study stems from clinical observations that the most stubborn tumors are often found in regions such as the peritoneal gutter, a common site of resistance and recurrence, and also a region that is subjected to fluidic stress from ascites. A new bioengineered system that integrates microfluidics and 3D tumor growth was developed to study hydrodynamic stress as a physical determinant of biological diversity in metastatic OvCa. OVCAR-5 human OvCa cells were introduced into microfluidic channels coated with growth factor reduced Matrigel. Adherent OvCa cells were successfully cultured under controlled and continuous laminar flow for 7 days and formed 3D tumors. Changes in the morphological, genetic, and protein profiles of biomarkers associated with aggressive disease were evaluated. A flow-induced increase in epithelial-mesenchymal transition (EMT) was observed in 3D nodules cultured under flow. A transcriptionally-regulated significant decrease in E-cadherin, a significant increase in vimentin, and significant increase in spindle-like morphology based on a custom image analysis framework was observed. A concomitant significant post-translational upregulation of epidermal growth factor receptor expression and activation was seen under flow. Future studies will evaluate the molecular characteristics of patient samples to establish the clinical relevance of these findings, and will integrate heterotypic partners to enhance the biological relevance of the preclinical model. The impact of this work will be to create a new treatment planning framework that accounts for regional, flow-induced molecular changes to deliver targeted PDT-based combinations that complement conventional regimens.

POS48

Classification of Neocortical Neurons

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Determining the circuitry of the neocortex requires an understanding of its components, making a classification of neocortical neurons necessary. The neocortex consists primarily of excitatory pyramidal neurons (~80% of neocortical neurons) and inhibitory interneurons (~20% of neocortical neurons). Though neocortical interneurons are ideally positioned to control circuit dynamics, they remain poorly understood. GABergic interneurons, in particular, largely contribute to the vast morphological and physiological variability of the cortex. A neuronal classification system is essential to organize such information and the knowledge that is derived from it. To better understand the diversity of neocortical neurons, we have used unsupervised learning methods to create classification schemes. First, we used PCA followed by k-means cluster analysis to create classify data based on detailed anatomical and electrophysiological characterizations of 59 GFP-positive interneurons from a somatostatin-positive mouse line. Each neuron was characterized by whole-cell recordings done by patch-clamping and complete 3D anatomical reconstructions. Cluster analysis revealed 3 groups of cells: one comprised of Martinotti cells, the other two composed of short asymmetric axons targeting layers 2/3. Subsequently, we expanded the data set to include pyramidal neurons in addition to known interneuron subtypes. To perform a quantitative classification of this diverse set of 337 neocortical neurons, we used affinity propagation. Affinity propagation is an exemplar-based method of cluster analysis that takes a similarity measure of data points as input. It outputs a set of data points representative of the data (*exemplars*) and assigns all non-exemplar points to one of the exemplars, thus partitioning the data set into unique clusters.

POS50

Elicitation of tumour-free long-term survival and long-lasting antitumor memory with novel non-immunosuppressive near-infrared PDT.

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TLD151982916, a novel small (< 2 kDa), water-soluble photosensitizer (PS) derived from the family of tunable, Os (II) and thiophene-based coordination complexes was investigated. The PS has absorption from 400 to 900 nm, is highly photostable and generates singlet oxygen ($^1O_2$) with a quantum efficiency ($\Phi(\Delta)$ ~0.05 at 808 nm. The phototoxic effects was quantified *in vitro*, in colon (CT26 WT, CT26 CL25) and glioma cell lines (F98, and U87) following incubation of 5 hrs prior to irradiation at concentrations up to 200 mM. Dark toxicity was negligible at concentrations <100 μM. Cells were irradiated using a Red LED (l =635 +- 25 nm, 90 J cm^{-2}, 125 $mWcm^{-2}$) or a NIR diode laser (l =808 +- 25 nm, 600 J cm^{-2}, 120 $mWcm^{-2}$). For U87cells the resulting LD50 was 0.0286 mM for the Red LED whereas it was 0.0324 mM for a NIR Light, so the mechanisms of the PS cytotoxicity is not fully understood. Photothermal effect do not play a role in the biologic effects or the therapeutic mechanisms of action of TLD151982916. The ability of the PS to initiate photochemical reactions was tested by exposing TLD151982916 treated U87 cells to the red LED source under hypoxic conditions (pO2<0.5%), resulting in a loss of the PDT effect. Experiments with hydroxyl radical and singlet oxygen scavengers indicate ROS mediate cytotoxicity and the low singlet oxygen quantum yield suggests that hydroxyl radicals may be involved in the TLD151982916 mediated phototoxicity. *In vivo* growth delay studies in the subcutaneous colon adenocarcinoma CT26CL25 murine model were performed at PS doses equal to ½; ¼; $^1/_6$ MTD50, administered intratumourly followed 4 hours later by NIR illumination at 300 $mWcm^{-2}$. All CT26CL25tumours showed a complete response which was maintained in 70% of the animals over 12 months. The tumour response was directly proportional to the PS dose and radiant exposure, demonstrating PDT activity in the NIR. An important observation made during this study was that NIR PDT results in 100% protection agenst rechallenge with CT26CL25 cancer cells, reingected 20 days after the initial treatment. Our findings demonstrate that NIR PDT leads not only to longstanding clearance of CT26CL25 tumors, but olso provides a longlasting protection against further tumour cell challenge in young (8-10 weeks) and aged (12-14 months) mice.

POS51

Reversing the cancerous glycolytic phenotype with dichloroacetate in vitro and its effects on photodynamic therapy.

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The Warburg Effect is a phenomenon observed in many cancers, including glioblastoma multiforme (GBM). Cells governed by this effect demonstrate a glycolytic phenotype coupled with a suppression of mitochondrial activity. Dichloroacetate (DCA) is a metabolic shunt drug that reverses this effect and increases pyruvate flux into the mitochondria.

GBM is the most common and regrettably the most aggressive primary brain tumor. Even with the selectivity achieved through photodynamic therapy (PDT) (owing to the preferential uptake of photosensitizer prodrug by tumor cells), PDT as a monotherapy has had limited success in substantially improving the prognosis for patients with deep-seated, highly invasive tumors such as GBM. Our group has investigated the relationship between the metabolic activity of GBM cells *in vitro* and the heme biosynthesis pathway to determine if DCA is a useful agent to improve the outcome of PDT treatment.

Western blotting demonstrated a decrease in the phosphorylation of pyruvate dehydrogenase with DCA treatment, establishing a plausible mechanism for the activity of DCA. DCA was found to decrease lactate production and increase oxygen consumption in a cell line specific manor.

POS52

Altered expression of PKCs leads to different response of human glioma cells (U87 MG) on photo-activated hypericin and switch apoptosis to necrosis

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We have previously shown that in human glioma cells (U87 MG) hypericin (Hyp) is mostly localized in endoplasmic reticulum and partially in mitochondria, lysosomes and Golgi apparatus. Photo-activation of Hyp affects mitochondrial function and induces apoptosis via mitochondrial apoptotic pathway. We have also described an interaction between Hyp and PKC and an activation and trans-localization of the enzyme after intracellular Hyp photo-activation.

Since PKCα is considered as “anti-apoptotic” and PKCδ as “pro-apoptotic”, we focus in the present study on modulation of their activities and related influence on cell survival and cell death induced by photo-activated Hyp.

Small interfering RNA (siRNA) was used to post-transcriptional silencing of *pkcα* and *pkcδ* gene expression. U87 MG cells were pre-incubated with siRNA prior to addition of Hyp and its photo-activation. Cell survival, type of the cell death, mitochondrial membrane potential depolarization ($\Delta\Psi_m$) and reactive oxygen species (ROS) generation were assessed by flow cytometry. Sub-cellular distribution of Bcl-2 family protein members and PKCs were monitored by confocal fluorescence microscopy and verified by Western blotting.

Post-transcriptional silencing of *pkcδ* gene expression (PKCδ-) does not significantly affect observed parameters in comparison with the effect of *pkcα* gene silencing. Post-transcriptional silencing of *pkcα* gene expression (PKCα-) affects cell death pathways after Hyp photo-activation. Our results show that ROS production is significantly increased in PKCα- cells treated with photo-activated Hyp which consequently leads to necrosis.

PKCα, as Bcl-2 kinase, supports stabilization of Bcl-2 in membranes and its antioxidant function and indirectly protects mitochondria/cells against oxidative stress and subsequent cell death.

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POS53

Kinetics of incorporation/redistribution of photosensitizer hypericin to/from high - density lipoproteins

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By means of fluorescence spectroscopy we have studied the kinetics of incorporation of hypericin (Hyp), a natural potent photosensitizing pigment, into high-density lipoproteins (HDL) and transfer of Hyp molecules between HDL particles. Hyp is incorporated into HDL molecule as monomer till ratio Hyp/HDL~8:1. At higher ratio (Hyp/HDL>8:1) Hyp forms non-fluorescent aggregates in these particles. Biphasic kinetics of Hyp association with HDL was observed when solutions of Hyp and HDL were mixed together at various concentration ratios. The rapid phase of Hyp incorporation is completed within seconds, while the slow one lasts several tens of minutes. This suggests that the process of Hyp interaction with HDL is non-trivial and the existence of various types of binding sites for Hyp in HDL is proposed. The kinetics of the incorporation of Hyp into HDL particles pre-loaded with Hyp (Hyp/HDL=12:1) was also investigated. The observed decrease of Hyp fluorescence is sign of the formation of aggregates as well as of the dynamic quenching of singlet excitation state of Hyp molecules inside HDL. The characteristic time for this process is comparable with the time of the slow phase of the Hyp

incorporation into LDL particles. To study the kinetics of the transfer of Hyp molecules between HDL particles, the time dependence of the fluorescence and absorbance of Hyp was followed after the mixing of the complex Hyp/HDL= 70:1 with appropriate amounts of free HDL. For each final Hyp/LDL ratio the increase of the fluorescence intensity of Hyp was observed. The half-time of this process is similar to that one of the slow phase of Hyp incorporation into HDL. All these experiments show that one phase of the incorporation of Hyp into HDL is a relatively slow process and this fact should be considered when Hyp is administered into a body. Differences between characteristics of Hyp association with HDL and low-density lipoproteins (LDL) are also discussed.

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POS54

Role of a Helix B Lysine Residue in the Photoactive Site in Channelrhodopsins

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Channelrhodopsins (ChRs) are light-gated cation channels used by algae as phototaxis receptors. They have helped revolutionize neural circuitry research by their use as genetically targeted tools enabling light-controlled neuron firing in a new technology called optogenetics. Understanding the mechanism of function of ChRs and residue determinants of their properties, such as color-tuning and channel kinetics, is needed to tailor ChRs for various optogenetic research and potential therapeutic applications. One approach is to study the diversity of their properties in homologs cloned from different algae. In nearly all microbial rhodopsins two conserved carboxylic acid residues and an arginine form a complex counterion to the protonated retinylidene Schiff base in the photoactive site, and neutralization of either of the negatively charged carboxylic acids causes a red shift of the absorption spectrum. In contrast, we found that the corresponding neutralizing mutations in some ChRs result in blue shifts. We also noted that they lack a particular lysine residue in the second helix that is unique to ChRs and is conserved in 8 of the 14 published sequences (Lys132, numbering from one of the first-found, *Cr*ChR1). To test the hypothesis that Lys132 plays a role in color tuning in ChRs, we measured the action spectra of photoinduced channel currents in HEK293 cells and absorption spectra of the pigments expressed and purified from yeast *Pichia pastoris*. We found that Lys132 controls the direction of spectral shifts of the mutants: red shifts occur when this lysine is present, whether naturally or by mutagenesis, and blue shifts occur when it is absent. Mutation of Lys132 itself also causes spectral shifts. Neutral substitution of Lys132 caused red spectral shifts in ChRs that contain it, whereas its introduction in a ChR that lacks it from *Chlamydomonas augustae* (*Ca*ChR1) caused a blue shift. Titration of the purified *Ca*ChR1 support a model in which Lys132 modulates the pKa values of the two carboxylic acid residues in the photoactive site, whose effective charges are a key factor in microbial rhodopsin spectral tuning. Additionally, a practical result of the study is that neutralization of Lys132 leads to longer wavelength absorption and faster channel kinetics, which are both desirable for optogenetics applications.

POS55

Light Mediated Toxic Effect of ZN Phthalocyanines on Hela Cells: A Comparison Using DPPC Liposomes and BSA as Delivery System

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Phthalocyanines have been proposed as photosensitizers in photodynamic therapy (PDT) for the treatment of various infectious diseases and cancer. However, the tendency of those compounds to form aggregates in solution, which reduces their efficiency as photosensitizers, presents a shortcoming for the clinical application of those compounds. Thus, the usage of nano- carriers (vesicles or bio-macromolecules) which are loaded with the phthalocyanine is required.

In a previous work we have demonstrated that phthalocyanine incorporation into DPPC liposomes decreases its aggregation, as revealed from absorption spectra, triplet quantum yield, and singlet oxygen quantum yield measurements for both Zinc phthalocyanine (ZnPc) and Zinc hexadecafluorophthalocyanine ($ZnF_{16}Pc$) [1]. Additionally, in an independent study we observed that the incorporation of ZnPc into bovine serum albumin (BSA) also promotes a decrease in the aggregation degree for those dyes [2].

In the present contribution, we study the photodynamic effect of red light on cultures of HeLa cells, using ZnPc and $ZnF_{16}Pc$ as photosensitizers that were delivered by using DPPC liposomes or bovine serum albumin (BSA).

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POS56

Comparative characterization of solar radiation-induced DNA lesions between *ex vivo* human skin and *in vitro* human hair follicle derived epidermis model

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Reconstructed human epidermis (RHE) is becoming used as surrogate to human skin for studying photobiology and photoprotection. However, little information is currently available concerning DNA damage induced by solar irradiation in these skin substitutes and their relevance to human skin. The aim of this study was to characterize solar-simulated radiation (*SSR*)-induced DNA lesions and cytotoxicity in a RHE model engineered from hair follicle keratinocytes and to compare the results with those obtained from *ex vivo* human skin organ culture. Liquid chromatography-Tandem mass spectrometry revealed that SSR induces a dose dependent cyclobutane pyrimidine dimer (*CPD*) formation and that the DNA lesion frequency depends on the nucleotide sequence (*TT>TC>CT>CC*). Both type and number of genomic lesions were similar to those determined in *ex vivo* skin model. DNA lesions persisted 24 hours after irradiation (*CPD staining)* and tissue viability was strongly altered (*sunburn cell detection, caspase-3 activity*). None of the above cellular responses was observed in non-irradiated epidermis. The topical application of a new broad-spectrum UVB+A photoprotective system (*a patented association of 4 filters*) at a dose of 2 mg/cm^2 1 hour before SSR exposure afforded an almost complete photoprotection. Thus, SSR-induced DNA damage has similar characteristics between RHE and native skin, suggesting that the skin substitute is suitable to mimic human skin *in vitro* and may be useful for the development of suncare products.

POS57

Novel targets for vitamin D in melanoma prevention, growth and metastasis.

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Australia has the highest incidence of melanoma in the world, where it is responsible for 75% of all skin cancer deaths. The relationship between melanoma and sunlight is interesting in that sunburn is causal while occupational sun exposure is not, and it has been suggested that patient outcome may be linked to vitamin D levels at diagnosis. The biologically active compound, 1,25-dihydroxyvitamin D3 (1,25D), which is produced in skin, can mediate its effects through either a well-established genomic pathway or a non-genomic pathway. We previously showed that 1,25D and a low calcemic non-genomic analog reduced UV-induced melanocyte cell death and DNA damage (thymine dimers), and that these effects were reversed by a non-genomic antagonist but not affected by a genomic antagonist. Moreover, we demonstrated that the UV-induced increase in tumor suppressor p53 was further enhanced when melanocytes were incubated with 1,25D immediately after UV. Thus, we have clear evidence that targeting of the non-genomic pathway in melanocytes prevents potentially mutagenic DNA damage that may lead to melanoma. Incubation of human melanoma cell lines with 1,25D significantly ($p<0.001$) reduced cell growth and migration. We speculate that the ability of 1,25D to inhibit growth and metastasis of melanoma cells may be associated with our finding in these melanoma cells of a 1,25D-induced increase ($p<0.05$) in PTEN, a known tumor suppressor and target of the metastasis suppressor, NDRG1. Non-genomic vitamin D analogs, which have no demonstrated toxicity in normal cells, may prove useful in preventing and inhibiting the growth and metastasis of melanoma cells.

POS58

On the natural function(s) of green fluorescent protein (GFP) in marine non-bioluminescent organisms

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The green fluorescent protein (GFP) is widely used in a large diversity of applications in molecular biology and biotechnology. The protein, and its color variants, was the core of the Nobel Prize in Chemistry in 2008. The protein was originally described from a bioluminescent jellyfish, and then subsequently largely found throughout most corals species, thus including non-bioluminescent ones. The prevalence of the protein in this group of organisms was so dominant that for long, it was thought that GFPs were strictly confined to these basal invertebrates. Despite the large popularity in biotechnology and the functional description of the commercially synthesized protein down to the atomic level, it is still unclear today what the possible biological and ecological functions of GFPs could be in the organisms that naturally harvest them. Assumptions range from photoprotection to spectral enhancement for the coral symbiotic algae, to biochemical antioxidative properties. Here, I will discuss research done in my laboratory where GFP was discovered in cephalochordates that are, much in contrast to cnidarians, the most evolved of the invertebrates. I will compare cnidarian and cephalochordate GFPs in term of sequences and spectral performances. I will also present data of experiments where changes in cephalochordates fluorescence are measured over time upon exposure to biochemically-induced oxidative stress. This will touch on addressing the possible natural function/s of GFPs in organisms, and will highlight the use of cephalochordates as new models for further understanding of fluorescence in Nature.

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Development of Folate-Targeted Photodynamic Therapy Agents Using Protein and PEG Carriers

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One of the limits of current photodynamic therapy (PDT) for cancer is that photosensitizers often accumulate in both tumor and healthy cells. Thus, the specificity of tumor cells killing is restricted. Targeted PDT combines the photosensitizer with a targeting moiety that is specifically taken up by the tumor cell. Within tumor cells, the excitation of the photosensitizer by light is coupled with the formation of singlet oxygen, which is phototoxic to the cells and induces tumor cell death. The cell membrane folate receptor (FR) can be used as a selective target for photosensitizer drug delivery. Conjugates of folic acid can be taken up by cancer cells via receptor-mediated endocytosis. Four folate-directed PDT agents have been synthesized and evaluated in cell culture and zebrafish. A direct linkage between folate and chlorin e6 was made by attaching them to the distal ends of the linker, 2,2-ethylenedioxy-bis-ethylamine (FA-CHLORIN). Two protein-based, folate-directed chlorin e6 derivatives have been made by attaching folate and chlorin e6 to the amino groups of lysine using carbodiimide chemistry. Fluorescein analogs can be made using similar chemistry. Both cross-linked bovine hemoglobin (FA-XLHb-CHLORIN) and bovine serum albumin (FA-BSA-CHLORIN) have been used as the carrier proteins. A second series of folate-dye complexes has been made using PEG carriers. Both linear and star PEGs have been used to make a new class of folate-PEG-chlorin PDT agents. The folate-directed PDT agents have been tested on HeLa cells, which have excess folate receptors. There was no cytotoxicity in the absence of light. The HeLa cells took up FA-XLHb-CHLORIN in a folate-specific manner. FA-Hb-fluorescein has been used to demonstrate the uptake of these agents in HeLa cells but not in A1V1 mouse cells that lack folate receptors. After exposure to light from a halogen bulb containing significant intensity at 660 nm, HeLa cells died. The use of hemoglobin as the carrier protein provides the potential for bringing additional oxygen into the cell, which is required for effective PDT in hypoxic tumors. Oxygen binding studies show that the FA-XLHb-CHLORIN complex had the same oxygen binding curve as XLHb. The uptake of FA-BSA-fluorescein has been demonstrated in zebrafish larva. The existence of folate receptors in zebrafish is being investigated. To try to move the wavelength range of the photoactive dye closer to 800 nm, we have used Buchwald-Hartwig chemistry to synthesize a phenothiazine trimer. This is currently being evaluated for its photophysical properties and its PDT potential.

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Harnessing of novel visible and near-infrared light photoactivated, Type II/Type I, tunable, metal-based, small molecule, coordination complexes in PDT.

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Small metal-based coordination complexes molecules recently have received attention as powerful photosensitizes (PS). We have found that Ru(II) dyads derived from organic units that impart low-lying 3IL excited states result in the most attractive features of these PS. Oligothiophenes participate in both energy and electron transfer reactions with appropriate acceptors to form $^1O_2$ and radical cations. The $^1O_2$ quantum yields (FD) increases for Ru(II) dyads derived with increasing number n of oligothiophenes. For n >3, FD is approximately 75%, so for n=2, oxygen-independent, Type I photochemistry persists. This dual Type II/I photosensitization has been quantified in glioblastoma U87 cells against Levulan® (δ-aminolevulinic acid) and PHOTOFRIN®. We have also documented that the low-lying 3IL states in Ru(II) dyads present the capacity for long wavelength excitation, so with very low molar extinction coefficients. An *in vitro* phototherapeutic index (PI) of 200 was calculated and remained persisting up to n=4 in this family of dyads. Moreover, the *in vitro* activity of these metal complexes translates to *in vivo* rodent models, with MTD50 values that are possibly superior to Photofrin. In BALB/c mice implanted subcutaneously with wild and/or antigenic colorectal carcinoma cells 632 nm light was used for excitation. These PSs offer a versatility beyond what can be achieved with traditional organic systems in clinical use for PDT. The lead drug candidates are currently undergoing the final stages of pre-clinical optimization for human Phase 1 study for this new class of PSs.

POS61

Photodynamic Therapy and Inflammatory Breast Cancer

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Inflammatory Breast Cancer is a rare and very aggressive type of breast cancer and is so called because the breast appears red and swollen. It presents in young women especially Middle Eastern and African American women. The five-year relative survival rate with treatment is about 34%. While conventional therapy currently involves surgery, chemotherapy or local radiotherapy, an approach to therapy that has the potential for increased selectivity is Photodynamic Therapy (PDT). PDT can preferentially eradicate malignant cells and their vasculature. PDT has three components: 1) a photosensitizing agent that can localize primarily in neoplastic cells, 2) dissolved oxygen in cells and tissues, and 3) light of an appropriate wavelength. The resulting photochemistry leads to formation of reactive oxygen species (ROS) that can cause cell death. The basic mechanisms of photokilling by PDT (e.g., type of ROS generated, drug kinetics and molecules involved) have been elucidated using tumor cells grown in 2D on plastic. Culturing cells in 3D has, in contrast to culturing in 2D, been shown to be more predictive of drug response and resistance and can be used to identify novel pathways for therapeutic intervention. PDT is a minimally invasive technique and its use has been explored for treatment of chest wall metastases of breast cancer. Moreover, IBC metastasizes to dermal (skin) lymphatics, an area accessible for photokilling by PDT. We are in the process of determining the efficacy of PDT in killing IBC cells grown in 3D reconstituted basement membrane (rBM) overlay cultures as IBC presents near the skin surface and would be easily accessible for treatment using PDT. IBC metastasizes to the dermal lymphatic vasculature and PDT causes vasculature shutdown so PDT might be more efficacious in photokilling cocultures of SUM149 and lymphatic cells. We are also interested in studying interaction of SUM149 cells with carcinoma-associated fibroblasts and efficacy of PDT in photokilling cells of respective type.

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