

ACCryo2013



American College of Cryosurgery * Society for Cryobiology
International Institute of Refrigeration * Cryogenic Society of America

CONFERENCE PROCEEDINGS

New Horizons for Cryomedicine

- *Cryoablative Therapies*
- *Clinical Outcomes*
- *Cryobiology*
- *Technological Advances*

HYATT REGENCY DOWNTOWN MIAMI
CRUISE CONFERENCE ON-BOARD CELEBRITY CONSTELLATION
JANUARY 2ND – 7TH, 2013

ACCryo2013
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Tel: (607) 687-8711

AMERICAN COLLEGE OF CRYOSURGERY
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American College of Cryosurgery

After a series of successful dermato-cryosurgical meetings in New Orleans, the American College of Cryosurgery (ACC) held its first annual meeting in New Orleans in March 1978. The membership was largely composed of dermatologists, but other specialists joined the ACC at succeeding meetings. When the American Academy of Dermatology (AAD) added cryosurgical training courses to its yearly program, the ACC began to shift focus to other specialties. Prior to this meeting, the last annual meeting, the 14th meeting was held in conjunction with the International Society of Cryosurgery (ISC) in Paris in June 1995. Following this period and commencing in 1997, a series of mini symposia on cryosurgery were held with a primary focus in urology.

In 1998, Dr. Gary Onik organized a combined the ISC - ACC meeting in Orlando FL. This meeting was very successful with participation by diverse specialists. It was, however, the last meeting of the ACC (15th). Importantly, the ISC has met every two or three years since 1971 featuring diversified programs representative of the many applications of cryosurgery. One should note also that the Society for Cryobiology often hosts a cryosurgical session at its annual meetings.

In considering the future status of cryosurgery in the United States we recognize that diverse specialties utilize cryoablative techniques. Most dermatologists likely use cryosurgery for some lesions. Use for diverse visceral tumors has been attractive for a number of years, but the freezing techniques for tumors are only a small part of the surgical armamentarium in current times. Other ablative techniques, such as RF, ultrasound, and laser, have become highly competitive to cryosurgery. Usage of cryoablation for tumors in Europe and Asia appears to be flourishing, as described in recent articles by Dr. Sajio Sumida in the journal Low Temperature Medicine. Further, use of cryoablative techniques for cardiac arrhythmias is gathering considerable interest and new equipment appears promising for further development of this use. Urological applications, especially for prostate and renal carcinomas, continue to grow.

We now face the matter of rejuvenation of the ACC. The Board of the ACC welcomes your comments at this, the 16th annual meeting. There are numerous options that the ACC might adopt to fulfill its mission ranging from annual meetings, symposia sponsorship, hosting satellite workshops at major medical meetings, conducting educational webinars, etc.

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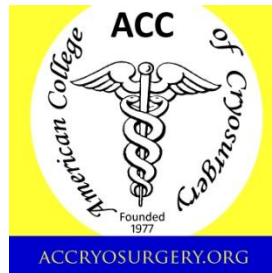
PARTICIPATING SCIENTIFIC SOCIETIES

American College of Cryosurgery

Society for Cryobiology

Cryogenic Society of America

International Institute for Refrigeration



ACCryo2013 CONFERENCE SUPPORTERS

The American College of Cryosurgery is indebted to the estate of Dr. Thomas A. Armao for his generous bequest to the college.

The American College of Cryosurgery would like to extend its appreciation and acknowledge the support from the following Gold Sponsors and Platinum Educational Grant Providers

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ACCryo2013 SCHEDULE AT-A-GLANCE

Date Time	January 2 WED	January 3 THU	January 4 FRI	January 5 SAT	January 6 SUN	January 7 MON
7:00 AM						Arrive at Port of Miami
8:00 AM				8:00a – 8:45a Keynote	8:00a – 8:45a Keynote	Depart Stateroom
9:00 AM				9:00a – 10:15a Session 4	8:45a – 10:15a Session 7	Disembark Ship
10:00 AM				10:30a – 11:40a Session 5	10:25a-11:30a Session 8	
11:00 AM		11:00 AM Coach Group #1				
12:00 PM		Boarding Cruise Ship		11:45a – 12:45p Session 6		
1:00 PM		12:30 PM Coach Group #2				
2:00 PM		Boarding Celebrity Constellation				
3:00 PM		Depart Port of Miami at 4:30pm	2:00p-2:45p Keynote			
4:00 PM		Muster Drill	3:00p – 4:40p Session 2			
5:00 PM		Registration Open			4:30p – 5:30p ACC Business Mtg.	
6:00 PM		5:30p – 6:15p Keynote	5:00p – 6:15p Session 3			
7:00 PM	6:00p - 9:00p President's Session: Poster Session & Exhibits	6:15p – 7:30p Session 1			7:00 - 8:00p Farewell Reception Reflections Bar, Deck 11	
8:00 PM	6:30p-7:30p Reception					
9:00 PM	7:30p -8:15p Keynote	8:30PM Seated Dinner Service	8:30PM Seated Dinner Service	8:30PM Seated Dinner Service	8:30PM Seated Dinner Service	
10:00 PM					Luggage Collection	
LOCATION	Hyatt Regency, Miami, FL	Celebrity Constellation – At Sea	Celebrity Constellation – Key West, FL	Celebrity Constellation – Cozumel, Mexico	Celebrity Constellation – At Sea	Return to Port of Miami

ACCryo2013 Scientific Sessions are located on Deck 3 (aft) in the Cinema Conference Center

Also on Deck 3: Guest Relations, Shore Excursion Desk & Concierge

San Marco Restaurant (Seated Dinner Service) is located on Decks 4 & 5; Medical Facility is on Deck 1

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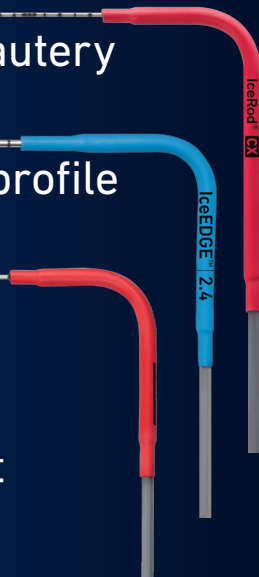
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insulated needle

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- Actively pursuing enrollment for 10 clinical trials and prospective registries

Providing comprehensive reimbursement support:

- Precertification and determination of coverage support
- Long-term strategies for establishing new indication reimbursement

Start	End		Wednesday, January 2, 2013 Hyatt Regency Downtown Miami
10:00	21:00		Registration & Cruise Document Pick-up <i>Japengo Room – Lobby Level</i>
18:00	21:00		President's Reception, Poster Session & Exhibits <i>Upper/Lower Promenade – Terrace Level, Jasmine & Hibiscus Rooms</i>
18:00	18:30		President's Welcome: Dr. John G. Baust, American College of Cryosurgery Dr. John H. Crowe, Society for Cryobiology <i>Jasmine & Hibiscus Rooms</i>
18:30	19:30		Welcome Reception – Upper/Lower Promenade, Terrace Level
19:30	20:15		Keynote – Dr. Jim Vaught, NIH, NCI Topic: NCI Cancer Specimen Biorepositories (K01) <i>Jasmine & Hibiscus Rooms</i>
20:15	21:00		Poster Session Presentations <i>Hibiscus Room</i>

POSTER PRESENTATIONS

- P01: Jill Davies**, Oxford University Hospitals NHS Trust "Evolution from NHS Heart Valve Bank to University Hospital Ovarian Tissue bank to Oxford Biomedical Research Centre Biorepository Biobank"
- P02: Birgit Glasmacher**, Pogozhykh, D., Hoffmann, N., Mueller, T. *Institute for Multiphase Processes, Leibniz Universitaet Hannover*, "Cryopreservation of primate mesenchymal stem cells with antioxidants as additional CPA"
- P03: Oleksandr Gryshkov**, Hofmann, N., **Glasmacher, B.** *Institute for Multiphase Processes, Leibniz Universitaet Hannover*, "Cell encapsulation into alginate micro-capsules provides living cells with a mild environment during cryopreservation"
- P04: Kimberly L. Santucci**, Snyder KK, Baust JM, Van Buskirk RG, Mouraviev V, Polascik TJ, Baust, JG. *Binghamton University*, "The use of 1,25[alpha] dihydroxyvitamin d3 as a cryosensitizing agent in a murine prostate cancer model"
- P05: Kenneth Baumann**, Baust, J.G., Van Buskirk, R.G., Snyder, K.K., Baust, J.M., *Binghamton University*, "Characterization of the Effects of Freeze Therapy on Pancreatic Cancer Cell Line PANC-1"
- P06: Ethel Rubin** and Sandra Schwartz. *Medtronic Inc.*, "Cryoablation as it applies to AF (namely balloon-based technology)"
- P07: John M. Baust**, Robilotto AT, Lueckge C, Guerra P, **Snyder K**, Cheeks R, Baust, JG. *CPSI Biotech*, "Development of a novel cryoablation device for the treatment of atrial fibrillation"
- P08: Kristi K. Snyder**, Baust J, Van Buskirk RG, Baust J. *CPSI Biotech*, "Investigation into cardiomyocyte response to cryoablation"
- P09: John M. Baust**, Klossner DP, Van Buskirk RG, Gage AA, Mouraviev V, Polascik TJ, **Baust, JG.** *Binghamton University*, "Targeted modulation of integrin expression increases freeze sensitivity of androgen-insensitive prostate cancer"
- P10: Anthony T. Robilotto**, Baust J, Van Buskirk R, Gage A, Baust J. *Binghamton University*, "Rapid induction of apoptosis at ultra low temperatures enhances the efficacy of prostate cancer cryoablation"
- P11: Anthony T. Robilotto**, Snyder, KK, Van Buskirk, RG, Baust, JG, Baust, JM. *CPSI Biotech*, "Development of a tissue engineered human prostate equivalent: evaluation of cryoablative techniques"
- P12: Xiule Yue**, Jin, M., Yang, N., Xiang, Y., An, L., Zhang, H. *Lanzhou University, School of Life Sciences*, "Identification and characterization of genes crucial for basal freezing tolerance in an alpine subnival plant *Chorispora Bungeana*"
- P13: William L. Corwin**, **Baust JM**, Van Buskirk RG, Baust JG. *Binghamton University*, "In vitro assessment of apoptosis and necrosis following cold storage in human corneal endothelial cells"
- P14: William L. Corwin**, **Baust JM**, Van Buskirk RG, Baust JG. *Binghamton University*, "In vitro assessment of apoptosis and necrosis following cold storage in human airway cell model"
- P15: Cheng-Wei Wu**, Bell, R.A.V., **Storey, K.B.** *Carleton University, Institute of Biochemistry & Department of Biology*. "Regulation of PTEN function and structural stability in hibernating thirteen-lined ground squirrels."
- P16: Shannon N. Tessier** and **Kenneth B. Storey**. *Carleton University, Institute of Biochemistry & Department of Biology*. "Muscle disuse atrophy: the expression of myocyte enhancer factor 2 in the skeletal muscle of *Spermophilus tridecemlineatus* during hibernation."
- P17: Andrew N. Rouble**, Tessier, S.N., **Storey, K.B.** *Carleton University, Institute of Biochemistry & Department of Biology* "Antiapoptotic signaling as a cytoprotection mechanism during mammalian hibernation."
- P18: Cheng-Wei Wu** and **Kenneth B. Storey**. *Carleton University, Institute of Biochemistry & Department of Biology*. "Roles of the mTOR signaling pathway in hibernating ground squirrels, a differential suppression of active protein synthesis."
- P19: Sergey Vasiliev**, **Pesnya-Prasolov, S.**, **Krylov, V.**, **Zuev A.**, **Kungurcev, S.** *Russian Research Center of Surgery*, "Cryosurgery of brain tumors with ultrasound neuronavigation control"
- P20: Azra Alizad**, Mitri, F.G., Davis, B.J., Greenleaf, J.F., Mynderse, L.A., Fatemi, M. *Mayo Clinic College of Medicine*, "Monitoring Prostate Cryotherapy by Vibro-acoustography"

Hibiscus Room Poster & Sponsor Exhibits Locations

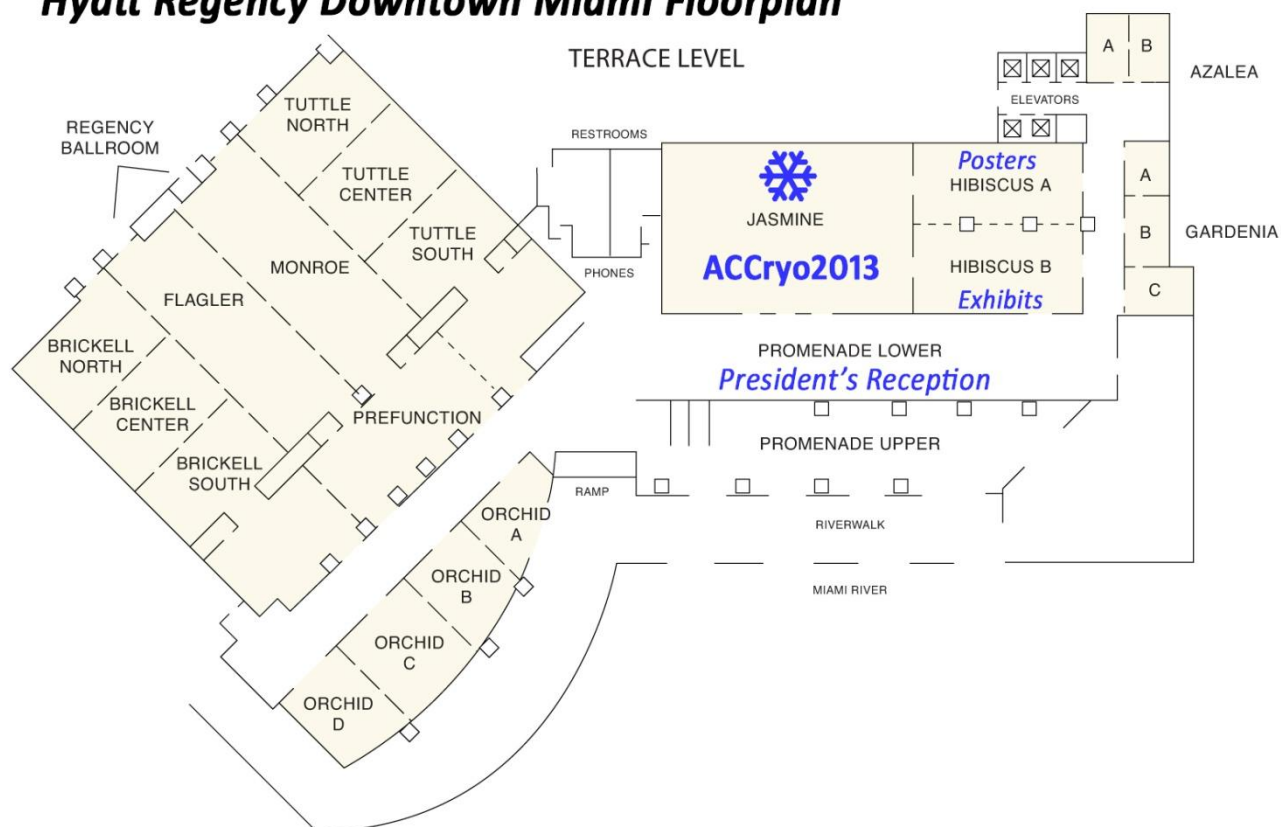
P03 P04	P09 P10	P14 P15	P18 P19
P02 P05	P08 P11	P13 P16	P17 P20
P01 P06	P07 P12		

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Hyatt Regency Downtown Miami Floorplan



Start	End	Thursday, January 3, 2013 Hyatt Regency Miami & Celebrity Constellation	
8:00	10:00	Registration & Cruise Document Pick-up <i>Japengo Room – Lobby Level</i>	
8:00	9:00	American College of Cryosurgery – Board Meeting <i>Lobby Level Restaurant</i>	
11:30	13:30	Motor Coach Transport from Hyatt Regency to Port of Miami	
12:00	15:00	BOARD Celebrity Constellation Cruise Ship – CHECK-IN NO LATER THAN 2:45PM <i>Lunch available on Deck 10 Seaside Café & Grill</i> <i>Free time to Explore Celebrity Constellation</i>	
16:00	16:30	Muster/Safety Drill for ALL Cruise Passengers <i>Guest Access to Staterooms</i>	
16:30	17:30	Speaker Preparation <i>Boardroom – Deck 3 Conference Center</i>	
17:30	18:15	Keynote – Dr. John G. Baust, State University of New York at Binghamton Topic: Advances in Cryoablation (K02) <i>Cinema Conference Center – Deck 3 aft</i>	
18:15	19:30	Session 1: Gastroenterology: Cryotherapy via flexible endoscopy in the GI tract <i>Conference Center Cinema – Deck 3aft</i> Session Chair: Dr. P.J. Pasricha, Johns Hopkins Medical Center Speakers: Anthony N. Kalloo , Johns Hopkins University School of Medicine "Overview of GI indications and cryo technology via flexible endoscopy" (A01) P. Jay Pasricha , Johns Hopkins Medical Center, "Experimental data on ablation responses in the GI tract" (A02) Marcia I. Canto , Johns Hopkins University School of Medicine "Barrett's Esophagus- a perfect target for cryo-ablation" (A03) Kristi K. Snyder , CPSI Biotech, "Multi-dimensional assessment of pancreatic cancer cell responses to cryoablation <i>In vitro</i> " (A04)	Speaker Ready Room & Internet Access <i>Cinema Conference Center Boardroom – Deck 3</i>
18:15	18:35		
18:35	18:55		
18:55	19:15		
19:15	19:30		
19:30	20:15	Networking Social	
20:30	22:00	Seated Dinner Service (8:30pm) <i>San Marco Restaurant, Deck 4 & 5</i>	

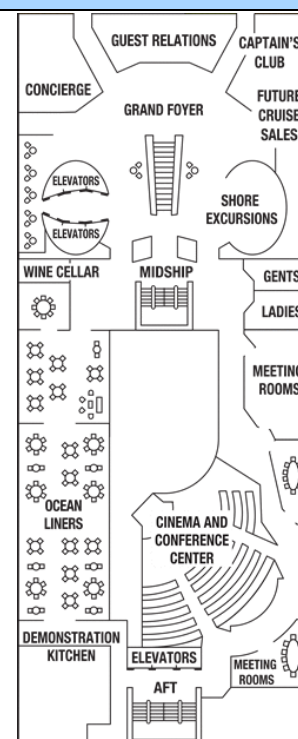
Celebrity Constellation

Ship Deck Plans – please see the map provided with your stateroom card key

ACCryo2013 Scientific Sessions will be held in the
Cinema Conference Center, Deck 3 aft

**ACCryo2013 Registration Desk, Speaker Preparation Area,
Sponsor Displays, Electronic Poster Display
& Conference Attendee Internet Access Computer**

Located in the Boardroom, Deck 3, Conference Center



Start	End	Friday, January 4, 2013 Celebrity Constellation	
6:00	10:00		Breakfast <i>Seaside Café (deck 10 aft) – Breakfast Buffet 6am – 10am AquaSpa Café 7:30am – 10am; San Marco Dining Room Open Seating 8am – 9:30am</i>
7:00	13:30		Port of Call: Key West, Florida Return to the ship by 1:15pm for departure
10:30	13:00		Speaker Presentation Ready Room Cinema Conference Center, Deck 3
12:00	14:00		Lunch <i>Seaside Café (deck 10 aft) Lunch Buffet & AquaSpa Café, Café al Bacio Sandwiches: noon – 2:30pm San Marco Dining Room Open Seating noon – 1:30pm; Riviera Grill (Deck 10 poolside) noon – 6pm;</i>
14:00	14:45		Keynote – Dr. Michael S. Sabel, University of Michigan Health Systems Topic: Cryo-Immunology: The Stimulatory and Suppressive Effects of Cryoablation on the Immune Response (K03) <i>Cinema Conference Center – Deck 3 aft</i>
14:45	15:00		Break
15:00	16:45		Session 2: Multi-Modal Treatments: Combinatorial Strategies and Immunology <i>Conference Center Cinema – Deck 3aft</i> Session Chair: Dr. John C. Bischof, <i>University of Minnesota</i> <u>Speakers:</u> John C. Bischof , <i>University of Minnesota</i> , “Nanoparticle Delivered Vascular Disrupting Agents (VDAs): A New Opportunity in Multimodal Cancer Treatment” (A05) Xiaoming He , <i>The Ohio State University</i> , “Effective Destruction of Cancer Stem Cells by Combing Freezing and Anticancer Drug Encapsulated in Nanoparticles” (A06) Yaron Har-Shai , <i>Carmel Medical Center</i> , “Intralesional Cryosurgery for Enhancing the Involution of Hypertrophic Scars and Keloid - A new fundamental adjunctive wound healing therapy based on experimental and clinical data” (A07) Patrick Le Pivert , <i>Interventional Drug Delivery Systems & Strategies</i> , “Image-Guided Concurrent Chemoablation-and Cryoablation for Lung Cancer: a Preliminary Clinical Investigation” (A08) Robert Griffin , <i>University of Arkansas for Medical Sciences</i> , “Role of Tumor Microenvironment in Cryosurgery: Opportunities for new approaches to multi-modality cancer treatment” (A09)
15:00	15:20		Speaker Ready Room & Internet Access <i>Cinema Conference Center Boardroom – Deck 3</i>
15:20	15:40		
15:40	16:00		
16:00	16:20		
16:20	16:40		
16:40	17:00		
17:00	18:15	Session 3: Clinical Cryosurgical Experience <i>Conference Center Cinema – Deck 3 aft</i> Session Chair: Dr. Kristi K. Snyder, <i>CPSI Biotech</i> <u>Speakers:</u> Kimberly L. Santucci , <i>Binghamton University</i> , “In Vitro Cryosensitization Approaches for Prostate Cancer Cryotherapy” (A10) Michael S. Sabel , <i>University of Michigan Health Systems</i> , “Cryoablation as an Alternative to Lumpectomy for Breast Cancer” (A11) Nikolai N. Korpan , <i>The Rudolfinerhaus, Vienna, Austria</i> . “Cryosurgery for Breast Cancer: A Challenge in Current and Future Invasive Oncology. Clinical Experience since 1995” (A12) Barlian Sutedja , <i>Gading Pluit Hospital, Jakarta, Indonesia</i> , “Laparoscopic Assisted Cryosurgery for Intra Abdominal Malignancy” (A13)	
17:00	17:15	Speaker Ready Room & Internet Access <i>Cinema Conference Center Boardroom – Deck 3</i>	
17:15	17:35		
17:35	17:55		
17:55	18:15		
19:00	20:00	Networking Social	
20:30	22:00	Seated Dinner Service (8:30pm) <i>San Marco Restaurant, Deck 4 & 5</i>	
For more details on Ship dining and activity options, please review the “Celebrity Today” Newsletter in your Stateroom Deck 3 Guest Relations. Shore Excursions & Concierge: Deck 1 Medical Facility			

Start	End	Saturday, January 5, 2013 Celebrity Constellation Time Change – minus 1 hour (Central Time)	
6:00	10:00	Breakfast Seaside Café (deck 10 aft) – Breakfast Buffet 6am – 10am AquaSpa Café 7:30am – 10am; San Marco Dining Room Open Seating 8am – 9:30am	
8:00	8:45	Keynote – Dr. Kenneth B. Storey, Carleton University Topic: Mammalian Hibernation – Clinical Implications (K04) Cinema Conference Center, Deck 3 aft	
8:45	9:00	Break	
9:00	10:30	Session 4: Hypothermic Medicine Conference Center Cinema – Deck 3aft Session Chair: Dr. Michael J. Taylor, Cell & Tissue Systems and Carnegie Mellon University Speakers 9:00 9:25 Michael J. Taylor, Cell & Tissue Systems, "Integration of time-honored principles of hypothermic cellular protection with modern day modalities of organ preservation" (A14) 9:25 9:50 John M. Baust, CPSI Biotech, "Molecular approaches to improving hypothermic biopreservation of cells and tissues" (A15) 9:50 10:15 Klearchos K. Papas, University of Minnesota, "Role of hypothermia for expanding the donor pool of transplant organs- Supplemented oxygenation during hypothermic organ storage: friend or foe?" (A16)	Speaker Ready Room & Internet Access Cinema Conference Room Alpha – Deck 3
10:15	10:30	Break	
10:30	11:45	Session 5: Blood Cell Preservation Conference Center Cinema – Deck 3aft Session Chair: Dr. John Crowe, University of California Davis Speakers 10:30 10:50 John H. Crowe, University of California Davis, "Freeze-dried Platelets: Moving Towards Clinical Use" (A17) 10:50 11:10 Jason Acker, Canadian Blood Services, "The Challenges and Limitations of Current Clinical Methods for the Hypothermic storage and Cryopreservation of human red blood cells" (A18) 11:10 11:40 Amir Arav, Core Dynamics, "Freeze drying red blood cells: Development of novel technologies" (A19)	Speaker Ready Room & Internet Access Cinema Conference Room Alpha – Deck 3
11:40	11:45	Break	
11:45	12:45	Session 6: Preservation of Biotherapeutic Products Conference Center Cinema – Deck 3 aft Session Chair: Anthony Robilotto, Binghamton University Speakers: 11:45 12:05 Gloria D. Elliott, University of North Carolina at Charlotte, "Dry Preservation of Biological Therapeutics"(A20) 12:05 12:25 David Gale, Cryolife, "Optimizing Tissue Cryopreservation Techniques for Cryopreservation Solution Modifications"(A21) 12:25 12:45 Birgit Glasmacher, Institute for Multiphase Processes, "Advances towards Standardised Freezing Protocols" (A22)	Speaker Ready Room & Internet Access Cinema Conference Room Alpha – Deck 3
11:30	20:30	Port of Call: Cozumel, Mexico Return to the ship by 8:15pm for departure	
12:00	14:00	Lunch Seaside Café (deck 10 aft) Lunch Buffet & AquaSpa Café, Café al Bacio Sandwiches: noon – 2:30pm San Marco Dining Room Open Seating noon – 1:30pm; Riviera Grill (Deck 10 poolside) noon – 6pm;	
17:00	18:30	Free Afternoon: Meeting Rooms in Conference Center available by Appointment	
20:30	22:00	Seated Dinner Service (8:30pm) San Marco Restaurant, Deck 4 & 5	
For more details on Ship dining and activity options, please review the "Celebrity Today" Newsletter in your Stateroom Deck 3 - Guest Relations, Shore Excursions & Concierge; Deck 1 - Medical Facility			

Start	End	Sunday, January 6, 2013 Celebrity Constellation Time Change – plus 1 hour (return to EST)	
8:00	10:30	Breakfast Seaside Café (deck 10 aft) – Breakfast Buffet 6am – 10am , AquaSpa Café 7:30am – 10am;	
8:00	8:45	Keynote – Dr. Truls E. Bjerklund Johansen, Topic: Whole gland cryosurgical ablation of prostate cancer (K05) Cinema Conference Center, Deck 3	
8:45	10:15	Session 7: Clinical Applications of Cryo-technology in Cardiac Arrhythmia Conference Center Cinema – Deck 3aft Session Chair: Dr. Neil Sanghvi, Lenox Hill Hospital Speakers: 8:45 8:50 Neil K. Sanghvi , Lenox Hill Hospital , Introduction (A23) 8:50 9:20 Neil K. Sanghvi , Lenox Hill Hospital , “Cryoablation of SVTs and Ventricular Tachycardia” (A24) 9:20 9:50 Boaz Avitall , University of Illinois , “Cryoablation of Atrial Fibrillation” (A25) 9:50 10:00 Panel Q&A 10:00 10:15 John C. Bischof , University of Minnesota, “Thermal Properties of Porcine Myocardial Tissue at Subzero Temperatures: Implications for thermal therapy studies” (A26)	Speaker Ready Room & Internet Access Cinema Conference Room Alpha – Deck 3
10:15	10:25	Break	
10:25	11:30	Session 8: Technology Advances Conference Center Cinema – Deck 3aft Session Chair: Dr. Yoed Rabin, Carnegie Mellon University Speakers: 10:25 10:45 Yoed Rabin , Carnegie Mellon University, “Computation Tools at the forefront of Cryosurgery Development” (A27) 10:45 11:00 Anthony T. Robilotto , Binghamton University and CPSI Biotech, “Assessing the Performance of a Novel Cryosurgical Platform” (A28) 11:00 11:15 Gary Onik , Center for Urological Advancement, “MR Guided Cryoablation” (A29) 11:15 11:30 Kenneth R. Diller , University of Texas at Austin, “Design and Testing of Above 0°C Cryo-Therapeutic Devices” (A30)	Speaker Ready Room & Internet Access Cinema Conference Room Alpha – Deck 3
11:30	13:30	Sea Day Brunch: San Marco Dining Room Open Seating 10:00am – 1:30pm	
		Free Afternoon	
15:00	16:00	Special Presentation: “Bethsaida – a City by the North Shore of the Sea of Galilee” Conference Center Cinema – Deck 3aft Dr. Rami Arav , Associate Prof. Department of Religion, University of Nebraska at Omaha, Director of the Bethsaida Excavations Project	
16:30	17:30	American College of Cryosurgery Business Meeting Conference Center Cinema, Deck 3 New Members Welcome	
19:00	20:00	Farewell Cocktail Reception & Closing Remarks Reflections Bar, Deck 11	
20:30	22:00	Seated Dinner Service (8:30pm) San Marco Restaurant, Deck 4 & 5	
	21:00	Luggage Collection outside Staterooms	

Monday, January 7, 2013 Celebrity Constellation & Port of Miami, Florida			
7:00	9:30	Ship Arrives at Port of Miami: Buffet Breakfast available on Deck 10 Depart Stateroom (by 9:30AM) and Disembark Ship (Final Departure by 10:00 AM)	

ABSTRACTS

Listed in Order of Presentation

Wednesday, Jan. 2, 2012, Hyatt Regency Hotel

7:30 PM Keynote K01: NCI Cancer Specimen Biorepositories

Jim Vaught, Ph.D.^{1*}

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High quality biospecimens with appropriate clinical annotation are critical in the era of personalized medicine. It is now widely recognized that biospecimen resources need to be developed and operated under established scientific, technical, business, and ethical/legal standards. To date such standards have not been widely practiced, resulting in variable biospecimen quality that may compromise research efforts. The National Cancer Institute (NCI) Biorepositories and Biospecimen Research Branch (BBRB) was established in 2005 to coordinate NCI's biospecimen resource activities, and address those issues that affect access to the high quality specimens and data necessary for its research enterprises, as well as the broader translational research field. BBRB developed NCI's Best Practices for Biospecimen Resources after consultation with a broad array of experts. A Biospecimen Research Network was established to fund research to develop additional evidence-based practices. Projects are in progress to study preanalytical variables that may affect biospecimen quality and suitability for proteomic and genomic analyses. BBRB is also engaged in the NIH Genotype Tissue Expression (GTEx) Project, for which we have implemented a complex biospecimen collection and processing infrastructure that will result in analyses of genetic variation and gene expression across many tissues, to aid in the interpretation of genome-wide association study hits, to help prioritize therapeutic targets, and to further our understanding of genome regulation.

Poster Presentations

P01 Evolution from NHS Heart Valve Bank to University Hospital Ovarian Tissue bank to Oxford Biomedical Research Centre Biorepository Biobank

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The Heart Valve Bank (HVB) based in the Heart Centre, Oxford University Hospital NHS Trust (OUH) procures and processes at least 150 cardiovascular tissues each year for transplantation. This HVB service has evolved now to process other tissue for transplantation and for research. The impetus for development was atypical. The HVB team reviews an average of 720 deceased patient notes per annum and interviews families of potential donors to obtain consent for transplantation. Of these, 80% families also consent to research in NHS or university setting (60% in commercial setting). The OUH is one of the largest acute teaching hospitals in the UK. Research is one of the OUH's three key strategic activities, alongside clinical services and teaching. HVB was encouraged to fulfill the wishes of the donors and to facilitate research within OUH.

HVB service has evolved from April 2011 as follows:

- 181 corneas retrieved and supplied to eye bank (per annum)
- Hearts unsuitable for transplantation used within OUH or Oxford University (OU) for research e.g. study to improve mitral isthmus ablation surgery (hearts placed in head coil inside MRI to enhance image clarity).

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- 101 aortic valves (calcified) supplied to Sir Magdi Yacoub's researchers at Harefield Hospital developing a tissue engineered valve and at Imperial College exploring the calcification of the aortic valve using state of the art materials analyses.
- More than 30 'control' & diseased brains and spinal cords supplied to OUH/OU brain bank. Brain tissue used to study effects of new therapies e.g. AD vaccine trial, PD cell transplantation, development of transgenic mouse models.
- Ovarian tissue cryopreservation set-up for 2013 with OU/OUH to preserve fertility in children and young females who survive cancer treatment (first UK clinical program).

HVB already had an embedded system within the OUH and the community for tissue donation for transplantation and a detailed consenting procedure (recorded), working within strict EU and UK legislation e.g. regarding 'DNA-theft' and 'privacy'. Prior to further development however, HVB required support and guidance from the Oxford Biomedical Research Centre (Ox BRC), funded by the National Institute for Health Research (NIHR), and aims to align excellence across the Strategic Partnership of the hospital Trust and the University. In practice this means bringing together the research expertise and skills of OU and OUH staff, with the aim of supporting translational research and innovation for maximum patient benefit. The biobank activities of the HVB are standardised and comply with Human Tissue Authority (HTA) licensing and ethical requirements. Access to samples and data is facilitated via a single point of access, open to all researchers via an online website enquiry form. Accessibility, quality control, documentation and distribution are efficiently managed. Public involvement and engagement and independent review are facilitated by OxBRC. HVB now considers specific or generic requests for any tissue required for research along with biofluids and associated medical, behavioural and social data and can create prospective sample collections. HVB requires feedback from researchers to encourage consent. The HVB is thus now acting as a hospital integrated BioResource.

P02 Cryopreservation of Primate Mesenchymal Stem Cells with Antioxidants as Additional CPA

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Objectives: Stem cells have potential use in clinical therapy and regenerative medicine. One of the major challenges regarding the application of these cells is the development of an efficient cryopreservation protocol, since currently used methods exhibit poor viability and high differentiation rates. A high survival rate is a function of optimal cooling rate, appropriate cryoprotective agent (CPA) and its adjusted concentration. The most widely used CPA, DMSO, however is toxic at high concentrations and has detrimental effects on the biological functioning of a cell. Therefore, it is of great interest to develop new cryoprotective strategies to replace currently used CPAs or to reduce their concentration. Since one of the major damaging factors is the occurrence of ROS during and after thawing we have investigated the addition of ascorbic acid and α -tocopherol as potential antioxidants. **Methods:** Mesenchymal stem cells (MSC) from the common marmoset monkey (*Callithrix jacchus*) were frozen in 200 μ l PCR-tubes (5×10^5 cells/ml) with optimal cooling rate (gained from a parallel study) in a μ -freezer device. Different concentrations of ascorbic acid (50, 100 and 250mM) and α -tocopherol (100, 200 and 500 μ M) were studied exclusive or in combination with ME2SO (2,5 and 5% v/v). Cells attached to the culture flask surface after 24 h recultivation were considered as viable, the survival rate was compared to that of cells frozen with ME2SO as positive control. Survived cells were further tested for differentiation capability. **Results:** The addition of 200 μ M α -tocopherol improved the survival of primate MSCs after cryopreservation even with 2,5% ME2SO. With 100 μ M α -tocopherol and 5% ME2SO survival was more than two fold higher than with 5% ME2SO alone. Ascorbic acid had no considerable effect on the survival rate of MSCs. Intracellular lipid vesicles were clearly stained by Oil Red O and proved the adipogenic differentiation capability of marmoset MSCs after cryopreservation with antioxidant addition. **Conclusions:** Accessory application of α -tocopherol improved survival and proliferation of MSCs from the marmoset monkey after cryopreservation. ME₂SO could be reduced to moderate concentrations. These findings will be transferred on the cryopreservation of iPS-cells in further studies. We thank L. Smits and B. Zingerov for their outstanding technical assistance. This work is supported by funding from the Deutsche Forschungsgemeinschaft for the Cluster of Excellence REBIRTH (EXC 62/1).

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P03 Cell Encapsulation into Alginate Micro-Capsules Provides Living Cells with a Mild Environment During Cryopreservation

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Cell encapsulation technology has been proposed to treat a variety of chronic disorders. However, the availability of some cell types such as human stem cells, in turn, promotes the need for such cells to be preserved for longer periods of time. For this, cryopreservation procedures are commonly used, which can cause injuries to the cells. A semi-permeable membrane might protect the cells during cryopreservation, serving as reservoir for cryoprotective agents (CPAs) and further additives [1]. Furthermore, smaller alginate-based micro-capsules (diameter 250µm) offer additional advantages over larger ones for transplantation and cryopreservation owing to higher specific surface area, less water content as well as improved heat and mass transfer [2]. In this work we indicate on the possibility to encapsulate living cells into semi-permeable alginate micro-capsules via electrospraying in order to improve viability of living cells post-cryopreservation as well as to support their normal proliferation rate after thawing. NIH 3T3 fibroblasts (5×10^6 cells/ml) were encapsulated into 1,5% or 2,0% (w/v) alginate micro-capsules (diameter 250µm) under sterile conditions using high-voltage processes based on previously optimized parameters. Cryopreservation was conducted under 2K/min to -30°C and 5K/min from -30°C to -80°C freezing protocol using DMEM, 20% FCS, 10% DMSO as freezing medium. Thawing was performed at 20°C using standardized equipment. Proliferation and viability of encapsulated cells before cryopreservation and after thawing was measured using MTT and Calcein AM/Ethd assays respectively. The change in morphology of alginate micro-capsules was observed under Carl Zeiss Axiovert 200M microscope using 5x or 10x magnifications and AxioVision V 4.8.2.0 built-in software. Alginate micro-capsules have been entrapped with NIH 3T3 fibroblasts cells at a density of 130 ± 24 cells per capsule. Preliminary results on cell survival after encapsulation showed that electrospraying is a suitable technique for living cells entrapment. Microscopic observations revealed that low temperature treatment and further thawing did not significantly affect the morphology of alginate capsules as they appeared to be stable and round in shape. The results of cell viability assays indicated an increase in viability of encapsulated cells post-cryopreservation as compared to non-encapsulated by 10%. The MTT proliferation assay showed that cells proliferate well after thawing. In order to further improve the viability of cells, the most suitable freezing and thawing protocol as well as the finding of alternative and less-harmful for the cells CPAs are of necessary and will supplement this study. Acknowledgements: This work was funded by the German Research Foundation (DFG) for the Cluster of Excellence REBIRTH (from regenerative biology to reconstructive therapy)(EXC 62/1) [1] B.C. Heng, H. Zu, S.C. Ng. *Biotechnology and Bioengineering* 2004, 85(2), 202-13 [2] J.T. Wilson, E.L. Chaikov. *Advanced drug delivery reviews* 2008, 60, 124-45

P04 The Use of 1,25[alpha] dihydroxyvitamin D3 as a Cryosensitizing Agent in a Murine Prostate Cancer Model

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Vitamin D3 has been identified as a possible adjunctive treatment for cancer therapies due to its antiproliferative and antitumorigenic properties. It has also been implicated in the inflammation process, which is critical to the initiation and progression of prostate cancer (CaP). Cryotherapy, the use of low temperatures to ablate tumorigenic tissues, has recently emerged as a primary treatment option for CaP. For these reasons we hypothesized that combining calcitriol with the sub lethal temperatures experienced in the periphery of the cryogenic lesion would result in an increase in apoptotic cell death as compared to either cryotherapy or calcitriol alone. This study aimed to identify the pathways activated when the biologically active metabolite of vitamin D3 (calcitriol) is combined with cryotherapy in a murine model of prostate cancer. The RM-9 cell line was treated with 1,25a dihydroxyvitamin D3 [25–150 nM] for 24 h prior to exposure to low temperature in a temperature controlled bath. Ice nucleation was initiated by liquid nitrogen vapor and cell viability was assessed using alamarBlue for 3 days post-freeze. Data indicate that pre-treatment with calcitriol sensitizes RM-9 cells to enhanced cellular injury at low temperature. Treatment with calcitriol alone [25–400 nM] shows no effect on cell viability. Similarly, a single freeze between -5 °C and -15 °C alone had little effect on survival. However, when cells are pre-treated with calcitriol and

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then exposed to low temperature, there is a synergistic effect of increased efficacy of cell death. At -15°C coupled with calcitriol [50–75 nM] cell death increased to 55% of day 1 controls following a single freeze exposure, compared to negligible cell death with freeze alone. Repeat exposure resulted in 90% cell death compared to 65% in dual freeze alone. Western blotting of signaling proteins during the calcitriol and cryotherapy sensitization regimes confirmed the activation of apoptosis. Specifically, pro-apoptotic Bid and pro-caspase-3 was found to decrease at 1h following combination treatment, indicative of cleavage to the active form. The survival protein Akt was found to be upregulated at 4 h post-cryo at higher doses of calcitriol ([150 nM]), which could be indicative of pro-survival signaling through the PI3-K pathway. Additionally molecular analysis of in vivo tissue tumor samples treated with calcitriol and cryotherapy also indicated the involvement of apoptosis through caspase-3 and caspase-9 cleavage. Previous studies have elucidated the in vitro benefits of cryosensitization strategies including combining drugs such as 5-fluorouracil and taxotere with cryotherapy; however, in vivo chemotherapeutic drugs present with toxic side effects as well as the likelihood of developing resistance. The need for the development of novel, non-toxic treatments for prostate cancer is essential, as many current strategies have negative side effects. The development of an adjunctive therapy combining calcitriol and cryotherapy represents a potentially highly effective, less toxic minimally invasive alternative treatment option.

P05 Characterization of the Effects of Freeze Therapy on Pancreatic Cancer Cell Line PANC-1

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Pancreatic cancer (PCC) is the fourth leading cause of cancer related deaths in the United States. Based on rates from 2007 to 2009, 1 in 68 individuals will be diagnosed with PCC in their lifetime. Although the incidence of PCC is lower than most cancers, the mortality rate is much higher with an average five year survival rate of approximately 6%. Current methods used to treat PCC include cryotherapy, radiofrequency ablation, and chemotherapy. The application of cryotherapy has been shown to be an effective, minimally invasive technique to treat PCC. However, wide spread utilization has been hindered due to limited clinical data and a void in our understanding of the cellular and molecular response of PCC to cryoablation. A more in-depth understanding of these molecular responses should provide a path to increasing cell death following freezing. In this study, we evaluated the effects of freezing on a human pancreatic cancer cell model, PANC-1, in an attempt to characterize the overall response of PCC to cryoablation, identify the lethal temperature, as well as investigate the mechanisms of cell death in PCC in response to a freezing insult. PANC-1 samples were exposed to a range of sub-freezing temperatures (-10 to -20°C) and compared to non-frozen controls in vitro. PANC-1 samples were frozen to a given temperature for 10 minutes, and then thawed at room temperature before returning to 37°C culture. Ice formation was initiated via LN_2 seeding when sample temperature reached -2°C to limit supercooling. Viability assessments were performed 24 hours post-freeze using Alamar Blue, a metabolic assay, and Calcein AM, a fluorescent membrane integrity indicator. Microfluidic flow cytometry was conducted using fluorophores YO-PRO-1 and Propidium Iodide to quantify the relative levels of apoptosis and necrosis, respectively, at 1, 4, 8, and 24 hours post-thaw. The data indicate that exposure to -10°C resulted in a minimal effect on PANC-1 cell viability one day post-thaw ($126 \pm 2\%$ vs. $133 \pm 2\%$ relative to pre-freeze control). Assessment of samples exposed to -15 and -20°C revealed a decrease in survival to 36% and 4%, respectively. Qualitative assessment via fluorescent microscopy revealed a peak in cell death between four to eight hours post-thaw. Quantitative analysis via microfluidic flow cytometry correlated with the fluorescent microscopy data showing a slight increase in apoptosis and secondary necrosis between four and eight hours at -15°C . Microfluidic flow cytometry also revealed a distinct rise in necrotic cell death throughout the post-thaw time course, peaking at 72% and 88% necrosis at -15°C and -20°C , respectively, 24 hours post-thaw. These data provide early indications that by increasing our understanding of the mechanisms of cryoablation, we may gain an improved understanding of ablative methods for pancreatic cancer, thereby potentially extending the life expectancy of patients with PCC.

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Po6 Worldwide Experience with the Arctic Front Cardiac CryoAblation System for Treatment of Atrial Fibrillation

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Introduction: Pulmonary vein (PV) isolation by percutaneous catheter ablation is a cornerstone for treatment of atrial fibrillation (AF). The 2012 HRS/EHRA/ECAS Expert Consensus Statement on Catheter and Surgical Ablation of Atrial Fibrillation made a Class 1 recommendation for catheter ablation for symptomatic paroxysmal AF refractory or intolerant to at least one Class 1 or 3 antiarrhythmic medication. The Arctic Front Cardiac CryoAblation System, a minimally invasive device that enables intravascular deployment of a cryoballoon to the left atrium, creates circumferentially transmural lesions at the PV antrum, blocking ectopic conduction to the LA from PV triggers that initiate AF. The device has been used to treat over 37,000 cases worldwide. A recent randomized, controlled clinical trial, the Sustained Treatment of Paroxysmal Atrial Fibrillation study (STOP AF), evaluated safety and efficacy of cryoablation (CRYO) in comparison with anti-arrhythmic drug therapy (AAD) for drug- refractory recurrent, symptomatic paroxysmal atrial fibrillation. Acute procedural success was achieved in 98.2 percent (160/163) of patients randomized to cryoablation. At 12 months, 69.9 percent of CRYO patients demonstrated treatment success compared to 7.3 percent of AAD patients. The overall rate of serious adverse events in the trial was 12.3 percent for the CRYO arm in comparison to 14.6 for AAD (p=0.69). Methods: To further explore worldwide experience with the device and cryoablation procedure, a literature search was conducted for all data published from June 14, 2005 to November 16, 2012. Only articles that contained both safety and clinical outcomes results for Arctic Front or the next generation Arctic Front Advance system were included. Results: 44 studies reporting on rates of acute PVI and chronic efficacy (3-21 months) from 2722 patients in 15 countries were identified. With the addition of STOP AF (n=163 CRYO) and an FDA approved continued access protocol (CAP, n=78), data from 2963 patients were analyzed. Fourteen of these studies, representing data from 13 countries, reported outcomes on 1293 patients at 12 months or longer. Twelve were nonrandomized, prospective observational studies and one was a randomized trial. In these studies, rates of acute PVI ranged from 100-67 percent while chronic efficacy for paroxysmal AF was 88-59 percent. The overall complication rate was low (6.1 percent) among all 44 studies, with 1 stroke, 2 TIA, 6 transient esophageal ulcerations, 3 delayed gastric emptying/gastroparesis and 7 pericardial effusions being the most serious adverse events in 2722 patients. As in the CAP, phrenic nerve palsy was the main complication reported in the literature, with an incidence of 4.66 percent compared to 11.2 percent in STOP AF and 3.7 percent in the CAP. Conclusions: Multiple studies, including data on over 1300 patients with at least one year followup duration, support the effectiveness and safety of the Arctic Front device and appear to be consistent with STOP AF results. Two ongoing, multi-center, nonrandomized postmarket studies sponsored by Medtronic continue to evaluate long term clinical outcomes of drug-refractory paroxysmal AF patients treated with the Arctic Front or the next generation Arctic Front Advance.

Po7 Development of a Novel Cryoablation Device for the Treatment of Atrial Fibrillation

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Cryoablation is an effective minimally invasive therapy for the treatment of atrial fibrillation offering patients a quicker recovery and reduced side effects. As the use of cryoablation increases, a need for the development of more powerful cryoablation devices has emerged. This need has been driven by technological limitations and early clinical outcomes, including lengthy periods to generate a single lesion (3-5 min), uncertain transmural and differential efficacy. As such, we have developed a novel cryogenic device (LEM) for the targeted ablation of cardiac tissue to treat atrial fibrillation (AF). The LEM technology employs liquid nitrogen (LN2) (-196°C) in a supercritical state to allow for the rapid and controlled application of ultra cold temperatures and efficient freezing of targeted tissue. Preliminary engineering and in vivo studies were conducted using 11 French flexible probes with 2 to 10 cm freeze zones. Test conditions included warm (37°C) water

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bath, heat load tissue and an in vivo canine model. Engineering research studies demonstrated ice formation at the tip of a cryoprobe within 5 sec and achieved a tip temperature of $<-150^{\circ}\text{C}$ within 10 sec. The LEM device repeatedly generated ice balls of 30mm x 40mm in less than 2 minutes in the water bath system. Heat load tissue model studies revealed the generation of a full thickness (8mm), 2 - 10 cm long cryogenic lesions (catheter/cryoprobe freeze zone dependent) within 1 min, achieving an opposite surface temperature of $<-100^{\circ}\text{C}$. Preliminary animal studies demonstrated the delivery of an ablative cryogenic "dose", producing a contiguous transmural linear lesion in less than 90 seconds. These studies confirm that the LEM supercritical nitrogen technology provides for rapid, effective, controllable freezing of targeted tissue. The ablative power, speed and directional freeze characteristics of the LEM system also offers the potential of improved safety via a reduction in procedural time compared to current cryoablation devices. Further, these technological developments may open new avenues for the application of cryo to treat other cardiac arrhythmogenic disorders.

Po8 Investigation into Cardiomyocyte Response to Cryoablation

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Atrial and ventricular tachyarrhythmias represent major cardiovascular diseases that may be treated utilizing thermal therapies. Given this there has been a palpable increase in technologies and approaches of therapeutic intervention, including cryoablation. While increased utilization of cryoablation continues, our fundamental understanding of the response of cardiac cells to the freezing process remains limited. As such we investigated the cellular and molecular response of cardiomyocytes to a range of subfreezing temperatures in an effort to provide further insight to help guide the application of cryoablation for the treatment of cardiac arrhythmias. Primary neonatal rat ventricular cardiac myocytes (NRVCM) and human cardiac myocytes (HCM) were cultured in vitro to study the cellular and molecular effects of thermal excursions. Cells were exposed to a range of temperatures from 37°C to -40°C for up to 1 min. Thermal profiles were generated to examine the cooling rates at each target temperature. Samples were then returned to culture and cell survival was measured via metabolic activity, membrane integrity and contractile function. Microfluidic flow cytometry was also conducted to investigate apoptotic involvement and confirm viability assessments of the various populations in a time-course manner. Exposure of NRVCM to temperatures ranging from 37°C to 0°C resulted in a minimal reduction in cell viability. Exposure to -5°C yielded 50% cell death. Temperatures ranging from -10°C to -20°C yielded less than 20% survival, whereas exposure to -40°C resulted in complete cell death. Regardless of the level of cell survival, exposure to temperatures below -5°C resulted in a loss of spontaneous contraction in surviving cells. HCM demonstrated moderate sensitivity ($<30\%$ loss) to temperatures above -10°C , however a steep decline in cell survival was observed from -12°C to -25°C , the point at which all cells were metabolically inactive. Flow cytometric analysis revealed the activation of apoptosis in cardiomyocytes in response to the freezing insult. The level and timing was found to vary with temperature, but not duration of the freeze. Although the utilization of cryoablation for the treatment of arrhythmias is established clinically, there remains a void in our knowledge base of the cellular and molecular responses of cardiac systems to thermal stress. This study, as well as others, are now providing mechanistic answers to the how cryoablation works at the cellular level as well as providing critical characterization data to help guide application and future development of therapeutic application.

Po9 Targeted Modulation of Integrin Expression Increases Freeze Sensitivity of Androgen-Insensitive Prostate Cancer

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Cryoablation has emerged as a primary therapy to treat prostate cancer. While effective, the assumption that freezing serves as a ubiquitous lethal stress is challenged by clinical experience and experimental evidence demonstrating time

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temperature related cell death dependence. The age-related transformation from an androgen-sensitive (AS) to an androgen-insensitive (AI) phenotype is a major challenge in the management of prostate cancer. AI cells exhibit morphological changes and resistance to many treatment therapies. This resistance has been linked with $\alpha\beta4$ integrin overexpression as a result of androgen receptor (AR) loss. As such, we investigated the influence of increased $\alpha\beta4$ integrin expression as a result of AR loss, on the reported increased freeze tolerance of AI prostate cancer. Further, we evaluated the targeted modulation of integrin expression in combination with cryoablation on human prostate cancer cell death. A series of studies using established AS (LNCaP LP and PC-3 AR) and AI (LNCaP HP and PC-3) cell lines were designed to investigate the cellular mechanisms contributing to variations in freezing response. Samples were frozen, thawed, and temporally assessed using fluorescence microscopy, flow cytometry and immunoblotting. Investigation into $\alpha\beta4$ integrin expression confirmed that AI cell lines overexpressed this protein, thereby altering cellular morphology and increasing adhesion characteristics. For instance, following freezing to -15°C , AI cells were found to exhibit increased resistance to freezing injury compared to AS cells (55% vs. 18%, respectively). Molecular investigations revealed a significant decrease in caspase 8, 9, and 3 levels in AI cells following freezing. Inhibition of $\alpha\beta4$ integrin in AI samples resulted in increased caspase activity and enhanced cell death. These studies demonstrate that integrin expression significantly influences cell tolerance to cryoablation. The data show that the inhibition of $\alpha\beta4$ integrin function results in a significant increase in freeze sensitivity of AI prostate cancer cells. Given these results, understanding the role of androgen-receptor related integrin expression in cell response to freezing may lead to novel options for neo-adjunctive approaches to treat prostate cancer.

P10 Rapid Induction of Apoptosis at Ultra Low Temperatures Enhances the Efficacy of Prostate Cancer Cryoablation

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Investigations into the molecular-based responses of prostate cancer following cold exposure have led to the discovery of delayed-onset, apoptotic cell death within the periphery of cryolesions. The apoptotic pathway typically attributed to this delayed death is the intrinsic/mitochondrial-mediated pathway characterized by a loss of mitochondrial potential, release of cytochrome c, and activation of caspase-9. Recent studies, however, have shown that at lower temperatures within the core of the cryogenic lesion ($<-20^{\circ}\text{C}$) a rapid programmed cell death response occurs. Using an engineered, 3-dimensional prostate tumor model, we investigated these events to determine the signaling pathway(s) responsible for the cell death as a means of developing improved molecular based approaches for the cryoablation of prostate cancer. Human prostate cancer cells (PC3) were cultured in the 3D matrices for 7 days prior to experimentation. The tumor models were then frozen to -30 or -15°C and analyzed at various times post-thaw using fluorescence microscopy, flow cytometry, and Western blots. Results demonstrated that the activation of apoptotic cell death occurred within 30 min of thawing at ultra low temperatures. At -30°C , $\sim 25\%$ of cells were apoptotic at 30 min and by 6 hr levels had dropped near those of controls. At elevated temperatures (-15°C), the activation and progression of apoptosis was considerably delayed, peaking at $\sim 20\%$ by 6 to 24 hr post-thaw. Additionally, it was determined that early onset apoptosis was regulated through a unique, caspase dependent process compared to that seen within the freeze margins. This induction was found to progress through a membrane mediated pathway associated with more severe thermal stressors as indicated by the activation of cas-pase-8 at low (-30°C) but not mild (-15°C) temperatures. These data suggest that an apoptotic continuum exists throughout the cryolesion whereby the more severe the cryogenic stress, the faster programmed cell death is manifested. The identification of this rapid-onset apoptosis within the core of the ablative zone represents a novel finding in a region previously thought to be only necrotic. Ultimately, it is our aim to decipher the signaling pathways involved in triggering rapid-onset apoptosis such that these events can be manipulated to enhance cell death, thus improving the overall efficacy of cryosurgical procedures.

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P11 Development of a Tissue Engineered Human Prostate Tumor Equivalent: Evaluation of Cryoablative Techniques

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The study of cancer biology is replete with debate and disagreement over the various analytical stratagems (*in vitro* vs. *in vivo*, animal vs. human) employed to develop therapeutic treatments. Yet, due to the highly variable nature of cancer cells and their acute sensitivity to extracellular microenvironments and culture conditions, it is critical that a culture model be chosen that closely resembles actual *in vivo* conditions in support of clinical relevance. As such, we hypothesized that the development of a three-dimensional (3D), tissue engineered human prostate cancer model would provide for a viable alternative, bridging the gap between issues with animal and *in vitro* models. In this study, we developed and tested the feasibility of such a model as well as a series of modified cell and molecular assays utilized to assess the effects of a given treatment on cell response. Human prostate cancer cells (PC3) were suspended in collagen matrices and variables such as gel thickness (1-3 mm), collagen density (0.5, 1, and 2 mg/mL), cell seeding density (1, 2.5, and 5 x 10⁶ cells/mL), and top-seeded vs. 3D-seeded, were evaluated for their influence on cell survival, proliferation, and response to a therapeutic stress. With the engineered model we were able to assess, using modified techniques, membrane integrity, metabolic function, and various cell death mechanisms. From our evaluations it was found that PC3 cells were able to survive and proliferate under several different model parameters, but a 2 mm thick collagen gel at 2 mg/mL seeded with 2.5 x 10⁶ cells/mL was chosen as the standard configuration. Additionally, the application of cryosurgical therapy to our 3D model revealed cell behavior characteristics similar to that of *in vitro* monolayer data. Through this study, we established a 3D, tissue engineered *in vivo*-like prostate cancer model along with a series of assessment techniques for utilizations in cancer research studies. Using this model, our aim is to better understand cancer cell responses to low temperatures, thus providing a new avenue for the improvement of cryosurgery.

P12 Identification and Characterization of Genes Crucial for Basal Freezing Tolerance in an Alpine Subnival Plant *Chorispora Bungeana*

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Cold acclimation improves freezing tolerance in plants. Many advances have been made toward identifying the signaling and regulatory pathways that direct the low-temperature stress responses. However, little is known about the mechanisms for naturally freezing tolerant species such as *Chorispora Bungeana* (*C. Bungeana*). This novel plant grows in high altitude regions with temperature ranging from 4°C to -10°C. We aim to isolate genes that are crucial for basal tolerance in *C. Bungeana* by suppression subtractive hybridization (SSH) with cold-treated (-4°C) and non-cold treated plant samples combined with microarray and real-time PCR analysis. A total of 85 genes including 52 up-regulated and 33 down-regulated genes were identified as candidates for basal freezing tolerance. These genes were sorted into broad functional categories including stress/ hormone stimulus response, photosynthesis, transcription, protein metabolism, ribosomal protein, etc. This suggests that a very complex series of molecular mechanisms are involved in the response to freezing stress in *Chorispora Bungeana*. Functional analysis indicates that a fibrillin protein FBN1a and an ACTIN cross-linking protein may act as the cold sensors by their physical changes caused by cold, and DREB2A signal pathway combined with ABA signal pathway may play a critical role in regulating the *COR* genes (cold responsive genes). Meanwhile, the downstream cold responsive genes, such as *KIN2* (*COR6.6*), *ZAT10* (*STZ*) and *GR-RBP8*, also play a very important role in freezing tolerance. In addition to that, some new genes were also identified successfully which indicated that there may exist some new signal pathway or mechanisms in *Chorispora Bungeana* in response to freezing stress.

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P13 In vitro Assessment of Apoptosis and Necrosis Following Cold Storage in Human Corneal Endothelial Cells

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Corneal endothelial cells serve as a limiting factor in the ability to preserve whole cornea for proper utilization, such as transplantation. Previous reports have demonstrated the importance of proper endothelial maintenance during preservation for improved corneal function. As such, analyses into the modes of cell death will allow for future in-depth investigations of the complex stresses and resultant changes cells experience during the entirety of the preservation process. To this end initial studies focused on assessing the extent of cellular demise via apoptosis and necrosis following cold exposure. We hypothesized that apoptosis would play a significant role in preservation-induced delayed cell death associated with exposure to hypothermic conditions. Human Corneal Endothelial Cells (HCECs) were stored for 18 hours to 8 days at 6°C in complete media, HBSS, ViaSpan and OptiSol. Viability was assessed 0, 24 and 48 hours post-storage using the metabolic indicator alamarBlue™ and flow cytometry (ViaCount Assay). Apoptotic and necrotic involvement was assessed via flow cytometry and fluorescent microscopy using the Vybrant® apoptosis assay at 1, 4, 8 and 24 hours post-storage. Mitochondrial potential was assessed via flow cytometry using the fluorescent dye JC-1. Western Blots were performed to evaluate alterations in apoptotic proteins including caspases, PARP, Bid, and the Bcl-2 family proteins. In addition to these analyses, the targeted inhibition of caspases using specific caspase inhibitors (8, 9 and pan) was conducted to evaluate their overall effects on cell tolerance to cold stress. Analysis of the sample sub-populations revealed that a peak in apoptosis was seen 4 to 8 hours after 1 day of hypothermic storage, approaching 10% under certain conditions. This analysis revealed that the necrotic population continues to increase in all conditions tested, peaking at 24 hours post-storage. Viability analysis demonstrated a significant impact by caspase inhibition on cell survival. Cells stored for 4 days in ViaSpan with and without a pan-caspase inhibitor revealed that while immediately post-thaw a similar level of viability remained between the two conditions (~65%), analysis at 24 hours post-storage revealed a ~30% improvement in cell retention post cold exposure. Samples stored in ViaSpan without inhibitor decreased to around 40-45% survival while the incorporation of a pan-caspase blocked delayed-onset cell death allowing cells to remain around 70% viable. Western blot analysis revealed a correlation between decreased viability and increased protein changes associated with apoptotic activation (i.e. cleavage of caspases, Bid, and PARP). These data are important because they are the first step in a more complete understanding of cold induced stress pathway activation.

P14 In vitro Assessment of Apoptosis and Necrosis Following Cold Storage in Human Airway Cells

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As advances in medical technology improve the ability and efficacy of cells and tissues to be transplanted, there remains a void in our knowledge of the specific molecular response of cells to low temperature storage. While much focus has been given to the formulation of solution to perfuse the tissues during storage to increase viability, investigations into the complex molecular changes that occur during cold exposure and their subsequent effect on outcome remain limited. An understanding of cellular stress on a molecular level is of fundamental importance as such information proves critical to tissue storage and transplantation as well as to the development of new technologies and processes in non-related areas of cell biology, bioprocessing, etc. The intent of this study was to begin to quantify the levels of cell death following hypothermic storage in a lung cell model in order to establish a foundation for future in depth molecular studies in support of improved lung transplantation. Normal human lung fibroblast (IMR-90) cells were stored statically for 1, 2 and 3 days at 4°C in basal media, complete media and ViaSpan®. Post-storage viability was assessed at 1, 2 and 3 days post-storage using the metabolic indicator alamarBlue. To determine the level of apoptotic and necrotic involvement, flow cytometry was performed and corroborated via visualization under fluorescent microscopy at 1, 4, 8, and 24 hours post-storage using the Vybrant apoptosis assay. Sample analysis revealed that cells stored in ViaSpan® were 68% (± 4%) viable after 24 hours of

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storage at 4C and repopulated to 103% ($\pm 6\%$) within 3 days. The other solutions resulted in complete cell loss after 24 hours of hypothermic storage with no re-growth observed. Extension of the storage interval to 3 days resulted in complete cell loss in all conditions. Analysis of the apoptotic and necrotic populations in the ViaSpan® stored samples revealed that 5% of the population was apoptotic at 8 hours post-storage while around 20% of the same population was found to be necrotic. The data revealed a high level of sensitivity to cold storage for this cell system, resulting in a significant amount of cell death, through both apoptosis and necrosis. These data highlight the critical need for a more in depth understanding of the molecular changes that occur as a result of cold exposure in cells.

P15 Regulation of PTEN Function and Structural Stability in Hibernating Thirteen-lined Ground Squirrels

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For many small mammals, survival over the winter months is a serious challenge because of low environmental temperatures and limited food availability. The solution for many species, such as thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*), is hibernation, an altered physiological state characterized by metabolic rate depression to achieve major energy savings, and a coordinated depression of non-essential ATP-expensive functions such as protein synthesis. This study examines the regulation of PTEN phosphatase, a negative regulator of the insulin receptor network, over the torpor–arousal cycle of hibernation in the skeletal muscle of *I. tridecemlineatus*. Immunoblots showed that the ratio of non-phosphorylated PTEN (Ser380, Thr382/383) to total PTEN levels were significantly elevated in (by 1.4-fold) during late torpor compared to euthermic controls; this was coupled with a significant decrease in Km for PIP₃ (by 59%) in late torpor. Pulse-proteolysis analysis of purified PTEN protein showed a decrease in structural stability of PTEN during late torpor compared to euthermic controls (decreased I₅₀ urea by 21%). Furthermore, the increase in PTEN activity observed was correlated with a decrease in the ratio of phosphorylated PDK-1 (Ser241) to total PDK-1 in late torpor, suggesting a downstream effect of PTEN activation during torpor. Transcriptional analysis showed that mRNA expression of both PTEN and PDK-1 was unchanged during the course of the hibernation cycle. Overall, the results indicate that post-translational modifications, more specifically phosphorylation, play a crucial role in regulating the enzymatic activity and half-life of PTEN, a major regulator of the insulin signaling pathway, during torpor. Funded by NSERC Canada. For more information go to: www.carleton.ca/~kbstorey.

P16 Muscle Disuse Atrophy: The Expression of Myocyte Enhancer Factor 2 in the Skeletal Muscle of *Spermophilus tridecemlineatus* During Hibernation

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Thirteen-lined ground squirrels, *Spermophilus tridecemlineatus*, are true hibernators that survive the winter by hibernating in underground burrows from August through to March. During hibernation, squirrels experience little or no muscle atrophy despite a reduced work load. Atrophy is minimized by changing the composition of muscle proteins to provide an optimal mix of isoforms. This study analyzed protein levels of Myocyte Enhancer Factor-2 (MEF-2) in skeletal muscle. MEF-2 proteins are transcription factors that regulate the expression of genes responsible for muscle remodeling in skeletal muscles. Western immunoblotting showed enhanced levels of MEF-2 and an increase in its nuclear localization in muscle of hibernating animals. Finally, Western blots of MEF-2 downstream genes were analyzed. An increase in protein levels of downstream genes supports the activation of MEF-2 transcription factors and the subsequent transcription of genes necessary for muscle remodeling. Funded by NSERC Canada. For more information go to: www.carleton.ca/~kbstorey.

P17 Antiapoptotic Signaling as a Cytoprotection Mechanism During Mammalian Hibernation

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Apoptosis (programmed cell death) is an essential natural biological process. A synergy between proapoptotic and antiapoptotic proteins, interacting at several signalling crossroads determines a cell's commitment to mitochondria-activated apoptosis. We proposed that changes in expression of antiapoptotic proteins may aid cytoprotection during

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hibernation of thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*) to cope with events including low body temperatures, changes in metabolic requirements, ischemia-reperfusion, muscle disuse, etc. Immunoblotting was used to analyze expression of proteins associated with apoptosis in three tissues. In brown adipose Bcl-2, cIAP and xIAP decreased significantly during torpor (compared with euthermia) whereas p-Bcl-2 (Thr 56), p-Bcl-2 (Ser 70), Bcl-X_L, Bcl-3, Mcl-1, and BI-1 were unaltered. In skeletal muscle only xIAP levels were altered during hibernation. In heart, most proteins increased significantly, except for Bcl-X_L (significant decrease) and xIAP/BI-1 (unchanged). The data show that antiapoptotic pathways have organ-specific responses in hibernators with a prominent potential role in heart. Funded by NSERC Canada. For more information go to: www.carleton.ca/~kbstorey

P18 Roles of the mTOR Signaling Pathway in Hibernating Ground Squirrels, a Differential Suppression of Active Protein Synthesis

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For many small mammals, survival during the winter months is a serious challenge because of cold environmental temperatures and limited food availability. The solution can be hibernation, an altered physiological state characterized by seasonal heterothermy and entry into long periods of torpor that are interspersed with short arousals back to euthermia. During torpor, metabolic rate is strongly depressed, often to just 1-5% of resting metabolic rate in euthermia. The 13-lined ground squirrel, *Spermophilus tridecemlineatus*, is a primary model species for hibernation research. A critical part of torpor is the coordinated strong suppression of nonessential ATP-expensive cell functions such as protein synthesis to achieve major energy savings. The mTOR signaling pathway is a critical component of the insulin receptor network and the primary cellular process that is responsible for regulating protein synthesis, through its direct interaction with P70S6K and 4E-BP, two regulators of ribosome assembly. Activity of the mTOR signaling pathway in skeletal and cardiac muscle was assessed at six different time points over the torpor-arousal cycle: active cold room (ACR), entrance of torpor (EN), early torpor (ET), late torpor (LT), early arousal (EA), and interbout arousal (IBA). Western blotting showed that phosphorylation of mTOR at the Ser2448 residue was strongly reduced in skeletal muscle (87%) during LT indicating reduced mTOR activity; however, phosphorylation of this residue in cardiac muscle was enhanced during EN and EA stages (by 2.9 and 3.2-fold, respectively), while remaining relatively constant during torpor. Evaluation of TSC2, an upstream inhibitor of the mTOR complex in its inactive form, showed reduced phosphorylation of TSC2^{Thr1462} in skeletal muscle (66%) during LT. Suppression of mTOR activity also lead to inactivation of selected downstream substrates; e.g. phospho-4EBP^{Thr46} and phospho-70S6K^{Thr389} contents decreased in skeletal muscle by 74% and 45% respectively during LT. Meanwhile, levels of phospho-S6 protein^{Ser235}, a component of the 40S ribosome in its active form, were also selectively reduced skeletal muscle (62%) during LT. Differential regulation of mTOR signaling during torpor suggests its alternative roles in tissue dependent responses. Funded by NSERC Canada. For more information go to: www.carleton.ca/~kbstorey

P19 Cryosurgery of Brain Tumors with Ultrasound Neuronavigation Control

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Goal of study: To estimate the results of brain tumor surgery with cryodestruction usage. **Materials and methods:** The cryodestruction of neuroepithelial tumors was performed in 18 patients. We used a new cryosurgical apparatus. This method of working is based on the passive liquid nitrogen supply and the active evacuation of nascent gaseous one. Tumors stereobiopsiya was performed before the cryoprobe introduction. Cryosurgery was performed with introducing the cryoprobe into the tumor with ultrasound neuronavigation control. There were MRI of brain before the operation and in 1, 3 and 7 days after it of all patients. The efficiency of the cryodesruction and the diagnostic value of ultrasound navigation to control the ice-ball generation and postoperative changes in cryodesruction and surrounding zones were estimated. **Results:** The ultrasound neuronavigation enabled to clearly visualize the tumor, the immersion of cryoprobe and ice-ball forming. With ultrasonography a cryoprobe looked like a hyperechoic structure with an acoustic shadow as in all cases with metal equipment. An iceball looked like a hypoechoic tissue near the cryoprobe with hyperechoic contour with 2-3mm

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thickness. Pre- and postoperative MRI permitted to compare zones of tumor and cryodestruction. The postoperative MRI showed that there was the intracerebral cyst formation in zone of cryodestruction of tumor. This cyst had the plain clear borders. There was no residual tumor tissue. The haemorrhage of cryodestruction and surrounding zones was not detected. According to MRI the clearest visualization of cryodestruction zone was up to 3 days. There was not any extension of perifocal edema within first 3 days however such insignificant one was found by the 7th postoperative day. The contrast accumulation at the border of destruction zone was marked by the 5th day, it was the effect of delimiting gliosis formation. **Conclusion:** Cryodestruction enables to destroy brain tumors efficiently in given volume. Ultrasound neuronavigation is an effective method of monitoring ice-ball formation in the brain during freezing and thawing in real time. This method allows to destroy the tumors which located in functionally significant brain areas and the attempts of removing ones is associated with a high risk of disability and death.

P20 Monitoring Prostate Cryotherapy by Vibro-acoustography

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Imaging frozen tissue with ultrasound is difficult because of strong reflection and refraction of ultrasound by frozen tissue. The goal of this study is to evaluate the feasibility and performance of vibro-acoustography (VA) in imaging the frozen tissue in prostate cryotherapy using an ex vivo model. VA is an acoustic imaging modality based on the radiation force of ultrasound. Using an experimental VA system, baseline VA scans of excised human prostate placed in a tank of degassed water are acquired. Then, alcohol and dry ice mixture are used to partially freeze prostate tissue samples. The frozen prostates are subsequently placed in the water tank at 27 °C. VA images are acquired at prescribed time intervals to characterize the image properties of the partially frozen tissue at different stages of thawing. The VA images show the frozen tissue as a uniformly solid region with enhanced borders. Once the tissue thaws, previously frozen regions show coarser texture than prior to freezing. The thawed cryolesions show a different contrast compared the normal unfrozen prostate. In conclusion, this pilot study shows that VA produces clear images of a frozen prostate tissue where the size of frozen region can be clearly identified at different stages. These characteristics suggest that VA may be a suitable tool for guiding prostatic cryosurgery. The promising results of this in vitro pilot study provide substantial motivation to further investigate VA modality for intraoperative imaging of prostate cryosurgery.

[not presented]

Thursday, January 3rd, 2013 – Deck 3, Cinema Conference Center

5:30 PM **Keynote K02: Advances in Cryoablation**

John G. Baust¹, Anthony Robilotto^{1,3}, Kimberly Santucci^{1,3}, A.A. Gage^{2,3} and John M. Baust^{1,3}

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Clinically-based cryoablation, grounded on well-recognized scientific principles along with the use of multiprobe devices and advanced imaging techniques, has experienced a rebirth in physician-managed destruction of diverse cancers. Cryoablative techniques have beneficially evolved over the past forty years with the development of successive generations of devices including cryoneedles, cryoballoons, intraoperative ultrasound and an expanded knowledge of the mechanisms by which cancer cells are challenged by low temperatures. A key discovery in 1998 identified the putative role of gene regulated cell death (apoptosis) in the management of the freeze zone. We now recognize three modes of cell death following a freezing insult: ice-dependent cell rupture in the tumor core, necrosis (primary and secondary) throughout the tumor and apoptosis. The AUA 2008 Best Practice Policy Statement on Cryosurgery for the Treatment of Localized Prostate Cancer recognizes that “prostate cancer cells experiencing multiple molecular-targeted stressors (cytotoxic agents) succumb more readily to low temperature exposure and that with the adoption of appropriately paired combinations, even freezing at -1 °C can be totally lethal.” This presentation will focus on the evolution of recent developments supportive of the use of combinatorial cryoablative strategies that may raise the ablative temperature to near -1 °C. Data will demonstrate the existence of an “apoptotic continuum” whereby the more severe cryogenic stress activates the extrinsic (membrane mediated) apoptotic pathway while less severe freezing activates the intrinsic (mitochondrial mediated) path. Ultimately, it is our aim to decipher the events and signaling pathways that are specifically involved in triggering these diverse cryo-induced mechanisms of cell death. Once known, cryosurgical procedures might be modified such that rapid-onset and delayed programmed cell death pathways would be selectively and preferentially induced in an effort to improve the overall efficacy of cryoablation. Early evidence suggesting that select cryo-sensitizers offer the potential to improve freeze sensitivity will be presented.

6:15 PM – 7:30 PM

**Session 1: Gastroenterology:
Cryotherapy via flexible endoscopy in the GI tract**

Session Chair: Jay Pasricha, M.D.

Speakers:

Dr. Anthony N. Kalloo, M.D.

Dr. P. Jay Pasricha, M.D.

Dr. Marcia Irene Canto, M.D., MHS

Dr. Kristi K. Snyder, Ph.D.

Clinical Session Overview: In this series of talks, we will provide an overview of the applications of cryotherapy in the GI tract, followed by preclinical experimental data on dosimetry and pathological effects. Then an update on the use of this therapy for Barrett’s will be provided.

A01 Overview of GI Indications and Cryo Technology via Flexible Endoscopy

Anthony N. Kalloo

The Johns Hopkins Hospital

Since the mid-1960’s, Cryotherapy was a technique in search of an indication for gastrointestinal diseases. In those early days, Cryotherapy although hampered by technical challenges was advocated for treating duodenal ulcers, gastric erosions, and hemorrhagic gastritis. By this time, Cryotherapy was showing great promise in other clinical areas such as dermatologic and prostatic conditions. Despite this initial enthusiasm, the results of clinical trials for these conditions were disappointing and when added to challenges of technically delivering freezing therapy to the gastrointestinal tract, Cryotherapy was soon

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forgotten as a modality to treat gastrointestinal disease. However the groundbreaking work by Pasricha and colleagues from Johns Hopkins in 1999 described for the first time an endoscopic device that was capable of delivering freezing therapy to the gastrointestinal mucosa. These initial experiments performed in an animal model demonstrated the feasibility of Cryotherapy with a compressed gas. This was soon followed by experiments demonstrating the feasibility of using low pressure liquid nitrogen by an endoscopic catheter. The initial clinical trials focused on diseases that would respond to ablating the superficial mucosa such bleeding arteriovenous malformations, radiation gastritis and proctitis and Barrett's esophagus. Using Cryotherapy for palliation of advanced gastrointestinal tumors has shown success in case reports but has been less well studied. Two different techniques have evolved for delivering cryotherapy. The initially described technique used compressed gas such as nitrous oxide and CO₂. Rapid expansion of the gases results in a significant drop in local temperature enabling cryogenic damage to the mucosa. The second technique involves direct application of low pressure liquid nitrogen to the mucosa. The techniques appear to have similar efficacy. Although lacking in prospective randomized trials, endoscopic cryotherapy is a promising modality for the treatment of gastrointestinal mucosal disease

A02 Experimental Data on Ablation Responses in the GI tract

P. Jay Pasricha

Johns Hopkins University School of Medicine

The development of flexible endoscopes has revolutionized the practice of gastroenterology, with the ability to both diagnose and treat a variety of lesions. Many of the current therapeutic approaches to endoscopically amenable lesions involve some form of thermal injury to produce either tissue ablation (for growths) or vascular coagulation (for bleeding). However, heat injury has several drawbacks, even with the use of newer devices such as argon plasma coagulation (APC). Present methods are either cumbersome and expensive (laser) or cannot truly be applied in a non-contact manner (heater probes) and thus require excellent visualization of the target lesion, (can be a problem in patients characterized by brisk bleeding). A further problem with thermal devices is the amount of time required to complete therapy when dealing with multiple lesions in the gut (such as arteriovenous malformations). Finally, the depth of injury with thermal methods is difficult to control and there is a risk of thermal injury to the intestine. Cryotherapy is a non-contact ablation method that has long been used clinically in the treatment of a wide variety of malignant and premalignant diseases. The relative ease of use and unique mechanisms of cellular destruction make cryotherapy particularly attractive for the eradication of dysplastic Barrett's esophagus. Currently, liquid nitrogen and carbon dioxide are the most common cryogens used. Preliminary data with these agents have shown high efficacy in the reversal of dysplastic Barrett's mucosa and excellent safety profiles. Intense investigation on cryotherapy ablation of Barrett's esophagus is ongoing. Currently there are two cryoablative systems for use in gastroenterology. This first, the CryoSpray Ablation System (CSA Medical, Inc, Baltimore, Maryland) uses a super-cold cryogen and uses a multi-layered 7F catheter coated with a special polymer on the outer sheath is warmed during delivery of the cooling agent to retain its flexibility. With this system, an orogastric suction tube is inserted into the stomach for luminal decompression. A distal port and side holes along the suction tube allow for both gastric and esophageal decompression. Luminal decompression is imperative to prevent perforation since liquid nitrogen can expand into 6 to 8 liters of gas during each 20 seconds of treatment. The endoscopist controls the cryoablation and suction decompression with a foot pedal provided with the system. The other system is the Polar Wand cryotherapy device (GI Supply, Camp Hill, Pennsylvania) and was developed utilizing carbon dioxide (CO₂) gas as a cooling agent. This system employs the Joule-Thompson effect whereby high pressured gas, in this case CO₂, is forced at or near ambient temperature through the catheter and upon reaching the distal tip, a sudden and rapid expansion of the gas from a higher pressure to atmospheric pressure causes a massive drop in temperature. At flow conditions of 6-8 L/min, end effector temperatures of -78 °C can be achieved. The ablation catheter is passed through the working channel and a suction catheter is attached to the tip of the endoscope for decompression. In contrast to liquid nitrogen based cryoablation system, the cryocatheter in the accessory channel remains at ambient temperature thus preserving normal endoscopic maneuverability. Furthermore, expensive cryogen holding tanks used to contain liquid nitrogen is also not necessary when using CO₂ based system. Finally, another major technical advantage of this system is the ability to spray the mucosa at will, producing rapid injury of large areas without the need for contact. However, because of the large volume of cryogen gas exiting the catheter, venting is still required.

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A03 Barrett's Esophagus- A Perfect Target for Cryo-ablation

Marcia I. Canto

Johns Hopkins University School of Medicine

Endoscopic cryotherapy is used for a variety of indications in the gastrointestinal tract, including treatment of chronic gastric antral vascular ectasia (GAVE), radiation proctitis, and Barrett's esophagus. Barrett's esophagus (BE) is a known precursor to esophageal adenocarcinoma resulting from chronic gastroesophageal reflux disease. Endoscopic treatment of high grade neoplasia (high grade dysplasia) and early adenocarcinoma in Barrett's esophagus is the preferred first line approach for most patients. This includes endoscopic mucosal resection (EMR) for nodular disease or focal neoplastic lesions, and mucosal ablation for flat neoplasia. Current ablative techniques include treatment with heat energy (such as radiofrequency ablation or RFA), cryogen (cryotherapy or cryoablation), and laser plus photosensitizing drug therapy (photodynamic therapy). Although less widely available and studied compared to RFA, endoscopic cryotherapy using liquid nitrogen or compressed gas such as carbon dioxide or nitrogen have excellent safety and efficacy profiles. The complete response rates for elimination of high grade neoplasia in Barrett's esophagus are comparable to those of RFA, whether it used for primary or salvage therapy. Cryotherapy can also be applied when RFA is contraindicated or associated with greater risk (such as in patients with esophageal strictures, radiation therapy, eosinophilic esophagitis, cardiac defibrillators, on chronic anti-platelet or anticoagulation therapy). Furthermore, a few studies of cryotherapy also demonstrate long term response rates for endoscopic treatment and palliation of esophageal adenocarcinoma, which cannot be treated with RFA. The incidence of side effects of cryotherapy, when used properly, is lower than that of RFA, particularly with less post-treatment pain and dysphagia. Patients can usually resume a normal diet, unlike RFA. Lastly, the current systems and cost of cryotherapy are significantly lower than that of RFA or photodynamic therapy. New methods of applying endoscopic cryotherapy are being developed to improve the portability and technical ease. Cryotherapy is an excellent mucosal ablative technique for the gastrointestinal tract.

A04 Multi-dimensional Assessment of Pancreatic Cancer Cell Responses to Cryoablation *In vitro*

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As the incidence of pancreatic cancer (PaCa) continues to increase worldwide with little improvement in the overall prognosis, there is a drive to identify alternative strategies for the treatment of PaCa to prolong patient survival. To this end, several reports have described the potential benefits of the use of cryoablation for the treatment of PaCa. While promising, few studies have been reported examining the physical and molecular response of PaCa to cryoablation. An understanding of these responses is critical to serve as supportive and guidance information if the use of cryo for the treatment of PaCa is to continue to grow. In an effort to detail the effects of freezing in pancreatic cancer (PaCa), a human PaCa cell line (Bx-PC3) was evaluated *in vitro* and using a *vivo-like* tissue engineered model (*PaCaTEM*). Bx-PC3 were exposed to a range of freezing temperatures from -10°C to -20°C and compared to non-frozen controls. *PaCaTEM* models were frozen *in situ* following a single 5min freeze protocol using the supercritical nitrogen (SCN) cryoablation system with a 1.5mm x 3cm cryoprobe. Following freezing, cell survival was assessed in the *in vitro* studies using the metabolic activity indicator, alamarBlue and microfluidic flow cytometry (MFC). Assessment of *PaCaTEM* samples included monitoring of iceball size and isothermal distribution during the freezing process as well as assessment of cell ablation at 1 and 24 hours post-freeze via fluorescence microscopy (Vybrant apoptosis assay). Results from *in vitro* experiments showed that freezing to -10°C did not affect Bx-PC3 viability, while -15°C and -20°C resulted in a significant loss of viability (90% and 98%, respectively). A complete loss of cell viability was evident at temperatures of -25°C and colder *in vitro*. Temporal analysis of the cell response to freezing via MFC revealed that following exposure, there was an initiation of both apoptotic and necrotic responses. Specifically, following freezing to -15°C, apoptotic cell death was found to peak at 4hrs and necrosis at 8hrs post-thaw. Analysis of the *PaCaTEM* samples revealed the creation of a 3.0cm (±0.2cm) diameter iceball with extension of the -25°C isotherm to a diameter of 2.0cm (±0.1cm). Fluorescence imaging of the *PaCaTEM* revealed complete PaCa cell ablation to a diameter of 1.9cm (±0.2cm) with a transitional zone of cell death extending to the margin of the iceball. This zone of ablation correlated to complete cell destruction below 25°C in *in vivo-like* tissue. The results of this study show that cryoablation is an effective tool for the targeted ablation of pancreatic cancer. Further, the data suggest that a temperature of -25°C may serve as a target temperature to be reached at the margin of a tumor to assure complete PaCa destruction.

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2:00 PM Keynote K03: Cryo-Immunology: The Stimulatory and Suppressive Effects of Cryoablation on the Immune Response

Michael S. Sabel, MD, FACS

University of Michigan, Ann Arbor, MI

Cryoablation as a cancer therapy dates back over one hundred years, however recent advances in technology have greatly expanded its clinical potential. Much of the interest in cryoablation centers around early observations that after freezing a primary lesion, distant, un-treated sites of disease began to regress. It was believed that this was secondary to cryoablation stimulating an anti-tumor immune response. This prompted multiple pre-clinical studies examining the impact of cryoablation on the immune response and the mechanisms involved. While most of these studies validated the ability of cryoablation to stimulate a tumor recognition by the immune system, some studies demonstrated the opposite; tumor bearing animals treated by cryoablation had diminished responses and increased tumor growth compared to controls. As our knowledge of the components of the immune system and their interactions in the generation, or suppression, of an immune response has increased, as well as our understanding of the mechanisms by which freezing leads to cell death, the immunomodulation seen with cryoablation is becoming clearer. Many questions still remain, but given the tremendous clinical potential in having a local therapy that could also have systemic benefits, further research in this area is strongly warranted. This talk will review the history of cryosurgery for the treatment of cancer; detail the mechanisms by which cryoablation leads to cancer cell death, and how this can be altered by variations in cryosurgical technique; and describe the pre-clinical data examining the relationship between cryoablation-induced cell death and both stimulatory and suppressive immune responses.

3:00 PM – 4:40 PM Session 2: Multi-Modal Treatments: Combinatorial Strategies & Immunology

Session Chair: John C. Bischof, Ph.D.

Speakers:

John C. Bischof, Ph.D.

Xiaoming He, Ph.D.

Yaron Har-Shai, M.D.

Patrick Le Pivert, M.D.

Robert Griffin, M.D.

A05 Nanoparticle Delivered Vascular Disrupting Agents (VDAs): A New Opportunity in Multimodal Cancer Treatment

Mithun M. Shenoi¹, Isabelle Iltis², Jeunghwan Choi³, Nathan A. Koonce⁵, Greg J. Metzger², Robert J. Griffin⁵, **John C. Bischof**^{1,3,4},

¹Departments of Biomedical Engineering, ²Radiology (CMRR), ³Mechanical Engineering and ⁴Urologic Surgery, University of Minnesota, Minneapolis, MN, USA;

⁵Department of Radiation Oncology, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Surgery, radiation and chemotherapy remain the mainstay of current cancer therapy. However, treatment failure persists due to the inability to achieve complete local control of the tumor and curtail metastatic spread. Vascular disrupting agents (VDAs) are a class of promising systemic agents that are known to synergistically enhance radiation, chemotherapy or thermal treatments of solid tumors. Unfortunately, there is still an unmet need for VDAs with more favorable safety profiles and fewer side effects. Recent work has demonstrated that conjugating VDAs to other molecules (PEG, NGR) or nanoparticles (liposomes, gold) can greatly reduce toxicity of one prominent VDA (tumor necrosis factor alpha, TNF- α). In this report, we show the potential of a gold conjugated TNF- α nanoparticle (NP-TNF) to improve multimodal cancer therapies with VDAs. In a dorsal skin fold and hindlimb murine xenograft model of prostate cancer, we found that NP-TNF

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disrupts endothelial barrier function and induces a significant increase in vascular permeability within the first 1-2 hours followed by a dramatic 80% drop in perfusion 2-6 hours after systemic administration. We also demonstrate that the tumor response to the nanoparticle can be verified using dynamic contrast-enhanced magnetic resonance imaging (MRI), a technique in clinical use. Additionally, multimodal treatment with thermal therapies at the perfusion nadir in the sub- and supra- physiological temperature regimes increases tumor volumetric destruction by over 60% and leads to significant tumor growth delays compared to thermal therapy alone. Lastly, NP-TNF was found to enhance thermal therapy in the absence of neutrophil recruitment, suggesting that immune/inflammatory regulation is not central to its power as part of a multimodal approach. Our data demonstrates the potential of nanoparticle-conjugated VDAs to significantly improve cancer therapy by preconditioning tumor vasculature to a secondary insult in a targeted manner with limited systemic toxicity or inflammatory response. We anticipate our work to direct investigations into more potent tumor vasculature specific combinations of VDAs and nanoparticles with the goal of transitioning optimal regimens into clinical trials.

Keywords: vascular disrupting agent; gold nanoparticles; TNF-alpha; DCE-MRI; thermal therapy; cancer; CYT-6091 **Abbreviations:** dorsal skin fold chamber (DSFC), Gadolinium-DTPA (Gd), high-temperature thermal therapy (HTT), tumor necrosis factor alpha tagged gold nanoparticle (NP-TNF), tumor necrosis factor alpha (TNF- α), vascular disrupting agent (VDA). **Acknowledgements:** Funding from NIH 2R01 CA075284 (JCB), a Distinguished McKnight University Professorship (JCB), a Grant in Aid seed grant (JCB) and an AHC Seed Grant (GM) are gratefully acknowledged. This work has been submitted to Molecular Pharmaceutics and is currently in review.

A06 Effective Destruction of Cancer Stem Cells by Combing Freezing and Anticancer Drug Encapsulated in Nanoparticles

Wei Rao¹, Xiaoming He^{1*}

¹Department of Biomedical Engineering, The Ohio State University, Columbus, OH, USA

Recent studies show that not all cancer cells in a tumor are equally tumorigenic, but only a subpopulation (up to ~3%) of cancer cells (the so-called cancer stem or tumor initiating cells) that have the capability of creating the heterogeneity of a solid tumor. The putative cancer stem cells have been proposed to be responsible for cancer metastasis, resistance to conventional therapies such as chemo and radiotherapy, and cancer recurrence after treatment. As a result, it is of utmost importance to destroy the putative cancer stem cells for effective cancer destruction. In this study, we investigated the effect of freezing at various temperatures (from -5 to -20°C) on destroying the putative cancer stem cells. Moreover, we further investigated the combination of freezing at the various temperatures and doxorubicin (an anticancer drug) that is either free or encapsulated in nanoparticles in augmenting destruction of the putative cancer stem cells. Our results show that the combination of freezing and doxorubicin encapsulated in nanoparticles is the most effective for killing cancer stem cells.

A07 Intralesional Cryosurgery for Enhancing the Involution of Hypertrophic Scars and Keloid - A New Fundamental Adjunctive Wound Healing Therapy Based on Experimental and Clinical Data

Yaron Har-Shai

The Unit of Plastic Surgery, Carmel Medical Center, Haifa, Israel

A novel cryoneedle (CryoShape, Etgar Group Ltd, Israel) is inserted into the hypertrophic scars and keloid (HSK). It is connected to a canister of liquid nitrogen, which causes the cryoprobe to freeze thereby freezing the HSK from the inside out. 95 Caucasian patients, with 112 HSKs more than 6 months duration were enrolled. The 18-month trial evaluated HSK volume reduction following a single cryo-session. Objective and subjective clinical parameters were evaluated. Pre- and post-treatment biopsies were taken for histo-morphometric studies of the collagen fibers and for collagen structure. Mast cells and blood vessels were identified and cell proliferation was estimated by proliferating cell nuclear antibodies. Surface thermal behavior measurements using thermocouples were executed to measure the thermal history. An average of 51 % of scar volume reduction was achieved following a single cryo- treatment. For ear HSK 67% reduction and for the upper back and shoulders 60%. In 8 scars no response has been documented. Significant alleviation of objective and subjective clinical symptoms was achieved. During the follow-up period there was no worsening or infection of the HSK and only minimal hypopigmentation. The histomorphometric analysis demonstrated rejuvenation of the treated scars. The collagen bundles became more compact. A clear distinct transition zone separated the cryo-treated from the unaffected area. The frozen tissue was devoid of proliferating cells and of mast cells whereas the number of blood vessels remained unaltered. Most of the fibroblasts expressed all tested myofibroblast markers. The surface thermal history showed a slow cooling and

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thawing rates, and less pronounced end temperature, which is "friendly" to the melanocytes. A significant long hold time was documented. A pain control protocol has been applied which involved oral pain-relief tablets and translesional local anesthesia. This protocol significantly reduced pain severity during the cryosurgery treatment to tolerable levels (VAS ≤ 3 cm). The intralesional cryosurgery technology is a new, evidence-based and fundamental adjunctive wound healing therapy which is responsible for the rejuvenation of post-cryosurgery HSK tissue by creating a normal wound healing environment.

A08 Image-Guided Concurrent Chemoablation-and Cryoablation for Lung Cancer: A Preliminary Clinical Investigation.

Patrick Le Pivert¹, Yueyong Xiao², Shi-Rong Liu², Xiao Zhang², Jin Lin Tian²

¹ *Interventional Drug Delivery Systems & Strategies, Jupiter, FL USA,*

² *Department of Radiology, Chinese PLA General Hospital, Beijing, China*

We report on the feasibility of CT guided percutaneous concurrent interstitial chemoablation-and-conformal Cryoablation therapy for inoperable lung tumors compared to conformal cryoablation only. The goal is to control and improve the cryo-kill zone at the margin of the target lesion with the intra-operative addition of cytotoxic free drug(s). The combination method is performed on selected patients as follows: Under CT guidance, depending on the size and location of tumors two or more cryoneedles are inserted into target along with injection needle. Injection needle is centrally located and cryoneedles shall freeze up to the tumor margins as in our conventional procedure (conformal cryoablation¹). The drug dosage is calculated on the tumor volume. Solution or emulsion of cytotoxic and/or chemical drug mixture with an imaging hydrosoluble (Hs) or liposoluble (Ls) contrast agent is prepared at time of intervention and injected a few seconds before conformal cryoablation. Intra lesional drug distribution and cryoablation is assessed by sequential CT for proper tumor drug filling and for adequate tumor freezing (ice coverage). Post operative CT is performed to assess the changes in tumor density, dimension and the effectiveness of cryo-chemoablation. Results show that drug Injection immediately preceding cryoablation is always doable with easy visualization of contrast agent distribution regardless of the associated drug characteristics. Ls contrast is filling tumor site better than Hs contrast but hinders somehow perfect visualization of ice ball margins. Ls contrast is trapped within tumor conversely to Hs contrast that disappears after a few minutes. Both contrast agents are not displaced by tumor freezing. Peripheral lung parenchymal reaction seems grander with drug-Hs contrast mixture than with drug Ls contrast mixture. Conclusion: per cutaneous CT guided concurrent interstitial chemoablation-and-cryoablation for lung tumors is feasible and allows controllable drug localization in this short series of patients. It does not add complexity or safety risks to the conformal cryoablation technique. The search for and demonstration by CT imaging of drug distribution and/or prolonged retention at cryotreated tumor margins needs improvements in the drug-to-contrast agent formulation, and timing of drug/contrast-to-cryo administration.

A09 Role of Tumor Microenvironment in Cryosurgery: Opportunities for New Approaches to Multi-modality Cancer Treatment

Robert J. Griffin¹, Gal Shafirstein², John Bischof³, Klressa Barnes¹

¹ *University of Arkansas for Medical Sciences, Department of Radiation Oncology, Little Rock, AR USA;*

² *Roswell Park Cancer Institute, Buffalo, NY US;*

³ *University of Minnesota, Department of Mechanical Engineering, Minneapolis, MN USA*

Tumor tissue that is damaged by thermal therapy may release a unique set of factors in an attempt to attract cells to repair and regrow the tumor, many times via an angiogenic/vasculogenic activation which also leads to changes in perfusion and oxygenation. The mechanisms and factors that are involved with this reaction suggest that blood supply and oxygenation is changed in tumor that remains viable after ablation and concomitant application of chemotherapy or radiation therapy may therefore improve tumor control. We have found that cryo- and thermal ablation affect the already extremely complex pathophysiology of solid tumor progression in similar ways. Cryoablation induces cellular membrane damage and cytotoxicity by the formation of intracellular ice crystals, solution effects, microvascular thrombosis and apoptosis. High temperature thermal ablation techniques can induce complete coagulative necrosis by subjecting the target tissue (tumor and margins) to enough heat to meet or exceed a threshold thermal dose. We have studied mouse and rat tumor models using various thermal ablation methods at an average peak temperature of 80-90C or -140C to ablate a portion of the tumor (50% or more) allowing us to study the remaining viable tumor tissue. Interestingly, freezing induces a transient nadir in tissue pH, which we have found can sensitize surviving cells to subsequent freeze cycles, or even be used to

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sensitize cells to hyperthermic temperatures. One approach that has been taken in our preclinical studies is to use an initial freezing cycle to cause thrombosis that will impair the ability of the vasculature to dissipate heat from the tissue during heat stress resulting in more uniform thermal dose patterns upon a subsequent application of high temperature therapy. The tumor oxygenation in viable tissue after thermal or cryoablation was found to increase compared to untreated tumor tissue. In control 4T1 tumors where 27% of the tumor was found to be severely hypoxic on average, only 5% remained hypoxic in thermally ablated tumors. In FSall fibrosarcoma tumors, the pO₂ increased substantially from 6-72 h after a cryoablation procedure. In our recent studies, increased tumor regression was observed in animals receiving combined thermal ablation and single dose 20 Gy irradiation in the MATBIII rat tumor model, suggesting that cryoablation combined with radiotherapy may have similar effects. Ultimately, the use of cryosurgical procedures in combination with single or several high dose, conformal radiotherapy treatments is expected to open up new multi-modality approaches to tumor control. Possible effects of cryosurgical procedures on imaging aspects and radiosensitivity for rational multimodality therapy will be discussed. This work was supported by NIH grant CA44114 and grants from the Focused Ultrasound Surgery Foundation, Winthrop P. Rockefeller Cancer Institute and the Arkansas Breast Cancer Research Program via funding from the Fashion Footwear Association of New York.

5:00PM – 6:15 PM

Session 3: Clinical Cryosurgical Experience

Session Chair: Kristi K. Snyder, Ph.D.

Speakers:

Kimberly Santucci.
Michael Sabel, Ph.D.
Nikolai Korpan, MD, Ph.D.
Barlian Sutedja, MD

A10 Cryosensitization Approaches in Cryotherapy

Kimberly L. Santucci^{1,2,3}, Kristi K. Snyder^{1,3}, John M. Baust^{1,3}, John G. Baust^{1,2}

¹*Institute of Biomedical Technology, State University of New York, Binghamton, NY 13902 USA*

²*Department of Biological Sciences, Binghamton University, Binghamton, NY 13902 USA*

³*CPSI Biotech, Inc., 2 Court Street, Owego, NY 13827 USA*

Cryoablation for the treatment of prostate cancer (CaP) is increasingly utilized in the clinical arena. Due to the sensitivity of surrounding tissues to freezing, implementation of a positive freeze margin often presents challenges. Co-morbidities coupled with the risk of a zone of incomplete ablation at the periphery of a cryogenic lesion may be reduced by increasing cryotherapy's total ablative potential via a combinatorial approach. Various agents have been utilized in vitro in an attempt to synergistically increase cancer cell death in conjunction with freezing. Previous studies have shown that chemotherapeutic drugs such as taxotere and 5-FU are effective cryosensitizers. More recently we have shown the combinatorial potential of calcitriol, the active metabolite of vitamin D₃. In both human and murine models, 24h calcitriol exposure prior to freezing to temperatures associated with the periphery (-10°C through -25°C) resulted in increased cell death compared to freezing alone. Specifically, in the androgen insensitive human CaP line PC-3, 24h calcitriol pre-treatment yielded a 36% increase in cell death at the -15°C isotherm compared to temperature-matched controls (60% viable vs. 96%, respectively). In addition to 2D in vitro modeling, studies were also conducted using a novel 3D prostate tissue engineered model (pTEM) system. CaP cells grown in this 3D matrix were frozen using a novel supercritical nitrogen (SCN) device in the presence or absence of calcitriol as a cryosensitizer. Results indicate that the zone of ablation within the iceball increased by 20% with the addition of calcitriol. These in vitro studies illustrate the potential benefits of calcitriol in combination with a cryosurgical treatment regime in androgen insensitive CaP. Additionally, we also investigated the "long-term" calcitriol cryosensitization potential using an in vitro dose escalation scheme. In order to model an oral VD₃ regime we utilized a 1nM initial dose of calcitriol followed by a 5nM increase every 2 days, resulting in a 32-day gradual escalation to 80nM. Calcitriol dose escalation yielded a significant increase in cell death over the 24h pre-treatment in all temperatures examined. Additionally, maintenance of 80nM calcitriol for 20 days after the escalation scheme resulted in complete cell death at the -25°C isotherm with no re-growth after 3 days. Interestingly, when calcitriol treatment was

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discontinued after the escalation scheme this sensitization benefit was lost. These results suggest that in a prolonged oral treatment regime, vitamin D3 may provide for improved CaP sensitivity to cryoablation thereby increasing cell death while reducing the need for an enlarged positive freeze margin. The results of this study suggest a potential “benchtop to bedside” framework for future clinical translation.

A11 Cryoablation as an Alternative to Lumpectomy for Breast Cancer

Michael S. Sabel

University of Michigan, Ann Arbor, MI USA

With improvements in technology, cryoablation as an alternative to surgery, has been used to treat cancers of the skin, prostate, liver, lung and bone. There is significant interest in cryoablation of breast cancer. Potential advantages over lumpectomy include the minimally invasive nature of the treatment, improved cosmetic results, patient comfort, decreased costs and the possible immune response to the ablated tissue. Several pre-clinical and clinical studies have demonstrated both the feasibility of breast cancer cryoablation and the generation of an anti-tumor immune response, and clinical experience with cryoablation of benign breast tumors is growing. The on-going American College of Surgeons Oncology Group Trial Z1072, a phase II trial examining the success of breast cancer cryoablation, is an important first step in possibly replacing lumpectomy with cryoablation for a subset of breast cancer patients. This discussion will review the breast cryoablation literature to date and discusses the directions the field needs to move in to realize that goal.

A12 Cryosurgery for Breast Cancer: A Challenge in Current and Future Invasive Oncology. Clinical Experience since 1995

Nikolai N. Korpan, MD, PhD, FICS

International Institute for Cryosurgery, The Rudolfinerhaus, Vienna, Austria

Background: Breast cancer is the most common type of cancer in women, with approximately one in nine women developing the disease in her lifetime. Breast cancer is also one of the leading causes of cancer death among women. **Materials and Methods:** In the past seventeen years our concept of cryogenic surgery was developed in the treatment breast malignant diseases namely breast cancer cryosurgery (BCC) for the first time and involved the use of own discovered universal cryosurgical system with multiple probes for combined surgical treatment breast cancer in different stages. The use of universal cryosurgical technology in surgical treatment patients with malignant diseases has tremendously expanded and has evolved into what is now considered an established discipline within the practice of surgical oncology, revolutionizing the surgical management of many malignancies, including breast cancer. We have attempted to comprehensively evaluate the history, technical aspects, and clinical applications of breast cancer cryosurgery using universal cryosurgical technology in the current presentation. Modern breast cryosurgery for primary breast carcinoma aimed at preventing local recurrence and distant metastases with preoperative mark and location of breast tumor through ultrasonography and MRI-mammography. The cryosurgical procedure was performed at -180 °C using original universal cryosurgical unit with cryosurgical instrument connected to cryoprobes in diameter from 5 mm to 55 mm. In patients with breast exulcerated cancer the cryospray was applied on the post-resected tumor loge. In patients with breast local recurrence the breast cancer cryosurgery was administered aimed at preventing re-local recurrence and distant metastases. As well, the patients with multiple skin metastases after the breast ablation were treated by universal cryosurgical unit using special cryosurgical probes at temperature of -180 C in two freeze-thaw cycles. When breast tumours were treated cryosurgically, monitoring was of practical concern. During these cryosurgical procedures, “iceball” growth was routinely monitored by ultrasound intraoperatively. **Results:** A new standard has been formulated for modern breast oncology based on our own results achieved in the field of cryoscience, cryomedicine and cryosurgery as well as modern cryotechnology using novel theoretical, experimental, clinical and technological research. **Conclusion:** The present research and clinical results have established a new level in breast cancer surgery. The innovative modern cryosurgery is challenged a novel standard for current and future invasive oncology.

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A13 Laparoscopic Assisted Cryosurgery for Intra Abdominal Malignancy

Barlian Sutedja, MD

Gading Pluit Hospital, Jakarta, Indonesia

Cryotherapy for cancer was first reported by James Arnott in England in 1850. But the widespread of the clinical applications started more than one century later along with the development of better instrumentations for cryosurgery, especially also due to the significant improvement of imaging techniques. Beside the open surgical procedure, recently the application of cryosurgery to the organ inside the body can also be done percutaneously or with laparoscopic technique as minimally invasive approach. Compare to the open surgical approach, the laparoscopic technique shows some advantages, namely: less pain, faster recovery, lower surgical site infections, better cosmetic result and also an excellent diagnostic tool. This technique has gained increasingly more acceptance to replace the open approach in many surgical procedures including the cryosurgery, known as laparoscopic assisted cryosurgery. It has been applied for malignancy in liver, kidney and other intra-abdominal solid organs with advantages of the minimally invasive technique and also improving the diagnostic, better monitoring of the process for the effectiveness and safety of the during the cryosurgery procedure.

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8:00 AM Keynote K04: Mammalian Hibernation– Clinical Implications

Kenneth B. Storey, Ph.D.

Carleton University, Institute of Biochemistry & Department of Chemistry, Ottawa, Ontario, Canada

Hibernating mammals have amazing capabilities that we, as humans, can only envy. They may spend up to 8 months of the winter in bouts of prolonged torpor with metabolic rates <5% of normal, core body temperature near 0°C, long periods of apnea, under-perfused organs, and skeletal muscles that go unused for weeks at a time. All of these “tricks” of hibernators have direct application to multiple medical concerns including preservation of organs for transplant, pushing the use of hypothermia as a treatment option to lower and lower temperatures, enhancing ischemia resistance of organs, minimizing skeletal muscle atrophy under conditions of extended immobility, and developing inducible torpor for use in multiple clinical settings. Studies in my lab focus on the molecular regulation of hibernation exploring both torpor regulation (biochemical mechanisms that coordinate transitions to/from the torpid state and/or reprioritize energy expenditures for extended survival in torpor) and the cytoprotective adaptations that stabilize macromolecules over days/weeks of dormancy. Using ground squirrels and bats as main models, our research has produced novel insights into hibernation regulation with studies of differential gene expression, microRNA and epigenetic controls, modification of signal transduction cascades, and adaptive regulation of enzymes often by posttranslational modification. Our newest work is a unique and exciting exploration of the biochemistry of primate hibernation in a lemur – the closest phylogenetic relative to humans that exhibits natural hibernation. Torpor and hibernation in lemurs is particularly exciting because it occurs with relatively small decreases in body temperatures, thereby allowing us to probe for key mechanisms that regulate torpor (as opposed to those that accommodate low body temperature) and providing an optimal model for translational studies to utilize inducible torpor as a treatment option for humans. For more information go to: www.carleton.ca/~kbstorey

9:00 AM -10:15 AM

Session 4: Hypothermic Medicine

Session Chair: Michael J. Taylor, Ph.D.

Speakers:

Michael J. Taylor, Ph.D.

John M. Baust, Ph.D.

Klearchos K. Papas, M.D.

A14 Integration of Time-Honored Principles of Hypothermic Cellular Protection with Modern Day Modalities of Organ Preservation

Michael J. Taylor

Cell and Tissue Systems, Inc. & Carnegie Mellon University, N. Charleston, SC USA

In recent years the role of hypothermia as the bedrock upon which techniques of organ preservation are based, has received renewed attention due to its importance in the procurement, storage and resuscitation of less-than-optimal donor organs. Clinical kidney and liver transplantation has evolved from an experimental procedure 50 years ago to the current treatment of choice for patients with end-stage organ disease where patient and graft survival rates exceed 90% per year. However, an ever increasing shortage of donor organs means that there is an increasing number of patients on the waiting lists for transplants such that future advances in this field are constrained by both the numbers of available organs and their quality. As a consequence, less-than-optimal donor organs are increasingly being used in an attempt to close the widening gap between supply and demand. The ex vivo perfusion of organs by hypothermic perfusion preservation (HPP) on a machine prior to transplant, as opposed to static cold storage (SCS) on ice, offers the prospect of making available more and better organs for transplantation. Studies have shown that expanded criteria donor (ECD) kidneys preserved by SCS had an overall increased risk of reduced graft viability. However, HPP was clearly associated with a reduced incidence of delayed graft function (DGF), an overall improved graft survival, and an increased rate of clinical use. Moers *et al* reported the

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results of a controlled, prospective randomized study involving 336 consecutive deceased donors from whom one kidney was randomly assigned to pulsatile perfusion and the contralateral kidney to SCS (N Engl J Med; 360(1):7-19 (2009) and 366: 770-1 (2012)). 1-3 year follow-up showed that HMP reduced the risk of DGF (43% less risk; $p=0.01$) and graft failure ($p=0.04$) and improved graft survival ($p=0.02$). HPP significantly reduces the risk of DGF in ECD ($p=0.047$) and kidneys donated after cardiac death (DCD; $p=0.025$), by 54% and 57% respectively. Very recently, the same HPP-approach has been reported for the first time for clinical liver transplantation and research continues to focus on other organs such as the pancreas. This presentation will re-visit the fundamental principles upon which hypothermia has proven to be a robust protective modality against ischemia and hypoxia. These basic principles will be considered in relation to the time-honoured interventional strategies that have been adopted for clinical organ preservation. In particular, the fundamentals of preservation solution design, to control the environment of cells to combat the deleterious events of ischemia and reperfusion injury, will be outlined. Integration of key modalities and technologies for hypothermic preservation will be further illustrated by recently reported extrapolation of clinical success in kidney transplantation to clinical liver preservation. Guarrera's group undertook an analysis of molecular stress markers using liver biopsies from the same groups of patients as the Phase I clinical study (Am.J. Transpl. 10:372-81 2010) and reported that expression of all mRNA markers examined, including inflammatory cytokines, was elevated in reperfusion biopsies from the SCS group compared with HPP. Upregulation of stress markers was significantly attenuated in livers preserved by HPP.

A15 Molecular Approaches to Improving Hypothermic Biopreservation of Cells and Tissues

John M. Baust¹, William Corwin², Robert Van Buskirk^{1,2}, John G. Baust²

¹ CPSI Biotech, 2 Court Street, Owego, NY 13827

² Binghamton University, Dept. of Biological Sciences and the Institute of Biomedical Technology, Binghamton, NY 13902

The utilization of biopreservation strategies continues to grow at a rapid pace as advances in fields such as cell therapy, stem-cell research, personalized medicine, organ transplantation, etc. drive the need for improved storage and shipment of living products. Given the complexity of biologics, hypothermic preservation (4°-10°C) remains the preferred approach for short term (hours to days) maintenance enabling holding and transport under controlled conditions. Numerous reports have detailed the benefits of hypothermia to suppress physiological activity at the cellular level thereby extending the window of viability. Pivotal to the success of hypothermic preservation has been an understanding of the metabolic, biochemical and physical characteristics of cells both in their normothermic environment and how these factors change during hypothermia. The translation of this understanding is best exemplified by the advancements in preservation solution design. Through the application of knowledge pertaining to cell system response to cold stresses, the first intracellular-like cold-perfusion solution, University of Wisconsin solution (UW or Viaspan), was developed and has become the gold standard hypothermic storage medium. The success of this approach evolved, leading to the development of improved solutions such as Celsior, HTS, and Unisol among others; however, there remains a significant void that solution design alone cannot bridge. The recent discovery of molecular components to preservation failure has served to complicate our understanding and slow technological progress due to the complex nature of the responses involved. To this end, several fundamental discoveries related to the molecular response of cells to cold stress have recently been reported, including the co-initiation of apoptotic cell death and cell survival responses, the characterization of specific genes and pathways involved and the identification of specific stress related trigger events resulting in cellular demise. As studies have begun to uncover the specific molecular responses involved, they have identified a wide array of targets whose modulation can improve preservation outcome. Numerous reports have described the benefit of the incorporation of protective agents, such as specific inhibitors, into the preservation medium to achieve targeted molecular control thereby improving outcome. This presentation will provide an overview of the molecular-based discoveries in the area of hypothermic preservation with focused discussion on the recent discovery of the differential activation of the UPR (unfolded protein response) pathway and its impact on the response of cells and tissues to cold storage. Given what is now known about the complexity of the preservation process and the multitude of stresses induced, it is clear that future advances will only be accomplished through multifaceted approaches. This is evident when examining the current literature, as reports on cell preservation have moved from classical cell biology to include aspects of other disciplines including molecular biology, biochemistry, genomics, proteomics, etc. With continued advancements in our understanding of the molecular response of cells to cold, a more individualized and targeted approach may be achieved moving us further along the path toward improving the quality, usability and extend the storage interval of highly complex and specialized biologics.

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A16 Supplemented Oxygenation During Hypothermic Storage: Friend or Foe.

Klearchos K. Papas

University of Arizona, Tuscon, AZ, USA

There is a shortage of donated organs for clinical allo-transplantation with potential recipients dying while on the waiting list. Despite that only a fraction of the donated organs are currently utilized. For example certain organs with prolonged cold ischemia times and organs from expanded criteria donors (ECD) are not routinely utilized. ECD organs may be more sensitive to stressful conditions during preservation and maintenance of the health of these organs during preservation is even more critical and challenging and may require more sophisticated preservation techniques. This has led to substantial interest and investment in improved methods for organ preservation. The lack of oxygen during hypothermic preservation is believed to be detrimental to cellular viability and maybe responsible for priming the organ for damage during normothermic reperfusion (ischemia reperfusion injury). As a consequence, oxygen supply is a major research focus in the field of organ preservation. It has been demonstrated that the simplest and most widely used technique for hypothermic organ preservation, static cold storage can only oxygenate to a maximum depth of a millimeter from the surface of an organ even if the organ preservation solution around the organ is fully saturated with 100% oxygen. In this case, even though the oxygen demand is substantially reduced by hypothermia, it still exceeds the supply by diffusion. Even hypothermic machine perfusion, which has been designed to deliver cold preservation solution into the organ via the circulation, may be incapable of delivering adequate oxygen to an organ during preservation, particularly when the perfusate does not have substantially higher oxygen carrying capacity than water and it is not saturated with a gas containing higher oxygen fraction than air. New and improved methods for oxygen delivery to organs during hypothermic preservation are clearly still needed. A major consideration in the development of such methods is that even though lack of oxygen can be detrimental, high levels of oxygen (hyperoxia) could be equally or more damaging. Emerging literature suggests that negative effects of hyperoxia may be more pronounced under hypothermia because the cellular antioxidant mechanisms in place may not be as effective at lower temperatures. The effects of high or low oxygen may be temperature as well as organ dependent and are currently not well defined. Further research is needed to define the fine balance between oxygen demand and delivery for the organ or tissue of interest at relevant temperatures. In this paper we will present data on the effects of high and low oxygen on pancreas and islet (beta-cell) health at various temperatures ranging from 4C-37C. Islets are known to be particularly sensitive to oxidative damage as they express lower levels of antioxidant enzymes typically found in other cell types under physiological conditions. Islets are also particularly sensitive to hypoxia because they express very low levels of LDH-alpha and they cannot effectively generate energy anaerobically, which makes them an interesting and challenging model system in the context of oxygen delivery optimization to avoid hypoxia induced damage without resulting to high oxygen toxicity.

10: 30 AM – 11:45 AM

Session 5: Blood Cell Preservation

Session Chair: John Crowe, Ph.D.

Speakers:

John Crowe, Ph.D.

Jason Acker, Ph.D.

Amir Avav, Ph.D.

A17 Freeze-dried Platelets: Moving Towards Clinical Use

John H. Crowe¹, Michael Fitzpatrick²

¹*University of California, Davis, CA, USA*

²*Cellphire, Inc., Rockville, MD*

It has been a dream of workers in the field of blood cell preservation to find a way to store the cells in a freeze-dried state. In this short symposium we will present results showing that we are getting close to realizing that possibility. We have been taking an unusual approach, utilizing what has been learned from the chemistry of organisms that survive drying in nature. We have applied some of that chemistry to blood cells, and I will report that platelets have been successfully freeze-dried.

Dr. Fitzpatrick will report on clinical trials with such platelets, and Drs. Acker and Arav will describe their progress with red blood cells, using related approaches. We suggest that this work will provide platelets long past their standard blood bank life of 3-5 days and will make platelets regularly available under field conditions for the first time. Similar statements will emerge concerning red blood cells in those presentations.

A18 The Challenges and Limitations of Current Clinical Methods for the Hypothermic Storage and Cryopreservation of Human Red Blood Cells

Jason Acker

Canadian Blood Services, Edmonton, AB Canada

One of the most important advances that led to the creation of blood banks and the widespread therapeutic use of blood products was the development of techniques for ex vivo blood preservation. All efforts to extend the storage of blood past a few days had failed until the discovery of the protective effects of glucose on citrated blood in 1916. Since then, the hypothermic storage of viable red blood cells (RBC) at 4°C has advanced to the point where these cells can be preserved ex vivo for up to 45 days with modern additive and extender solutions. Cryopreservation of RBCs for transfusion is one of the most successful applications of the cryobiology research that followed the discovery of glycerol as a cryoprotectant. This presentation will review the challenges and limitations of the current methods used in blood banks for the hypothermic storage and cryopreservation of human RBC used in transfusion medicine. Emerging data on the RBC storage lesion and RBC cryoinjury will be discussed with a focus on changes to cell metabolism, oxidative injury and membrane microvesiculation. Current RBC quality control methods and international regulations will be briefly described and details on the Canadian Blood Services RBC Quality Monitoring program will be presented. Recent clinical data on the effects of hypothermic red blood cell storage and cryopreservation on recipient outcomes will be reviewed. While RBC transfusions have been used in the treatment of the anemic patient for almost 100 years, clinical data indicates that there is a need for additional improvements. Applying emerging principles and techniques in the preservation sciences would have important clinical impact on the quality and efficacy of hypothermic stored and cryopreserved RBC.

A19 Freeze Drying Red Blood Cells: Development of Novel Technologies

Amir Arav, DVM, Ph.D.

Core Dynamics

Long term preservation of blood products for transfusion purposes has been of great scientific and practical interest over the past decade. In modern blood banking system red blood cell (RBC) units are refrigerated (2-8°C) in a liquid state and stored up to 42 days. However, degradation is demonstrated as early as 2 weeks after collection. A freeze dried RBC unit that could be stored at room temperature and readily rehydrated and processed for transfusion will be a lifesaving solution in combat scenarios, in undeveloped countries and where refrigeration and freezers are difficult. Additionally, autologous use would be very simple since any individual will be able to request to freeze dried his own unit of blood to be stored for future use. The purpose of this work was to achieve above 70% recovery rate of functional RBCs after drying, demonstrate their ability to survive and carry oxygen in-vivo and in an animal model. The scope of the study encompasses finding the optimal parameters for each step of the processes involved: (1) prior freezing- freezing solution components, concentrations, volume and cell concentration, (2) freezing- freeze/thawing method and all its parameters, (3) Freeze drying- freeze-drying method and all its parameters, (4) storage- temperature and duration and finally (5) rehydration-rehydration solution, its volume, temperature, etc. All aforementioned steps must be synchronized and targeted in such a way that will stabilize the cells (particularly the membrane) and reduce ice damage effects during freezing and drying. Therefore, we have tried several methods for introducing trehalose into RBCs, since the paradigm was that it is necessary to stabilize the membrane from both sides and that it is best done with trehalose which replaces the water molecules that have been dehydrated. One of our findings is that this is not the case; on the contrary, introducing trehalose damages the cells and results with decreased viabilities. Furthermore, our latest solutions indicate that the presence of trehalose, even extracellularly, is redundant. Our best results were achieved with a new solution which resulted with up to 70% recovery of functional cells with 0.4% Hct, these cells were able to undergo dilution to physiological osmotic conditions, demonstrate nearly normal oxygen carrying capacity and osmotic fragility.

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11:45 AM - 12:45 PM

**Session 6: Preservation of Biotherapeutic Products
Cryopreservation Processes**

Session Chair: Anthony Robilotto, M.S.

Speakers:

Gloria D. Elliott, Ph.D.

David Gale, Ph.D.

Birgit Glasmacher, Ph.D.

A20 Dry Preservation of Biological Therapeutics

Gloria D. Elliott¹

¹*University of North Carolina at Charlotte*

The fields of tissue engineering, cellular therapeutics, and transfusion medicine have matured considerably over the past decade, thus accelerating the need for cost-effective preservation methods to enable the ready supply of biological therapeutics. The current state of the art for preserving biological samples is by cryopreservation, either by slow cooling or vitrification, followed by storage at cryogenic temperatures (-196°C). Using innovative processing approaches and formulations, advances are being made towards stable dry state preservation at non-cryogenic temperatures. This work impacts on many practical problems related to improving the cost, availability, and environmental impact of bio-banking technologies. We have recently developed a versatile microwave based drying process, which enables rapid and controllable isothermal vitrification of carbohydrate-based preservation solutions. We have been applying this technology to the preservation of viruses, sperm and eggs, as well as macrophages, a representative nucleated mammalian cell line. Depending on the end use of these biological materials, different levels of functionality are required to achieve what might be considered a viable product for therapeutic use. The challenges and successes of dry preservation as a bio-banking strategy for biological therapeutics will be discussed.

A21 Optimizing Tissue Cryopreservation Techniques for Cryopreservation Solution Modifications

Stacy Arnold,¹ **David Gale¹**

¹*CryoLife, Kennesaw, GA, USA*

The purpose of this study was to observe how minor changes to cryopreservation solutions affect the success of cryopreservation controlled-rate freeze cycles. Cryopreservation is an important technique utilized in multiple industries. In the medical industry, specifically when used in tissue preservation, there are several components critical to the success of the cryopreservation process including maintaining a constant freeze rate. A typical cooling rate appropriate for vascular tissue, as evaluated in this study, is 1°C/min. Due to exothermic reactions occurring during phase change, a constant freeze rate is not able to be achieved unless accounted for in a controlled-rate cryopreservation program. Different additives included in cryopreservation media may drastically alter the time and temperature at which these exothermic reactions occur. Unless modifications are made to the cryopreservation freeze program, a constant freeze rate is not achievable. This study evaluated controlled-rate cryopreservation responses to cryoprotectant modifications. Minor changes to a vascular tissue control cryoprotectant containing DMSO were made to determine the effects on the controlled freezing rate. A total of six solutions were evaluated using a controlled-rate freeze program developed to maintain a 1°C/min freeze rate for vascular tissues cryopreserved in the control cryoprotectant. Solution compositions tested included: (1) Control cryoprotectant (CCP); (2) CCP+15g Dextran 40; (3) CCP+15g Dextran 40 and 1.0g Mannitol; (4) CCP+9.5g Trehalose Dihydrate; (5) CCP+47.3g trehalose Dihydrate; (6) DMEM with DMSO. The freeze program utilized accommodates for the exothermic phase change occurring when the control cryoprotectant gives off heat as it changes from a liquid to a solid. Cryopreservation reference pouches were filled with each cryoprotectant. Thermocouples were inserted in each of the pouches to monitor the temperature of the solution throughout the controlled-rate freeze. During this process, graphical data reported the temperature inside the cryoprotectant pouches and cryopreservation chamber. Observations were recorded for all solutions to determine if the freeze program was able to accommodate the exothermic phase change.

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The two parameters assessed were linearity over the entire controlled-rate freeze duration and cryoprotectant temperature increases during the exothermic phase change. All additives resulted in a deviation from the programmed freeze rate of 1°C/min. The control solution, Solution 1, behaved as expected having a linear, constant freeze rate of 1°C/min with no temperature increases during the phase change. The results for Solution 2 and Solution 3 demonstrated that they were unable to maintain a 1°C/min controlled-rate freeze and had visual temperature increases during the exothermic phase change. Solutions 4, 5 and 6 did not have visual temperature increases during the phase change period, but were not able to maintain a constant controlled-rate freeze of 1°C/min. For all test solutions evaluated, minor changes to the cryoprotectant resulted in cryopreservation runs unable to maintain a constant 1°C/min freeze. In conclusion, controlled-rate freezing programs are sensitive to changes in the cryopreservation solution. It is important that new cryopreservation freeze programs be developed to accommodate solution changes thereby ensuring optimal cryopreservation technique.

A22 Advances Towards Standardised Freezing Protocols

Birgit Glasmacher¹, Ralf Spindler¹, Denys Pogozykh^{1,2}, Nicola Hofmann¹

¹*Institute for Multiphase Processes, Callinstrasse 36, DE-30167 Hannover, Germany*

²*Institute for Problems of Cryobiology & Cryomedicine of the National Academy of Sciences of Ukraine*

In the field of cryopreservation cells are stored at cryogenic temperatures in order to maintain their quality after thawing. Lowering the temperature reduces the metabolism of cells and therefore can prevent their loss of quality over time. During the freezing process different parameters have been identified as cell damaging mechanisms [1]. Thus, a correct setting of process parameters including the cooling and warming rate, nucleation temperature (T_n), sample geometry, as well as type and concentration of cryoprotective agents can reduce cellular damage and result in high quality output after freezing. For comparison of the qualities of different biological specimens, freezing protocols and viability analyses need to be standardised. Most of the earlier studies did not account for the control of all process parameters, especially the nucleation temperature [1]. Therefore, the aim of this study was to control all possible process parameters during freezing of human endothelial cells (ECs) and mesenchymal stem cells (MSCs) of the marmoset monkey using different experimental methods. A standardised freezing protocol was developed which is based on our earlier experimental results [2]. The protocol includes a cooling rate of 5 K/min, heating rate of 100 K/min, 5 % (v/v) DMSO and a nucleation temperature of -8°C. The first method used was a biological freezer (CM2000, Carbueros Metalicos) where 1 mL cell suspensions were frozen in cryovials. For the control of the nucleation temperature a new setup was developed which is based on nucleation via Peltier elements [3]. A second method used a commercially available cryomicroscopic setup to visualize the cells in suspension which involves a LINHAM cryostage FDCS 196. and a digital camera (Retiga, QImaging). The temperature of the samples was controlled from 40 to -196°C. The manual cell seeding process required the removal of the lid of the cryostage when a cooled copper rod touches the supercooled sample. To overcome this limitation, we developed a new seeding device for cryomicroscopic investigations of aqueous samples. This device applies a copper rod which is cooled via liquid nitrogen flushed heat exchanger. A USB camera is used to identify the position of the copper rod tip during an initial local calibration procedure which is electronically stored. Thus, the system can automatically calculate the distance to the sample. nucleation is induced by a liquid nitrogen cooled copper rod which is positioned towards the specimen by a step motor system. The device allows for the control of the nucleation temperature at cooling rates up to 10K/min with an accuracy of ± 0.2 K. Both construction designs allow the control of the nucleation temperature and contribute to a further standardisation of freezing protocols. After cell freezing, the quality of the protocol was determined using staining methods based on trypan blue, calcein AM and ethidium homodimer. As a general quality test the *absolute viability* was determined which is defined as the ratio of viable cell number after freezing to the total cell number before freezing. Using this method, the viability of ECs after freezing was 90%. After thawing, MSCs were tested for proliferation and differentiation into the osteogenic and adipogenic lineage. To achieve a standardisation of freezing protocols we propose to control all process parameters including the nucleation temperature and the use of the *absolute viability* as a general method for quality comparison of different cellular specimens after freeze/thawing.

[1] P. Mazur, S.P. Leibo, E.H.Y. Chu: A two-factor hypothesis of freezing injury, *Experimental Cell Research* 71 (1972) 2, 345-355

[2] I. Bernemann, N. Hofmann, R. Spindler, A. Szentivanyi, B. Glasmacher: Strategy to improve cryopreservation protocols, *CryoLetters* 29 (2008) 1, 73-88

[3] R. Spindler, B. Rosenhahn, N. Hofmann, B. Glasmacher: Video analysis of osmotic cell response during cryo-preservation, *Cryobiology* 64 (2012) 3, 250-260

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8:00 AM Keynote K05: Whole Gland Cryosurgical ablation of Prostate cancer

Truls E. Bjerklund Johansen, MD, Dr. Med. Sci.

Urology Department and Institute of Clinical Medicine, Aarhus University Hospital, 8200 Aarhus, Denmark

Introduction and background: Prostate cancer may be a lethal disease causing significant suffering, usually for men of older age. Unfortunately, our understanding is limited when it comes to growth rate and the time when metastases develop. Radical treatment may be effective in properly selected patients, but side effects are significant and contemporary treatment demands big resources. In spite of overtreatment, failure rates are high and there is great need of salvage treatment. Cryosurgical ablation (CSA) is a minimally invasive treatment option which may be offered as day-surgery and is a preferred modality for focal treatment of prostate cancer. The aim of this presentation is to provide an overview of contemporary evidence for CSA as primary and salvage treatment in prostate cancer, and to discuss how the technique of CSA may be improved. **Materials and methods:** A Medline/Pubmed search using Scopus and Science direct for articles published in the English language was undertaken. The following search terms were used: Prostate cancer, primary treatment, salvage radiation treatment or therapy, salvage brachytherapy, salvage prostatectomy, salvage cryoablation or cryotherapy, salvage HIFU (high intensity focused ultrasound), and permutations of these procedures. Papers were selected for scrutiny based on their pertinence, the study size and overall contribution to the field. **Results: Salvage CSA.** Primary treatment fails in up to 40% of patients after radical prostatectomy (RP) and up to 67% after external beam radiation (EBRT). 10year data after salvage CSA show biochemical disease free survival between 40-60%, incontinence rates 3-13%, rectal fistula rate of 1-2% and need of TURP in 3%. Published results depend heavily on patient selection. Unfortunately, most patients with radiation failure are not offered salvage treatment but are given ADT which might increase the risk of cardiovascular events. **Primary CSA.** 10 year results of primary CSA are similar to EBRT and RP in terms of evidence for progression. A positive biopsy rate of 7.7% at 36months was found in a RCT of CSA versus EBRT, and a 10year negative biopsy rate of 77% was found in a prospective case series. The incontinence rate is 0.9% but erectile dysfunction is still high with 64% reported in a recent series. The need for TURP after CSA is about 1% and the risk of rectal fistula is almost negligible. **Discussion:** CSA may kill prostate cancer in primary and salvage settings. The procedure is technically demanding as there is a delicate balance between freezing hard enough for cell death and not causing damage to surrounding structures. The challenge is to achieve killing conditions in all regions of the prostate. A cryosensitizer which gives killing potential to ice near the freezing point should be aimed at. Vitamin D-3 is an interesting alternative for further studies. The importance of slow thawing also needs further exploration as there seems to be differences in practice between US and European colleagues.

9:00 AM -10:20 AM Session 7: Clinical Applications of Cryo-technology In Cardiac Arrhythmia

Session Chair: Neil K. Sanghvi, M.D.

A23 This session will aim to review the application of cryoablation in the management of common cardiac arrhythmias. We will discuss an overview of the technology and its current application in the treatment of common cardiac arrhythmias including supraventricular tachycardias, ventricular tachycardia and atrial fibrillation. Participants will be able to appreciate the efficacy and future direction of cryoablation for patients presenting with cardiac dysrhythmias.

Speakers:

Neil K. Sanghvi, M.D.

Boaz Avitall, M.D.

John C. Bischof, Ph.D.

A24 Cryoablation of SVTs and Ventricular Tachycardia

Neil K. Sanghvi, MD

Lenox Hill Hospital

Cryotechnology has been employed in the management of cardiac arrhythmias ever since IS Cooper invented the hand-held cryoprobe. In 1967, we witnessed the first clinical application of cryoablation with the surgical destruction of the AV node in a patient with refractory atrial fibrillation. It was not until 1991 that a similar feat was achieved with an 11F percutaneous endovascular cryocatheter. Since then, cryoablation has been used to address most cardiac arrhythmias. This review summarizes the efficacy of cryoablation in managing cardiac arrhythmias.

Cryoablation has three major advantages:

- Cryomapping allows assessment of response to ablation with reversibility
- Cryoadherence facilitates catheter stability during ablation
- Minimal levels of pain during ablation

Cryoablation is predominantly employed for arrhythmias that would otherwise be high-risk for collateral damage when using traditional radiofrequency (RF) ablation including complete heart block, phrenic nerve injury, or excessive pain.

Cumulatively, cryoablation is estimated to offer a 95% cure rate and an 11% recurrence rate when used for atrioventricular nodal reentrant tachycardia (AVNRT). Success rates of 95-100% have been reported for atrioventricular tachycardia (AVRT); however, there is a higher recurrence rate of 20-30%. There is limited data on ablation of atrial tachycardia (AT), but reported rates are similar to AVNRT – 96% success and 12% recurrence. Studies of cryoablation for typical atrial flutter describe success rates of 87-100% with recurrence averaging 5-10%. Long-term success is estimated to be 91% for an average of 27 months. There is very little data regarding cryoablation of ventricular tachycardia (VT). Limited series suggest equivalent success to RF ablation in treating RVOT VT with greater than 90% success. Cryoablation performs very well in treating parahisian VT foci. However, when considering all forms of VT it is successful 45% of the time. Amazingly, there have been no reported cases of complete heart block requiring pacemaker implantation with cryoablation when treating AVNRT, AVRT, or AT. Cardiac cryoablation continues to remain an important tool for the invasive electrophysiologist. A 2010 survey estimates that approximately 10-15% of all ablations for tachy-arrhythmias are performed using cryoenergy. This is easily an underestimate given the explosion of cryoablation in treating atrial fibrillation. Advances in catheter design and size as well as potentially coupling with RF energy will likely increase adoption of cryoablation in the future.

A25 Cryoablation of Atrial Fibrillation

Boaz Avitall MD, PhD, FACC, FHRS

University of Illinois, Chicago, IL, USA

It is now well established that paroxysmal atrial fibrillation triggers are often found to originate from the atrial muscular sleeve wrapped around the Pulmonary Veins (PV). The introduction of Cryo technology for the treatment of atrial fibrillation took place in 1998 where a Nitrous Oxide Cryo balloon technology was first utilized for the isolation of the PVs. Since it is well established that Radio frequency applications for the isolation of the PVs can result in PV stenosis as well as life threatening damage to the esophagus, it was necessary to first establish the safety of Cryo applications for the isolation of the PVs. A 22mm balloon was used to ablate the PVs antrum once, and for up to 4 consecutive times to establish the tissue response to multiple applications of the Cryo applications. The tissues were allowed to heal for 5-6 month followed by remapping and histopathology. In these experiments we were able to show that the use of the Cryo balloon technology is both safe and effective for the electrical isolation of the PVs. The Cryo balloon was quickly adopted and developed as an extension of the catheter based Cryo ablation, initially developed by CryoCath, that was already being utilized for the ablation of focal tissues such as AV nodal modification as well as accessory pathways and atrial tachycardias. The current Cryo balloon technology consists of 22 and 28mm balloon sizes. Furthermore, a PV mapping lasso type catheter is used to monitor the PV electrical isolation post Cryo application. A critical aspect of the ablation success is the ability of the balloon to occlude the blood flow from the vein into the atria insuring efficacious freezing. To-date over 20,000 patients were treated using this technology. Recent Meta-Analysis of 23 studies 1349 patients the acute PV isolation success rate was 98.8%, one year freedom from AF in patient with paroxysmal atrial fibrillation was 72.8% (vs. RF 66%). The complications include acute right phrenic nerve paralysis in 6.38% (vs. RF 0.2%) with only 0.37% experiencing symptoms 1 year later. Pulmonary vein stenosis was reported in 0.17%. Other complications include pericardial effusion in 1.46% (vs. RF 6%) and

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in 0.57% (vs. RF 2.6%) requiring pericardial tap. Microembolic monitoring of the blood flow to the brain revealed that the rate of micro emboli was 1/3 of that noted with RF ablation. Future Cryo technologies for the ablation of AF include low pressure flexible balloons that adopt to the PVs, linear lesions Cryo-technology. **In conclusion:** Cryo balloon PV isolation is now proven to be effective and safe technology for the isolation of the PVs and the treatment of paroxysmal AF.

A26 Thermal Properties of porcine myocardial tissue at Subzero Temperatures: Implication for Thermal Therapy Studies

Jeunghwan Choi¹, Michael Etheridge¹, Dushyant Mehra¹, John Bischof¹

¹ University of Minnesota, Minneapolis, MN, USA

Cryoablation for atrial fibrillation is a clinically approved technique used to control irregular heart rhythms due to abnormal electrical signals conducted from the pulmonary veins to the atria. The ablation catheter rapidly cools the cardiac tissue to freezing temperatures creating a discrete lesion which terminates these abnormal electrical pulses. Benefits of this approach include decreased risk of thrombus formation, well demarcated lesions, and preservation of extracellular matrix and endothelial integrity. However, possible complications include phrenic nerve injury and pulmonary vein stenosis (although these are not unique to the cryoablative approach). Therefore, further understanding of the mechanisms and thresholds of cryoinjury are required. This study begins to address these issues by improving predictive model accuracy through demonstrating the importance of accurate, temperature-dependent tissue properties. The direct relationship between thermal history and treatment outcome, combined with the difficulties of realtime, clinical thermometry, make heat transfer modeling and numerical predictions important aspects of cryoablation studies. The accuracy of the models depends on numerous factors including the kinetics and energy release during phase change phenomena and knowledge of thermal properties as a function of temperature. However, insufficient data for tissue thermal properties in the subzero domain results in a reliance on property estimations, generally based on water-ice data or weight averaged values from known materials. This study focused on expanding the thermal properties database for biological tissue in the subzero regime for use in cryoablation modeling. Results for porcine myocardium as well as ultrasound gel (a substitute phantom used for cryoprobe characterization) are reported. A differential scanning calorimeter was used to measure the specific heat and to observe latent heat effects. The thermal conductivity was measured using self-heating thermistors, employing both transient pulse decay and quasi-steady constant power methods. Specific heats in porcine myocardium were slightly less than those for ice, ranging from 0.93 J/gK at -150 °C to 2.35 J/gK at -21 °C. Specific heat behavior for ultrasound gel could be bracketed into two ranges, in which the values measured were less than those for ice at temperatures below the glass transition temperature (around -110 °C), and progressively higher than those for ice with temperatures above the glass transition. Thermal conductivities of porcine myocardium rose gradually at lower temperatures, ranging from 1.52 W/mK at -10 °C to 2.31 at -148 °C, and were about 50% less than those values for ice. Thermal conductivities of ultrasound gel were similar to those of porcine myocardium, but with an inflection point at the glass transition temperature after which the rise in thermal conductivity was attenuated. The importance of accurate, temperature-dependent thermal properties is demonstrated by comparing the predicted thermal history versus those estimated from constant property values.

10:30 AM – 11:30 AM

Session 8: Technology Advances

Session Chair: Yoed Rabin, Ph.D.

Speakers:

Yoed Rabin, Ph.D.

Anthony Robilotto, M.S.

Kenneth Diller, Ph.D.

Gary Onik, M.D.

A27 Computation Tools at the Forefront of Cryosurgery Development

Yoed Rabin¹

¹*Department of Mechanical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA*

Cryosurgery practices have evolved over the years in close relationship with concurrent technology developments. Well recognized milestones along the path of development are the beginning of commercial production of liquefied gases at the beginning of the 20th century; leading to the widespread of cryosurgery as a medical treatment, the development of the cryostat by Cooper and Lee in the early 1960s; leading to the dramatic development of cryosurgery as an invasive procedure, and advances in medical imaging; leading to the expansion of cryosurgery as a multi-cryoprobe and minimally invasive procedure in the 1990s. The new minimally invasive procedure has presented additional technical challenges associated with visualization of the process, correlating medical imaging with the thermal history, optimizing the geometry and the cooling capability of each cryoprobe, and optimizing the total number of cryoprobes and their layout. Recent developments in computer hardware and computation techniques are now defining the next milestone in the evolution of cryosurgery. The current presentation focuses on recent developments in computation tools for purposes of training, planning, and monitoring of the cryoprocure. This presentation reviews the key building blocks of computation in the service of cryosurgery, including geometric modeling of the target region, integration of clinical data on the progression of the disease, bioheat transfer simulation techniques, optimization methods for cryosurgery planning, incorporation of real-time temperature data, and integration of the thermal field with medical imaging. Examples are drawn from prostate cryosurgery, which has been extensively studied over the years. In conclusion, this presentation provides a broader view on the role of computation tools in the service of cryosurgery, while discussing directions for future developments.

A28 Assessing the Performance of a Novel Cryosurgical Platform

Anthony T. Robilotto^{1,2}, John M. Baust^{1,2}, Robert G. Van Buskirk^{1,2}, Andrew A. Gage^{2,3}, and John G. Baust¹

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³*Department of Surgery, State University of New York at Buffalo, Buffalo, NY 14260 USA*

The cryogens employed in current cryosurgical devices are almost exclusively a gas, typically argon, undergoing Joule-Thomson (JT) cooling. These devices utilize small diameter probes with near instantaneous ice generation. The effective cryoablation zone, however, is small thus requiring multiple probes and/or multiple freeze and extended freeze application intervals to provide a given size cryolesion. In contrast, liquid nitrogen systems provide high levels of heat extraction with low nadir temperatures, but given its boiling point (-197 °C) and ratio of thermal expansion, probe sizes are often larger and procedure durations longer. Recently, we have developed a novel cryosurgical platform utilizing nitrogen in the super critical state (SCN). In this study, we conducted a quantitative comparison of the performance of the SCN system to that of a JT device. Evaluation of the isothermal gradient generated during the freeze procedure was assessed with thermocouple arrays in various tissue approximations such as water, ultrasound gel, and porcine muscle sections. Additionally, a series of feasibility *in vivo* studies were conducted to assess the performance of the SCN system for endocardial and epicardial ablation applications in a canine model. The data revealed that the SCN system created significantly colder nadir temperatures (-170 °C as compared to -140 °C) and broader spread of critical isotherms compared to that of the JT system. Further, it was found that the SCN generated this larger and colder iceball in approximately half the time. In a comparison of surface freezing in an *ex vivo* porcine tissue model (5-10 mm thick muscle) under thermal load to model epicardial cardiac ablation, it was found that the SCN platform created transmural lesions that were colder and narrower than that created by JT devices. *In vivo* endocardial cardiac ablation canine studies revealed that the SCN system was capable of generating transmural lesions with complete electrical block in fully beating heart in ≤60sec. The results of these studies demonstrated the SCN system provides for rapid, effective, controllable freezing of targeted tissue. The use of the SCN system allowed for the miniaturization and speed of JT devices, yet maintains the ultra cold nadir temperatures and cooling potential of a liquid nitrogen system. The power, speed and freeze characteristics of the SCN system offers the potential of a reduction in procedural time compared to current cryo devices. Further, these technological developments may open new avenues for the application of cryo to treat other cardiac arrhythmogenic disorders.

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A29 MR Guided Cryosurgery, A Preferred Method?

Gary Onik

Center for Urological Advancement, Florida Hospital/Celebration Health

Imaging guidance for cryosurgery, has revolutionized the field and allowed cryosurgery to be effectively applied to organs such as the prostate and liver. With each new advance in imaging this application of new technology has been applied to cryosurgery to advance the field. MRI has had great promise for the monitoring of cryosurgery but has never reached its promised potential. In this lecture we will explore the factors that effect the efficacy of an imaging modality as applied to cryosurgery and how to possibly overcome the barriers to the use of MRI in this process. There are a number of factors that must be examined to determine the applicability of an imaging modality for monitoring cryosurgery. The first is the ability of that modality to image the pathology you are trying to treat. Not all imaging modalities see a particular pathology equally well and within one modality different organ pathologies can be seen with different efficacies. For instance, prostate cancer currently is best imaged using multi-parametric MRI, while liver tumors are most efficaciously discovered using intra-operative or laparoscopic US. Next to be considered is the ability of the imaging to guide the cryoprobes into the correct position. MRI has challenges in this regard due to the relatively slow image acquisition time compared to CT or US. Also to be considered is the actual imaging of the freezing process, how that might be correlated to temperature, freezing rates and the ultimate reliable destruction of the abnormal tissue. The best example of this is the hypoechogenic change in thawed liver tissue after it has been frozen and its excellent correlation with complete tissue destruction. Lastly, integral to this whole choice of imaging cryosurgery is cost effectiveness. The particular technical advantages of an imaging modality to monitor cryosurgery has to be weighed against its ultimate cost to use in that setting, this perhaps is one of MRI's greatest challenges in becoming widely used in monitoring cryosurgery.

A30 Design and Testing of Above 0°C Cryo-Therapeutic Devices

Kenneth R. Diller

University of Texas at Austin, Biomedical Engineering Department Austin, TX USA

This presentation will describe the design of several new classes of devices for low temperature therapeutic procedures and the results of initial tests on humans. The devices are used for cryotherapy and for induction of core temperature reduction. Their operation is based on new methods to induce targeted behaviors of the human thermoregulatory system. More than 300 human trials have been conducted that will be presented and discussed. Extensive data records include skin and core temperature measurements, and superficial and deep cutaneous blood flow. Computer simulation models for local and systemic heat transfer behavior during therapeutic cooling include integrated effects such as regional modulation of blood flow, local thermal boundary conditions, and thermoregulatory control schemes. This research was sponsored by National Science Foundation Grants CBET 0828131 and CBET 096998, and the Robert and Prudie Leibrock Professorship in Engineering at the University of Texas at Austin.

Sunday, January 6th, 2013

3:00 PM – 4:00 PM, Deck 3, Cinema Conference Center

Special Presentation: Bethsaida – a City by the North Shore of the Sea of Galilee

Dr. Rami Arav

Associate Prof. Department of Religion, University of Nebraska at Omaha, Director of the Bethsaida Excavations Project

Bethsaida is a fairly well known place-name for anyone interested in the Bible. It is the third most frequently mentioned town in the Gospels; it was the birth-place of three disciples of Jesus, Peter, the first Pope, Andrew, his brother, the founder of the Orthodox Church, and Philip. Bethsaida witnessed Jesus's ministry and miracles. However, the location of Bethsaida was a mystery until 1987 when we first launched our archaeological probes. Bethsaida is among the four places most scholars believe Jesus's ministry took place and the only site available to archaeological excavation. Anyone interested in the historical Jesus quest, the results of the dig at Bethsaida are most valuable. Moreover, we not only managed to decode its location, but to our amazement, we discovered that beneath the town that dates from the time of Jesus there are the monumental remains of a forgotten capital city of a kingdom neighboring the kingdom of Israel by the name of Geshur. The Geshurites maintained peaceful relationships with the Israelites. King David married Maacha the daughter of Talmai the king of Geshur, and the couple bore children, Tamar and her rebellious brother Absalom. When Absalom murdered the crown-prince and his half-brother Amnon he found refuge with his grandparents in Geshur. Absalom's daughter, also named Maacha, married Rehoboam the son of Solomon. According to the Bible she was his most beloved wife. Despite these detailed biblical narratives we had no clue exactly where neither the kingdom nor its capital city was located. The excavation at Bethsaida is conducted by Dr. Rami Arav on behalf of the Consortium for the Excavations of Bethsaida housed at the University of Nebraska at Omaha. It has revealed a monumental capital city, and shed light on this forgotten kingdom. To understand this discovery more completely we need to add a little context and perspective. In the region between Damascus and Egypt, there were no less than seven ancient kingdoms that flourished between the tenth to the eighth centuries BCE. Each one of these kingdoms had a capital city. However, during the course of time, most of the remains that date from this period virtually disappeared due to later destructions and construction activities. Cities such as Damascus, the capital city of Damascus of the Arameans, Tyre the capital city of Phoenicia, Samaria the capital city of the kingdom of Israel, Jerusalem the capital city of Judah, Rabat-Ammon the capital city of the Ammonites, Dibon, the capital city of the Moabites, Sela the capital city of the Edomites, all either left meager, or no remains. Bethsaida is the only capital city in the region that was preserved. The excavation revealed some of the monuments of the city such as the city walls, a palace, and the largest and the best preserved city gate known from the biblical period. This presentation, illustrated by power point, will tell the story of the city that has been a mystery for so many years.

4:30 PM – 5:30 PM

Business Meeting, American College of Cryosurgery

New Members Welcome

Cinema Conference Center, Deck 3

7:00 PM – 8:00 PM

Farewell Reception and Closing Remarks

Hosted by the American College of Cryosurgery and ACCryo2013

Reflections Lounge, Deck 11

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