22nd Annual Conference of the German Society for Cytometry

October 10. - 12. 2012

CAESAR
Center of Advanced European Studies and Research
Bonn

Topics
Advanced Microscopy
Cancer, Rare Cells
Nano-, Micro- and Biotechnology
New Materials and Methods
Immunology

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Welcome to the 22nd Annual Conference of the German Society for Cytometry (DGfZ)

Dear Colleagues,

The program committee is delighted to welcome you at the

22nd Annual Conference of the DGfZ 2012

hosted at the Center of Advanced European Studies and Research, Bonn, Germany.

Before we talk about this year's conference, we would like to take the opportunity to look back for a moment. For those, who attended last year's conference, it was a lively and informative exchange of both scientific, technical and personal ideas and information. Both the lectures and the industrial exhibition were characterised by high quality presentations, as well as profound experts on site. The broad spectrum of topics gave attendants the opportunity to talk about nearly all aspects of cytometry. And whenever we asked people afterwards, talking was the main characteristic of the conference and what they enjoyed most. The center of advanced european studies and research in Bonn was an excellent venue for this purpose, and we would like to thank the people there that supported us, not only by providing excellent catering.

We therefore will organise this year's conference around the same topics and continue with the discussions that were already started. Beside the traditional topics in Immunology and Oncology, Nano- and Microtechnology as well as Micro- and Biotechnology are by now an integral part of the conference. Following the success of last year's microscopy session, we

also hope to have set up another impressive session on topics in advanced microscopy. We will again organise the *Meet the Expert* lecture, to give young people the opportunity to get in touch with experts in the field and we are happy that Prof. Andreas Radbruch took the time to come to our meeting. Moreover we would like to say thank you to all the speakers for sharing their knowledge with us.

Additionally, in order to support young scientists, the Klaus Goerttler Prize (1.000 €) was awarded by the DGfZ for extraordinary research of cytometric relevance. The 2012 Prize winner Susann Günther will present her data in the Biotechnology Session on Thursday morning.

Enough said about the spirit of a DGfZ conference, it is time now to get your own impressions and memories of the conference, as well as to make new contacts that might be old friends in the future.

Yours

Elmar Endl and Frank Schildberg
(Conference Organisers)

Scientific Committee

Wolfgang Beisker
Lars Blank
Hyun-Dong Chang
Elmar Endl
Wolfgang Fritzsche
Thomas Kroneis
Susann Müller
Josef Neumüller
Leoni Kunz-Schughart
Frank Schildberg
Stephan Schmid
Frank Schmidt
Torsten Viergutz

Organising Committee

Elmar Endl
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Local Organisers

Andreas Dolf
Peter Wurst

Keynote Speakers 22nd Annual Meeting DGfZ, Bonn 2012

June 21st, 2012 DGfZ Posted in Bonn 2012 | Edit | No Comments »

Meet the Expert Lecture

Andreas Radbruch

Cytometric Monitoring of Immunity and Inflammation

Deutsches Rheuma-Forschungszentrum (DRFZ)

10117 Berlin

E-Mail: raulfs@drfz.de

Free access for all students in Bonn and the members of the SFB704

Advanced Microscopy

Chair: Leoni Kunz-Schughart, Elmar Endl

Jan Huisken

Visualizing zebrafish development with light sheet microscopy and real-time image processing

Max Planck Institute
of Molecular Cell Biology and Genetics
Pfotenhauerstr. 108
01307 Dresden
Germany

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Kay Grossmann

Applications and advantages of laser-induced cryogenic fluorescence microscopy

Helmholtz-Zentrum Dresden-Rossendorf
Institut für Ressourcenökologie
Abteilung Biogeochemie
Postfach 51 01 19
01314 Dresden

Cancer

Chair: Stefan Schmid, (University Hospital Regensburg), Leoni Kunz-Schughart (OncoRay-Radiation Research in Oncology, Dresden)

Wolfgang Müller-Klieser

Biological and Clinical Relevance of Induced Metabolic Bioluminescence Imaging (imBI) in Cancer

Johannes Gutenberg-Universität
Institut für Physiologie und Pathophysiologie
Duesbergweg 6
D-55128 Mainz

E-Mail: Wolfgang.Mueller-Klieser@uni-mainz.de

Gabriele Multhoff

Detection of membrane-bound Hsp70 on tumor cells by flow cytometry and by in vivo imaging in tumor mouse models

Das Munich-Centre for Advanced Photonics
Klinik und Poliklinik für Strahlentherapie und Radiologische Onkologie
Klinkum rechts der Isar, Technische Universität München
D – 81675 München

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Rare Cells

Chair: Thomas Kroneis (Medizinische Universität Graz, Austria)

Klaus Pantel

Circulating Tumor Cells: Current State and Future Perspectives

Department of Tumor Biology
Universitätsklinikum Hamburg-Eppendorf
20246 Hamburg

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Anders Stahlberg

Understanding cell heterogeneity using single-cell gene expression profiling

University of Gothenburg
The Tumor heterogeneity group
Gothenburg, Sweden

Email: anders.stahlberg@gu.se

Core Facility Workshop

Rui Gardner

Core Facilities and their role in Research

UIC Imaging Unit
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Portugal

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Alfonso Blanco

Remote Training, Assisted Sessions and Standardization of Protocols

UCD Conway Institute of Biomolecular and Biomedical Research
Dublin 4
Ireland

E-Mail: alfonso.blanco@ucd.ie

Biotechnology

Chair: Lars M. Blank (Institute of Applied Microbiology, RWTH Aachen)

Lothar Eggeling

Metabolite sensors for HT single-cell isolation of producing bacteria

Forschungszentrum Jülich
IBG-1: Biotechnology
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New Materials and Methods

Chair: Elmar Endl (University Bonn)
Chair: Wolfgang Fritzsche, (ipht Jena)

Günter Mayer

Fluorescence Activated Cell Sorting for aptamer identification and validation

University of Bonn
Life and Medical Sciences (LIMES)- Institute
53121 Bonn

gmayer@uni-bonn.de

Stefaan Soenen

The Cytotoxic Effects of Inorganic Nanoparticles: A Multiparametric Methodology Exemplified with Quantum Dots and Gold Nanoparticles.

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Bernd Ebert

A new RNAi-based detection mechanism for imaging of tumors

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Innate Immunity

Chair: Frank Schildberg, IMMEI, Bonn, Eike Latz, Institute of Innate Immunity, Bonn

Eicke Latz

Assessing innate immune activation with fluorescence techniques

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Microbiology

Chair: Frank Schmidt (Competence Center- Functional Genomics, Greifswald), Susann Müller (Helmholtz Zentrum für Umweltforschung, Leipzig)

Nico Boon

Flow cytometry community fingerprinting to detect quickly stress in drinking water systems

The Laboratory of
Microbial Ecology and Technology (LabMET)
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Belgium

Immunology

Chair: Hyun-Dong Chang, Deutsches Rheuma Forschungszentrum, Berlin, Frank Schildberg, IMMEI, Bonn

Enric Esplugues

How TH17 cells are controlled in the gut

NeuroImmunology Lab
Deutsches Rheuma-Forschungszentrum (DRFZ)
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Session Overview

Date: Wednesday, 10/Oct/2012

12:15pm	**Welcome**
12:30pm - 2:00pm	**Advanced Microscopy** *Location:* ***Lecture Hall*** *Chair:* ***Elmar Endl*** *Chair:* ***Leoni Kunz-Schughart*** **Visualizing zebrafish development with light sheet microscopy and real-time image processing** **Jan Huisken** Max Planck Institute of Molecular Cell Biology and Genetics, Germany **Applications and advantages of laser-induced cryogenic fluorescence microscopy** **Kay Großmann[1], Christoph Tondera[2], Birgit Mosch[2], Christin Wimmer[2], Jens Pietzsch[2]** 1: Institute of Resource Ecology, Helmholtz-Zentrum Dresden-Rossendorf, Germany; 2: Institute of Radiopharmacy, Helmholtz-Zentrum Dresden-Rossendorf, Germany
2:00pm	**Coffee Break**
2:30pm - 4:30pm	**Cancer** *Location:* ***Lecture Hall*** *Chair:* ***Stephan Schmid*** *Chair:* ***Leoni Kunz-Schughart*** **Biological and clinical relevance of induced metabolic bioluminescence imaging (imBI) in cancer** **Wolfgang Müller-Klieser** Johannes Gutenberg-Universität Mainz, Germany

In vivo but not in vitro CD133 and CD44 expression pattern correlate with the engraftment of LS1034 colorectal cancer cells

Thiemo Dinger, Claudia Dittfeld, Melanie Huether, Marit Wondrak, Leoni A. Kunz-Schughart
OncoRay - National Cneter for Radiation Research in Oncology, TU Dresden, Germany

Detection of membrane-bound Hsp70 on tumor cells by flow cytometry and by in vivo imaging in tumor mouse models

Gabriele Multhoff[1], Alan Pockley[2]
1: Department of of Radiation Oncology, Klinikum Rechts der Isar, TU München, Germany; 2: John van Geest Cancer Research Centre, Nottingham Trent University, UK

Cell-based pre-selection of therapeutic antibody-conjugates for radionuclide-complexation

Mirjam Ingargiola[1], Claudia Dittfeld[1], Roswitha Runge[2], Jörg Steinbach[3], Leoni Kunz-Schughart[1]
1: Tumorpathophysiologie, OncoRay – National Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, TU Dresden; 2: Department of Nuclear Medicine, Medical Faculty Carl Gustav Carus, TU Dresden; 3: Institute of Radiopharmacy, Helmholtz-Zentrum Dresden-Rossendorf, Germany

microRNA-33: Cycling forward

Daniel Cirera
Deutsches Rheuma-Forschungszentrum (DRFZ) & NeuroCure, Germany

4:30pm **Coffee Break**

5:00pm - 6:30pm

Rare Cells

Location: ***Lecture Hall***
Chair: ***Thomas Kroneis***

Circulating tumor cells: Current state and future perspectives

Klaus Pantel
Institute of Tumour Biology, University Medical Center Hamburg Eppendorf

Understanding cell heterogeneity using single-cell gene expression profiling

Anders Stahlberg
University of Gothenburg, Sweden

Characterization of rare circulating tumor cells in patients with renal tumors

Amin El-Heliebi[1], Peter Sedlmayr[1], Thomas Kroneis[1], Johannes Haybäck[2], Katja Fischereder[3], Karin Kampel-Kettner[3], Richard Zigeuner[3], Berthold Huppertz[1], Carolin Lackner[2]

1: Institute of Cell Biology, Histology & Embryology, Medical University of Graz, Austria; 2: Institute of Pathology, Medical University of Graz, Austria; 3: Department of Urology, Medical University of Graz, Austria

Single cell next generation sequence - Creating digital heterogeneity profiles

Nicolò Manaresi

Silicon Biosystems SpA

6:30pm — **Coffee Break, Get Together**

7:00pm - 10:00pm

Core Managers Workshop

Location: ***Lecture Hall***
Chair: ***Elmar Endl***

Core Facilities and their role in Research

Rui Gardner

Instituto Gulbenkian de Ciência (IGC), Portugal

Remote Training, Assisted Sessions and Standardization of Protocols

Alfonso Blanco

UCD Conway Institute of Biomolecular and Biomedical Research, Ireland

Date: Thursday, 11/Oct/2012

9:00am - 10:30am

Biotechnology

Location: ***Lecture Hall***
Chair: ***Lars M. Blank***

Metabolite sensors for HT single-cell isolation of producing bacteria

Lothar Eggeling, Stephan Binder, Georg Schendzielorz, Dietrich Kohlheyer, Michael Bott

Forschungszentrum Jülich GmbH, Germany

	Towards a flow cytometry-based high throughput screening and selection platform for directed evolution and protein engineering **Ronny Martinez, Georgette Wirtz, Christian Pitzler, Ulrich Schwaneberg** RWTH Aachen University, Germany **Subpopulation-proteomics of bacterial cultures in biotechnology** **Michael Jahn, Jana Seifert, Martin von Bergen, Susann Müller** Helmholtz Centre for Environmental Research, Germany **Polyphosphate accumulating organisms in complex microbial communities – From fixation to community dynamics** **Susanne Günther (Klaus Goerttler Awardee 2012)** Helmholtz Centre for Environmental Research - UFZ, Germany
10:30am - 11:00am	**Coffee Break**
11:00am - 12:00pm	**Meet the Expert Lecture** *Location:* ***Lecture Hall*** **Cytometric monitoring of immunity and inflammation** **Andreas Radbruch** Deutsches Rheuma-Forschungszentrum Berlin (DRFZ), Germany
12:00pm	**Lunch**
1:00pm - 2:30pm	**New Materials and Methods** *Location:* ***Lecture Hall*** *Chair:* ***Elmar Endl*** *Chair:* ***Wolfgang Fritzsche*** **Fluorescence Activated Cell Sorting for aptamer identification and validation** **Günter Mayer** University of Bonn, Germany, LIMES Institute **The cytotoxic effects of inorganic nanoparticles: A multiparametric methodology exemplified with quantum dots and gold nanoparticles** **Stefaan Soenen[1,3], Wolfgang Parak[2], Stefaan De Smedt[1], Kevin Braeckmans[1,3]** 1: Faculty of Pharmacy, University Ghent, Belgium; 2: Biophotonics Group, Philipps Unniversity Marburg, Germany; 3: Nano- and Biophotonics Group, University Ghent, Belgium

A new RNAi-based detection mechanism for imaging of tumors

Bernd Ebert
Physikalisch-Technische Bundesanstalt, Germany

Nanoparticle-based single cell detection in biological fluids - Approaches and challenges

Roland H. Stauber
University Hospital of Mainz, Germany

2:30pm **Coffee Break**

3:00pm - 5:00pm

Emerging Technologies

Location: ***Lecture Hall***
Chair: ***Wolfgang Beisker***
Chair: ***Elmar Endl***
Chair: ***Frank A. Schildberg***

Droplet digital PCR: Molecular biology in high resolution

Daniel Burdziak
Bio-Rad Laboratories GmbH, Germany

Massively informative: Fundamentals and applications of mass cytometry

Ted George
DVS Sciences, Inc., United States of America

Apoptosis and associated assays for flow cytometry

Thomas M. Bauer
eBioscience, Germany

Simultaneous analysis of cell death mechanisms and oxidative stress using Molecular Probes® next generation reagents for imaging and flow cytometry

Bjoern Biedermann
Life Technologies, Switzerland

Standardized enumeration of human endothelial progenitor cells (EPCs) based on flow cytometry with the MACSQuant Analyzer

Andrea Völkel, Kathrin Pütsch, Leonie Wegener, Jürgen Schmitz
Miltenyi Biotec GmbH, Germany

	Put a handle to the individual cell - a new way of sorting with the Deparray system **Nicolò Manaresi** Silicon Biosystems SpA, Italy
5:00pm	**Coffee Break**
5:30pm - 7:00pm	**Meeting of DGfZ Members** *Location:* ***Lecture Hall*** *Chair:* ***Elmar Endl*** *Chair:* ***Leoni Kunz-Schughart***
7:00pm	**Break and Departure for the Dinner**
8:00pm	**Conference Dinner** The Zoological Research Museum Alexander Koenig (ZFMK) is one of the largest natural-history-research museums in Germany. The museum will host this years conference dinner and give the attendants the opportunity to visit the permanent exhibition, presenting only parts of these rich resources.

Date: Friday, 12/Oct/2012

9:00am - 10:15am

Innate Immunity

Location: ***Lecture Hall***
Chair: ***Eicke Latz***
Chair: ***Frank A. Schildberg***

Assessing innate immune activation with fluorescence techniques

Eicke Latz
Institute of Innate Immunity, University of Bonn, Germany & Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, USA

NLRP3 inflammasome activity is negatively controlled by miR-223

Anna Rieger[1], Franz Bauernfeind[1,2], Frank A. Schildberg[3], Percy A. Knolle[3], Jonathan L. Schmid-Burgk[1], Veit Hornung[1]
1: Institute for Clinical Chemistry and Pharmacology, Unit for Clinical Biochemistry, University Hospital, University of Bonn, Germany; 2: Department of Internal Medicine III, University Hospital, University of Bonn, Germany; 3: Institutes of Molecular Medicine and Experimental Immunology (IMMEI), University Hospital, University of Bonn, Germany

Influence of RNA secondary structure on the activation of Toll-like receptors 7 and 8

Thomas Zillinger[1], Vera Wimmenauer[1], Eicke Latz[2], Gunther Hartmann[1], Winfried Barchet[1]

1: Institute of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Germany; 2: Institute of Innate Immunity, University of Bonn, Germany

10:15am **Coffee Break**

10:30am - 12:00pm

Microbiology

Location: ***Lecture Hall***
Chair: ***Frank Schmidt***
Chair: ***Susann Müller***

Flow cytometry community fingerprinting to detect quickly stress in drinking water systems

Karen De Roy[1], Yingying Wang[1,3], Lieven Clement[2], Olivier Thas[2], Nico Boon[1]

1: Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Belgium; 2: Department of Applied Mathematics, Biometrics and Process Control, Ghent University; 3: Key Laboratory of Pollution Processes and Environmental Criteria, Nankai University, China

Monitoring microbial population dynamics using community flow cytometry

Christin Koch, Ingo Fetzer, Thomas Hübschmann, Hauke Harms, Susann Müller

Helmholtz Centre for Environmental Research - UFZ, Germany

3D visualization of bacterial uptake by human platelets using fluorescence microscopy and electron tomography

Josef Neumüller[1], Christof Jungbauer[2], Renate Renz[2], Adolf Ellinger[1], Margit Pavelka[1]

1: Medical University of Vienna, Center for Anatomy and Cell Biology, Department of Cell Biology and Ultrastructure Research, Vienna, Austria; 2: Blood donation Center of the Austrian Red Cross for Vienna, Lower Austria and Burgenland, Vienna, Austria

Preoteomic characterisation of host pathogen interactions during internalisation of Staphylococcus Aureus HG001 by A549 cells

Kristin Surmann

University of Greifswald, Germany

1:00pm - 2:30pm

Immunology

Location: ***Lecture Hall***
Chair: ***Hyun-Dong Chang***
Chair: ***Frank A. Schildberg***

How TH17 cells are controlled in the gut

Enric Esplugues
Deutsches Rheuma-Forschungszentrum (DRFZ), Germany

Antigen-reactive T cell enrichment for direct high resolution analysis of the human naïve and memory T helper cell repertoire

Petra Bacher[1], Janka Teutschbein[2], Olaf Kniemeyer[2], Mario Assenmacher[1], Axel A. Brakhage[2], Alexander Scheffold[3]
1: Miltenyi Biotec GmbH, Germany; 2: Institute for Natural Product Research and Infection Biology - Hans Knoell Institute; 3: Clinic for Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin

Differentiation of human leukocyte subsets by flow cytometry

Dorothee Köhler, Manuela Herber, Petra Bacher, Martin Büscher, Mario Assenmacher
Miltenyi Biotec, Germany

Identification of cell type-specific type I interferon signatures as biomarkers in viral infection and autoimmunity

Chieko Kyogoku[1], Joachim R Grün[1], Biljana Smiljanovic[1], Tobias Alexander[2], Robert Biesen[2], Falk Hiepe[2], Thomas Häupl[2], Andreas Radbruch[1], Andreas Grützkau[1]
1: German Rheumatism Research Centre Berlin (DRFZ), an institute of the Leibniz Association, Berlin, Germany; 2: Charité University Hospital Berlin, department of Rheumatology and Clinical Immunology, Berlin, Germany

Blocking type I IFN receptor signaling pathway during IDDM clearly restored the perturbation in the B and T lymphocyte, antigen-presenting cells (APCs) through the pro-inflammatory cytokines (IL-1α, IL-1β and TNFα and IL-6)

Heba Mohamed Saad Eldien Abd El ALL[1], Ibrahim Abd elhamid El-Elaimy[2], Hany Mohammed Ibrahim[2], Mohamed Badr[2], Gamal Badr[3]
1: Faculty of Medicine, Assiut University, Egypt; 2: Faculty of Science, Mnoufia University, Egypt; 3: Faculty of Science, Assiut University, Egypt

2:30pm **Farewell Coffee**

Advanced Microscopy

Time: **Wednesday, 10/Oct/2012: 12:30pm - 2:00pm**

Location: Lecture Hall
Session Chair: Elmar Endl
Session Chair: Leoni Kunz-Schughart

1

Visualizing zebrafish development with light sheet microscopy and real-time image processing

Jan Huisken
Max Planck Institute of Molecular Cell Biology and Genetics, Germany

In the past the recording speed of a time-lapse experiment has ultimately been limited by the amount of light the specimen could tolerate. Lately, it has been shown that light sheet microscopy such as Selective Plane Illumination Microscopy (SPIM, reviewed in Weber 2011) reduces photo-toxic effects to a minimum. Due to the illumination of the sample in a thin volume around the focal plane no tissue outside the plane of interest is exposed and bleached. In addition, the fluorescence is collected with very high sensitivity cameras. SPIM benefits from the latest camera technology and is therefore constantly improving in speed and sensitivity.

Experiments have become possible that run at full speed using the best possible hardware, without being limited by the fragility of the sample. The speed advantage of the SPIM over other fluorescence technique can be utilized to image rapid events in developing tissue or to record a large number of views for multi-view reconstruction. The large amount of data that is accumulated when modern cameras are run at high-speed for hours or days is enormous. Combining light-sheet microscopy with automated image processing opens the door for quantitative high-throughput developmental biology in real-time.

One key application of this emerging technology includes the multi-dimensional imaging of the developing zebrafish larvae over extended periods of time (Kaufmann 2012). I will give some examples of the unique capabilities of SPIM, especially for monitoring the development of the zebrafish heart (Arrenberg 2010) and the early endoderm.

2

Applications and advantages of laser-induced cryogenic fluorescence microscopy

Kay Großmann[1], Christoph Tondera[2], Birgit Mosch[2], Christin Wimmer[2], Jens Pietzsch[2]
[1]Institute of Resource Ecology, Helmholtz-Zentrum Dresden-Rossendorf, Germany; [2]Institute of Radiopharmacy, Helmholtz-Zentrum Dresden-Rossendorf, Germany

Fluorescence light microscopy has many advantages for the study of cells. Specimen preparation is easy and relatively inexpensive, and the use of appropriate tags gives scientists the ability to visualize specific cells, cell organelles, proteins of interest. However, recent trends in cell analytics tends to use label free methods. In this context it is well known, that the most organic molecules are able to emit fluorescence light after an excitation with light at a characteristic wavelength. At room temperature the fluorescence is frequently minimized by different quenching effects. Some of these quenching effects are strongly influenced by the temperature of the specimen. Normal temperature dependence of fluorescence shows an increasing fluorescence intensity and lifetime when the temperature is lowered. For label free fluorescence analytics we developed a combined system for laser induced fluorescence spectroscopic and microscopic measurements at temperatures down to 20 K. The system consists of a confocal laser scanning microscope, a very sensitive detector including

spectrograph and CCD, and a special cryogenic measuring cell. The cell is characterized by a closed cycle Gifford McMahon-based cryostat and a device for active insulation of cryostat-based vibrations. The design of the measuring cell is constructed for easily adapting on common light microscopes without time consuming reconstructions. Currently microscopic measurements with an up to 630-fold magnification are possible. The use of the novel technique was evaluated in two representative applications.

First experiments demonstrate an increment in the intensity of the fluorescence spectrum of different uranium VI species in biological samples by decreasing the temperature down to 20 K. Some of these uranium species show no detectable fluorescence at room temperature (RT), however, at 20 K a characteristic spectrum of uranium was visible. Comparable results show experiments on lactate, citrate, pyruvate and glucose.

Second, continuative microscopic experiments in melanoma cell lines demonstrate improved sensitivity in detection of fluorescent dyes at cryogenic conditions. In this regard, DAPI and other fluorescence dyes could be detected in a melanotic mouse melanoma cell line with 100-fold increased

sensitivity at 20 K compared to RT. As an additional benefit a lower photobleaching was observed at 20 K compared to RT. Furthermore, the use of laser-induced cryogenic fluorescence microscopy allowed visualization of COX-2 protein expression in amelanotic human melanoma cells using a novel, autofluorescent selective COX-2 inhibitor. The compound did not require additional chemical modification, e.g., by coupling fluorophor substituents.

These results show that the new cryogenic measuring chamber represents an interesting and gainful tool for fluorescence based investigations.

Cancer

Time: **Wednesday, 10/Oct/2012: 2:30pm - 4:30pm** ·

Location: Lecture Hall
Session Chair: Stephan Schmid
Session Chair: Leoni Kunz-Schughart

1

Biological and clinical relevance of induced metabolic bioluminescence imaging (imBI) in cancer

Wolfgang Müller-Klieser

Johannes Gutenberg-Universität Mainz, Germany

Malignant transformation is frequently associated with an increase in cellular glucose uptake by one order of magnitude. As a consequence, elevated glucose consumption and lactate production even under normoxic conditions are common features of cultured cancer cells, which is often termed "aerobic glycolysis" or the "Warburg effect". Database analyses suggest that over-expression of glycolysis-related genes is present in 26 tumor entities, i. e. in 70 % of all human cancers worldwide. Although Warburg metabolism frequently occurs in malignant tumors in the clinic, its effectiveness can be rather variable. This may be reflected by a pronounced inter-individual variability in the lactate accumulation in human cancers which is even true for equal tumor stages and pathohistological grades.

Metabolites can be detected quantitatively in relation to the tissue structure by induced metabolic bioluminescence imaging (imBI), a technique that has been developed in our laboratory and has been exploited for the generation of a number of clinically relevant results on tumor metabolism (1). Realistic metabolite concentrations in situ are obtained with imBI in snap frozen tissue as a "snap shot" of the momentary metabolic situation at the time of specimen removal.

Using imBI in several clinical studies on different tumor entities, we could show that the degree of pre-therapeutic lactate accumulation in primary tumors can predict for metastases, as well as overall and disease-free survival of patients, as summarized in (1). These significant findings may be explained, at least in parts, by a lactate-mediated enhancement of random migration of single cancer cells and cancer cell clusters, and by a lactate-induced secretion of hyaluronan by tumor-associated fibroblasts creating a milieu which is favorable for migration. Findings on interactions of tumor metabolites with immune cells indicate a contribution of lactate to the immune escape of tumors, e. g. by impeding migration and cytokine release of dendritic cells or cytotoxic T cells (1). Furthermore, lactate plays a crucial role in wound healing, chronic inflammation, and cancer development. Tumor cells ensure sufficient oxygen and nutrient supply for proliferation through lactate-induced secretion of VEGF, resulting in the formation of new vessels. In recent studies, radioresistance has been positively correlated with lactate concentrations, which may at least be partially based on the well-documented anti-oxidative capacity of glycolytic metabolites, such as pyruvate and lactate (1). In summary, accumulation of lactate in solid tumors is a pivotal and early event in the development of cancer. Pre-therapeutic tissue levels of lactate in primary tumors are closely correlated with the prospective malignant behavior of the disease. As a conclusion, the determination of lactate should enter further clinical trials to confirm its relevance in clinical oncology. – A summary of lactate biology in cancer has been published recently by Hirschhaeuser et al. (1).

(1) Hirschhaeuser, F., Sattler, U. G. A. and Mueller-Klieser: Lactate: A metabolic key player in cancer - Invited review; Cancer Res. 71(22): 6921-6925 (2011).

Supported by the DFG: PAK124; Mu576/14-1, /14-2, 15/-1, /15-2, /17-1; SA1749/3-1

2

In vivo but not in vitro CD133 and CD44 expression pattern correlate with the engraftment of LS1034 colorectal cancer cells

Thiemo Dinger, Claudia Dittfeld, Melanie Huether, Marit Wondrak, Leoni A. Kunz-Schughart

OncoRay - National Cneter for Radiation Research in Oncology, TU Dresden, Germany

Background & Aim

CD133 and CD44 have both been described as surrogate biomarkers to enrich colorectal cancer cell (CRC) subpopulations from primary tissue with enhanced tumorigenic potential. However, the relevance of these markers to define similar subpopulations in established CRC cell lines is under debate. We showed earlier, that CD133 expression does not define a highly tumorigenic subpopulation in various CRC cell lines *in vitro.* One cell lines of interest was LS1034 which is negative for CD44 surface expression *in vitro.* In LS1034 xenografts, however, a CD44/CD133 double-positive population was identified. We therefore aimed to elucidate the impact of CD44 induction on tumorigenicity.

Materials & Methods

CD133/CD44 antigen pattern were studied by flow cytometry and Western blotting. Xenograft formation and growth were monitored after injection of LS1034 cells into the hind limbs of NMRI(nu/nu) mice with Matrigel. Cell numbers of 10 - 10,000 were applied per injection site and mouse, respectively. CD133-positive and CD133-negative subpopulations were separated by fluorescence-activated cell sorting (FACS) and analyzed for their tumorigenic potential and marker expression in the resulting xenograft tumors. Selected primary xenografts were dissociated, stained for HLA, CD44, CD133 and PI, sorted by FACS and re-injected *in vivo.*

Results & Conclusion

Exponential LS1034 cells under standard conditions do not express CD44 on their surface. In xenograft tumors, a clear CD44-positive fraction was identified. This fraction was always positive for CD133. Tumor take of CD133-negative versus CD133-positive LS1034 only showed a tendency. However, when LS1034 xenografts were dissociated and the human cancer cells were sorted according to their *in vivo* CD133/C44 expression pattern to be re-injected for secondary engraftment, the CD133/CD44 double-positive population showed highest tumor formation rate, CD133-positive/CD44-negative LS1034 cells were intermediate and the CD133/CD44 double-negative fraction turned out to not produce secondary xenografts upon injection of up to 10,000 cells per mouse. We conclude that the expression pattern of the markers and the behavior of the cells are critically affected by the *in vivo* microenvironment.

This work was supported by DFG grant KU 971/7-1.

3

Detection of membrane-bound Hsp70 on tumor cells by flow cytometry and by in vivo imaging in tumor mouse models

Gabriele Multhoff[1], Alan Pockley[2]

[1]Department of of Radiation Oncology, Klinikum Rechts der Isar, TU München, Germany;
[2]John van Geest Cancer Research Centre, Nottingham Trent University, UK

The Munich laboratory has generated the IgG1 mouse monoclonoal antibody (mAb) termed cmHsp70.1 by immunizing mice with a 14-mer Hsp70 peptide TKDNNLLGRFELSG (TKD). This antibody uniquely detects Hsp70 on the cell surface of human and mouse tumor cells

(Stangl S et al. 2011a). In contrast to normal tissues, a variety of tumor types frequently present Hsp70 on their plasma membrane. By screening of a large panel of different tumor entities we have demonstrated that overall survival of patients with membrane Hsp70 positive lower rectal cancer, non small lung cell carcinomas, sarcoma and leukemia is significantly lower than that of patients bearing membrane Hsp70 negative counterparts. Furthermore, Hsp70 exhibits a rapid turn-over rate at 37°C and is also secreted into the extracellular milieu by tumor cells. Evidence relates circulating Hsp70 levels to disease status/progression in patients with cancer. Ongoing work at the Nottingham Trent University is assessing the relationship between the membrane Hsp70 status of tumors with serum Hsp70 protein levels in patients with cancer.

In addition to the above, *in vivo* studies reveal that fluorophor-labeled cmHsp70.1 mAb binds to membrane Hsp70 positive tumors in syngeneic tumor mouse models (CT26 colon and 4T1 mammary carcinoma models) after intravenous injection of the antibody into the tail vein of mice (Stangl S et al. 2011b). Intraoperative *in vivo* imaging and fluorescence molecular tomography (FMT) demonstrate the tumor specificity of cmHsp70.1 mAb, *in vivo.* As therapeutic interventions such as radiochemotherapy enhance the tumor-specific, cell surface expression density of Hsp70, these data suggest that the cmHsp70.1 mAb could be used for *in vivo* tumor imaging, monitoring of clinical outcome and when coupled to toxins as a theranostics in the future (Gehrmann M et al. 2012).

Stangl S et al. Targeting membrane Hsp70 on tumors by cmHsp70.1 antibody PNAS 108: 733-738, 2011a

Stangl S et al. *In vivo* imaging of CT26 mouse tumors by using cmHsp70.1 antibody. J Cell Mol Med 15(4): 874-887, 2011

Gehrmann M et al. Immunotherapeutic targeting of membrane Hsp70 expressing tumors suing recombinant human granzyme B. PLos One accepted, 2012

4

Cell-based pre-selection of therapeutic antibody-conjugates for radionuclide-complexation

Mirjam Ingargiola[1], Claudia Dittfeld[1], Roswitha Runge[2], Jörg Steinbach[3], Leoni Kunz-Schughart[1]

[1]Tumorpathophysiologie, OncoRay – National Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, TU Dresden; [2]Department of Nuclear Medicine, Medical Faculty Carl Gustav Carus, TU Dresden; [3]Institute of Radiopharmacy, Helmholtz-Zentrum Dresden-Rossendorf, Germany

Background & Aim: Radionuclide-conjugated target-specific antibodies (Abs) in combination with external irradiation present a promising theragnostic strategy for solid tumors. Binding of radionuclides to an Ab-carrier requires chemical modification, which may lead to a critical loss in binding affinity. We used the therapeutic anti-EGFR Ab Cetuximab (C225) as carrier conjugated to several chelator molecules. We aimed at developing a cell-based flow cytometric binding assay to pre-select a suitable construct for 90Y-complexation. The construct of choice was then further validated in a competitive radioactive binding assay using 2-D and 3-D culture models. Materials & Methods A flow cytometric test protocol was established to compare EGFR-binding capacities of C225-conjugates using A431, FaDu and SAS cells. Exponential monolayer cultures were dissociated, and single cells were incubated with different concentrations of the Ab-conjugates. Unconjugated C225 served as control. Flow cytometric measurement of a minimum of 10.000 viable cells per condition was performed after staining with a fluorescent-labelled secondary Ab and addition of propidium iodide.

For the competitive radioactive binding assay, monolayer and spheroid cultures were

incubated with 90Y-complexated C225-conjugate supplemented with increasing amounts of unconjugated C225. Cell and spheroid-bound activity was measured after defined times of exposure using a gamma counter.

Result & Conclusions

Flow cytometric analysis revealed that cell surface EGFR is highest in A431, intermediate in FaDu and lowest in SAS cells. Antigen saturation by unconjugated C225 was cell line-dependent (3.3 – 33 nM). For the evaluation of C225-conjugate binding capacities, a concentration below saturation was required and thus defined at 0.33 nM. The application of one cell line in the flow cytometric assay was found to be sufficient for evaluating EGFR-binding. Examination of various C225-conjugates indicated that only (CHX-A´´-DTPA)5-C225 exhibited a C225-analogous binding capacity. The candidate was then complexated with 90Y and tested in monolayer culture and tumor spheroids in a competitive radioactive binding approach. Cell and spheroid-bound radioactivity of the [90Y]Y-C225-conjugate showed to be directly proportional to the binding of unconjugated Ab. The Ab construct thus complies with the basic requirements for therapeutic application. The flow cytometric assay turned out to be an easy-handling tool for integration in an Ab test platform.

For more details see Ingargiola et al., Cytometry A, Aug 2012 online.

This work was supported by the BMBF via grant 02NUK006B.

5

microRNA-33: Cycling forward

Daniel Cirera

Deutsches Rheuma-Forschungszentrum (DRFZ) & NeuroCure, Germany

Cholesterol metabolism is tightly regulated at the cellular level and is essential for cellular growth. Cellular imbalances of cholesterol and fatty acid metabolism lead to pathological processes, including atherosclerosis and metabolic syndrome. MicroRNAs (miRNAs), a class of noncoding RNAs, have emerged as critical regulators of gene expression acting predominantly at posttranscriptional level. Recent work from Fernandez-Hernando´s group and others has shown that hsa-miR-33a and hsa-miR-33b, miRNAs located within intronic sequences of the sterol regulatory element-binding protein (SREBP-2 and SREBP -1) genes, respectively, regulate cholesterol metabolism in concert with their host genes. Similarly, miR-33 targets key enzymes involved in the regulation of fatty acid oxidation including carnitine O-octanoyltransferase (CROT), carnitine palmitoyltransferase 1A (CPT1A), hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (HADHB), sirtuin 6 (SIRT6) and AMP-activated protein kinase (AMPK)α, likewise, the insulin receptor substrate 2 (IRS2), an essential component of the insulin-signaling pathway in the liver. This study shows that hsa-miR-33 family members not only regulate genes involved in cholesterol and fatty acid metabolism and insulin signaling, but in addition modulate the expression of genes involved in cell cycle regulation and cell proliferation. Thus, miR-33 inhibited the expression of the cyclin-dependent kinase 6 (CDK6) and cyclin D1 (CCND1), thereby reducing cell proliferation and cell cycle progression. Over-expression of miR-33 induced a significant G1 cell cycle arrest and most importantly, inhibition of miR-33 expression using 2'fluoro/methoxyethyl-modified (2'F/MOE-modified) phosphorothioate backbone antisense oligonucleotides improved liver regeneration after partial hepatectomy (PH) in mice, suggesting an important role for miR-33 in regulating hepatocyte proliferation during liver regeneration. Altogether, these data establish that miR-33 regulates pathways controlling three of the risk factors of metabolic syndrome, namely levels of HDL, triglycerides and insulin signaling, and suggest that inhibitors of miR-33 may be useful in the treatment of this growing health concern. Furthermore, Srebf/miR-33 locus may co-operate to regulate cell proliferation, cell cycle progression and may also be relevant to human liver regeneration.

Rare Cells

Time: **Wednesday, 10/Oct/2012: 5:00pm - 6:30pm**

Location: Lecture Hall
Session Chair: Thomas Kroneis

1

Circulating tumor cells: Current state and future perspectives

Klaus Pantel
Institute of Tumour Biology, University Medical Center Hamburg Eppendorf

Microscopic spread of cancer cells is usually undetected by current imaging technologies. Therefore, a variety of sensitive methods have been developed to detect circulating tumor cells (CTC) in the peripheral blood. The capture of CTC that are surrounded by millions of blood cells is based on the physical and/or biological properties of cancer cells. Thus far, only the automated CellSearchTM system has been cleared by the FDA. However, many assays including the CellSearchTM system rely on EpCAM for CTC capture and cytokeratins for CTC detection but these epithelial marker proteins can be downregulated during epithelial-mesenchymal transition.

Besides assessing the clinical utility of CTC for assessment of prognosis, monitoring of CTC during and after systemic therapy can provide unique information for the clinical management of individual cancer patients. In particular, the molecular analysis of CTC as “liquid biopsy” will give new insights into therapeutic targets and the selection of tumor cells under specific therapies. In addition, molecular and functional characterization of CTC opens a new avenue for understanding metastatic spread of tumor cells with important implications for future therapies.

Implementation of CTC analyses in clinical trials testing new anti-cancer agents as companion diagnostics will speed up the cumbersome and expensive drug validation process in oncology.

2

Understanding cell heterogeneity using single-cell gene expression profiling

Anders Stahlberg
University of Gothenburg, Sweden

Individual cells in organism are unique units that can work both independently and together in tissues and organs. Single-cell studies on both the protein and mRNA level show large cell-to-cell variation in both resting and stimulated states. This implies that data obtained from large pools of cells does not, and indeed, cannot, accurately reflect the behavior of the individual cell.

Here, we discuss technical and biological aspects of single-cell analysis using qPCR. Single-cell data from tumor cell lines, embryonic stem cells, astrocytes and beta-cells will be presented to visualize how different stimuli affect cell differentiation and specific cell functions. We will also discuss how single-cell data can be used to gain detailed information about cell types, subpopulations and biomarkers.

3

Characterization of rare circulating tumor cells in patients with renal tumors

Amin El-Heliebi[1], Peter Sedlmayr[1], Thomas Kroneis[1], Johannes Haybäck[2], Katja Fischereder[3], Karin Kampel-Kettner[3], Richard Zigeuner[3], Berthold Huppertz[1], Carolin Lackner[2]

[1]Institute of Cell Biology, Histology & Embryology, Medical University of Graz, Austria; [2]Institute of Pathology, Medical University of Graz, Austria; [3]Department of Urology, Medical University of Graz, Austria

Background:

Malignant tumors may release tumor cells into the blood either singly (circulating tumor cells, CTCs) or as cellular aggregates (tumor cell microemboli, CTM). Detection of CTCs/CTMs is an important prognostic factor but has hitherto not been analyzed in detail in patients with renal tumors. We therefore aimed to isolate and characterize CTCs/CTMs from the blood of patients with renal tumors.

Methods:

Whole blood of 40 patients with malignant (30) and benign (10) renal tumors was filtered through 8µm pore size ScreenCell® filters. Epithelial cells are mostly larger than 8µm and are trapped on the filter while most blood cells pass through. Large cells and cellular aggregates were analyzed by light microscopy using cytopathological criteria which have been issued by expert cytopathologists for the classification of cells isolated by whole blood filtration. The filtered cells were classified as malignant (CTCs/CTMs), uncertain malignant or benign, isolated by laser capture microdissection and the DNA was analyzed by array-CGH. DNA from the respective renal tumors served as control.

Results and conclusion:

Large cells were found in 10/37 blood samples (27%). While the amount and quality of DNA from single large cells was not sufficient for array-cgh we detected genetic aberrations in CTMs and cellular aggregates with uncertain malignant features which, however, did not correspond to the aberrations present in the respective renal tumors. Although speculative, these differences might be explained by tumor heterogeneity.

4

Single cell next generation sequence - Creating digital heterogeneity profiles

Nicolò Manaresi

Silicon Biosystems SpA

Next Gen Sequencing (NGS) has made multiplexed sequence analysis more cost effective and easier to obtain. When combined with appropriate sample preparation technology it is possible to obtain clear mutational profiles at the single cell level. In this talk Silicon Biosystems will show NGS results obtained from the recovery of single circulating tumor cells.

Core Managers Workshop

Time: **Wednesday, 10/Oct/2012: 7:00pm - 10:00pm**

Location: Lecture Hall
Session Chair: Elmar Endl

1

Core Facilities and their role in Research

Rui Gardner

Instituto Gulbenkian de Ciência (IGC), Portugal

Core Facilities are becoming more and more professionalized. They evolved from the need of researchers to answer questions requiring high-end technology that only a few highly self-trained people could cope with, and from the need of research centers to optimize shared resources. In the beginning, most core facilities were created and ran by principle investigators who had acquired knowledge in the respective technique. But as research centers grew in number and in demand for quality, the need for specialized and highly trained staff became evident. This process evolved at a considerable fast rate in the last decades, from which a new group of research staff has spawned, known today as core facility technicians or staff. This staff is composed both by researchers who found they could apply their specialized skills and capabilities to increase their contribution to science, or by people that have never had experience as researchers. This mixed population, and other aspects such as the requirements of these staff to provide technical service, has led to a growing confusion towards the contribution and importance of core facilities in science and in research centers.

In my talk, I would like to briefly present a few key points, some of which were discussed fervently in the Core Management Workshop that took place last July in Lisbon, that I feel are important to help us communicate to our peers, fellow researchers, and institutions, the role of core facilities in research centers, and their contribution towards science. There is a growing feeling in the community of core managers and staff in general, that we need to find common ground, and more and more societies and associations are joining to help us in in this goal, allowing us to reach what should be our most significant objective, which is to make better science.

2

Remote Training, Assisted Sessions and Standardization of Protocols

Alfonso Blanco

UCD Conway Institute of Biomolecular and Biomedical Research, Ireland

In September 2011, during the 7th European Course on Clinical Cytometry, it took place the first hands on cell sorting training course using remote sites, 14 places in Europe and USA). After the success of this course, this idea has been maturated and discussed and it has been converted into a proposal for the 3 main cytometry societies: International Society for Advancement of Cytometry (ISAC) – European Society of Clinical Cytometry Analysis (ESCCA) – International Clinical Cytometry Society (ICCS).

This proposal is the creation of a network of labs and expertise in different fields and aspects of cytometry (flow cytometry and imaging) for the development of new educational tools, running hands-on training courses, remote assisted sessions at basic and advance level, standardization of clinical protocols,… This proposal can go as far as people want to develop it and we are looking for fresh ideas, different criteria about how it should be established it, as well as volunteers all around the World.

Biotechnology

Time: **Thursday, 11/Oct/2012: 9:00am - 10:30am** ·

Location: Lecture Hall
Session Chair: Lars M. Blank

1

Metabolite sensors for HT single-cell isolation of producing bacteria

Lothar Eggeling, Stephan Binder, Georg Schendzielorz, Dietrich Kohlheyer, Michael Bott

Forschungszentrum Jülich GmbH, Germany

We have developed a general tool to visualize at the single cell-level of E. coli or C. glutamicum the cytosolic concentration of small-molecules. This enables numerous new applications. The system developed is based on transcriptional regulators sensing the molecule of interest and which in response to this signal drives transcription of an autofluorescence protein. We have developed individual sensors to monitor in C. glutamicum the concentration of L-lysine, O-acetyl-serine, or L-serine, and in E. coli L-arginine. I will present this new single cell technology together with three examples on its application.

In one example we identified new chromosomal mutations leading to L-lysine synthesis. The wild type of C. glutamicum carrying the L-lysine sensor was treated with mutagen. Out of 6.5 x 106 cells 270 cells were selected via FACS, of which 225 accumulated 3-38 mM L-lysine. Targeted sequencing identified 13 new chromosomal mutations in the known targets lysC and hom. From 10 mutants with no mutation in known targets the entire genome was sequenced using Illumina HiSeq 2000 technology. As a new target a murE mutation was identified which when introduced into existing L-lysine producers improved the L-lysine titers significantly.

In another example we used our technology to successfully screen in vivo via FACS a plasmid encoded library of a key enzyme of arginine synthesis to isolate enzymes no longer controlled in their activty.

In an even further example we used our technology together with a microfluidic chip device to follow lysine synthesis in single cells by time-lapse microscopy.

Since transcriptional regulators exist for sugars, sugarphosphates, vitamins, oxoacids, mevalonate, antibiotics, and further small-molecules our technology is expected to boost metabolic engineering substantially.

Binder S, Schendzielorz G, Stäbler N, Krumbach K, Hoffmann K, Bott M, Eggeling L. A high-throughput approach to identify genomic variants of bacterial metabolite producers at the single-cell level. Genome Biol. 2012, 13(5):R40.

2

Towards a flow cytometry-based high throughput screening and selection platform for directed evolution and protein engineering

Ronny Martinez, Georgette Wirtz, Christian Pitzler, Ulrich Schwaneberg

RWTH Aachen University, Germany

Enzyme reengineering for research and industrial applications has revealed many challenges in the fields directed enzyme evolution. While the genetic diversity generation has been addressed by the development of novel methods such as SeSaM, pTRec and Omnichange, researchers still struggle to effectively screen the immense theoretical sequence space available in the generated libraries. This is especially true when the standard format for

enzymatic activity screening is still the well microtiter plate. Based in the concept presented by Tawfik and Griffiths, we are working in the development of a flow cytometry-based screening to provide an ultra-high throughput platform for directed enzyme evolution. We have proven, in the case of proteases, monoxygenases and glucose oxidases by employing bacterial whole-cell flow cytometry screening systems that is possible to identify and isolate improved enzyme variants by flow cytometry.

The next step towards a reliable flow cytometry screening platform is to optimize the genotype-phenotype link through microcompartmentalization. In that front we are investigating, aside from the classic water-in-oil-in-water emulsion, new polymer-based encapsulation systems suitable for flow cytometry analysis.

In parallel, we are addressing the diversity loss associated to the subcloning and transformation of the genetic libraries into the protein expression host. In vitro protein production allows skipping those steps, by directly producing and screening enzyme variants inside microcompartments.

By integrating these developments, it is possible to accelerate directed evolution campaigns, in two aspects, the amount of screened variants, and number of iterative rounds per campaign. Such acceleration would open new research possibilities and strategies in protein engineering, such as evolutive behavior studies, genetic drift, evolutive statistics and directed evolution at high mutational loads.

3

Subpopulation-proteomics of bacterial cultures in biotechnology

Michael Jahn, Jana Seifert, Martin von Bergen, Susann Müller
Helmholtz Centre for Environmental Research, Germany

Clonal microbial cells do not behave in an identical manner and form subpopulations during cultivation. Besides varying micro-environmental conditions, individual cellular features like cell cycle stage or epigenetic modifications cause cellular heterogeneity. Furthermore, transcription and translation are processes prone to molecular noise. A promising tool to unravel these causes for cell heterogeneity is cell sorting followed by subpopulation proteomics of sorted cells. As a proof of principle, cultures of *Pseudomonas putida* were sorted by flow cytometry regarding EGFP expression (yes/no) and inclusion body formation (yes/no). The resulting three subpopulations were analyzed by mass spectrometry identifying around 750 different proteins. The comparison of the three subpopulations revealed only minor changes in metabolic pathways but a strong induction of single stress response proteins. The presented method is therefore useful to characterize cell heterogeneity on the protein level with high accuracy and coverage.

4

Polyphosphate accumulating organisms in complex microbial communities – From fixation to community dynamics

Susanne Günther
Helmholtz Centre for Environmental Research - UFZ, Germany

Susanne Günther was awarded the Klaus Goerttler Prize 2012

Wastewater treatment often suffers from instabilities and the failure of specific functions such as biological phosphorus removal by polyphosphate accumulating organisms. Since most of the microorganisms involved in water clarification are unknown it is challenging to operate the process accounting for the permanent varying abiotic parameters and the complex composition and unrevealed metabolic capacity of a wastewater microbial community.

Flow cytometric analyses of cellular DNA and polyphosphate content were used to create patterns mirroring dynamics in community structure in a primary clarifier and two activated sludge basins. These patterns were resolved in up to 15 subcommunities, the presence and abundances of which correlated with abiotic data. Biostatistics were applied to determine the stability of the microbial community.

The stability of microbial community structure was found to be high in the basins and low in the primary clarifier. Despite major abiotic changes certain subcommunities were dominantly present (up to 80% stability), whereas others emerged only sporadically (down to 3% stability, both according to equivalence testing). Additionally, subcommunities of diagnostic value were detected showing positive correlation with substrate influxes (blackwater and brewery inflow). A high percentage of polyphosphate accumulating organisms were observed whose abundance was positively correlated with nitrate and the presence of denitrifying organisms (Rhodocyclaceae).

In essence, a monitoring tool was developed which is quick, cheap and causal in its interpretation. It allows reliable process monitoring and control in wastewater treatment plants.

Meet the Expert Lecture

Time: **Thursday, 11/Oct/2012: 11:00am - 12:00pm** ·

Location: Lecture Hall

Cytometric monitoring of immunity and inflammation

Andreas Radbruch

Deutsches Rheuma-Forschungszentrum Berlin (DRFZ), Germany

A biologist by education, Andreas Radbruch did his PhD with Klaus Rajewsky at the genetics institute of the Cologne university, working on genetic variants of hybridoma cell lines. He then became assistant and later associate professor there, and developed a line of research aiming at a molecular understanding of immune reactions and immunological memory.

Initially, he showed that antibody switch recombination in B lymphocytes is targeted to the same switch region on both IgH loci of a cell, and controlled by cytokine-induced and processed “switch transcripts”. His group then addressed expression of the cytokines by T lymphocytes, in particular, how a "cytokine memory" is imprinted in effector and memory T lymphocytes.

At that time, the group of Andreas Radbruch had developed high-gradient magnetic cell sorting, the MACS-technology, and the cytometric secretion assay, which were instrumental to analyze cytokine gene expression in T cells, but also to analyse plasma cell biology. In 1996, Andreas Radbruch became professor of rheumatology at the Charité and director of the German Rheumatism Research Center in Berlin.

The Radbruch group could show that plasma cells can survive long time periods in the bone marrow, as “memory” plasma cells, in survival niches organized by dedicated stroma cells. It appears that this principle of organisation of memory also applies to T cell memory. The “resting” memory of bone marrow contrasts with the longlived effector T cells of inflamed tissue. The Radbruch group has described several microRNAs and transcription factors involved in the clonal expansion and persistence of inflammatory, rheumatic T cells.

New Materials and Methods

Time: **Thursday, 11/Oct/2012: 1:00pm - 2:30pm** ·

Location: Lecture Hall
Session Chair: Elmar Endl
Session Chair: Wolfgang Fritzsche

1

Fluorescence Activated Cell Sorting for aptamer identification and validation

Günter Mayer

University of Bonn, Germany, LIMES Institute

Aptamers are short nucleic acids that fold into defined 3D- structures. Upon these they can interact with defined target molecules with high affinity and selectivity. In vitro selection schemes, also coined as SELEX (systematic evolution of ligands by exponential amplification) allow the identification of aptamers in highly diverse nucleic acid libraries. Recently, aptamers have been described that recognize specific cell-surface marker proteins. These aptamers have a great potential to be applied in diagnostics and the development of novel therapies.

We have established a flow-cytometry and cell sorting-based selection scheme (FACS-SELEX) that enables the identification of population-specific aptamers targeting Burkitt's lymphoma cells. Upon cell recognition the target cells endocytose these aptamers thus, enabling the delivery of attached cargo molecules. These properties allow the cell-specific delivery of therapeutically relevant agents thereby diminishing potential side effects. For example, we have identified a series of pan-tumour selective aptamers that enable the intracellular delivery of siRNA molecules and, thus, the induction of apoptosis and cell death. Imoprtanty, non-targeted cells were not affected by the aptamer-siRNA assemblies.

2

The cytotoxic effects of inorganic nanoparticles: A multiparametric methodology exemplified with quantum dots and gold nanoparticles

Stefaan Soenen[1,3], Wolfgang Parak[2], Stefaan De Smedt[1], Kevin Braeckmans[1,3]

[1]Faculty of Pharmacy, University Ghent, Belgium; [2]Biophotonics Group, Philipps Unniversity Marburg, Germany; [3]Nano- and Biophotonics Group, University Ghent, Belgium

The interest in using nanoparticles for a wide range of biomedical applications is vastly increasing. However, for most particles, their use in live cells and animals remains limited, which is largely due to the potential toxicity of these particles which remains an issue of debate. This uncertainty is caused by large differences between nanoparticle characteristics and cellular parameters tested, resulting in large discrepancies in the available literature data.

Here, a recently established multiparametric protocol is used to investigate the cytotoxic effects of polymer-coated gold nanoparticles and core-shell quantum dots, of which the cytotoxic effects are determined using multiple cell types, including murine neural progenitor cells and primary human umbilical vein endothelial cells. Parameters studied include the effects of the particles on cell viability, generation of reactive oxygen species (ROS) and secondary effects, cell cycle progression, intracellular quantum dot localization and concentration, cell morphology and cell functionality. For quantum dots, their stability against the degradative endosomal environment and long-term effect on toxicity and particle functionality are given special focus. Using this extensive panel of tests allows to reveal the mechanisms involved in particle toxicity and to define the No Observed Adverse Effects Levels (NOAELs: concentrations at which no negative effects are observed).

Gold particles were found to affect cells mainly through the induction of ROS and alterations in cell cytoskeleton, which resulted in DNA damage and impeded cell functionality. Using lysosomal buffer systems and proliferation-restricted cells, intracellular quantum dots were found to localize in endosomes, generating reactive oxygen species, interfering with cell cytoskeleton and leaching free cadmium ions due to quantum dots dissolution, resulting in increased toxicity and impeded quantum dot fluorescence. Furthermore, asymmetric partitioning of quantum dots upon recurrent cell division results in the sacrifice of heavily-loaded cells and a rapid loss of particles in live cells.

These data highlight the importance of a multiparametric methodology to assess nanoparticle cytotoxicity as particles were found to affect cell homeostasis by various mechanisms. Also, the behaviour of the particles in their final biological micro-environment must be studied carefully as well as the effect of the particles as a function of time.

1) Soenen SJ, Rivera-Gil P, Montenegro JM, Parak WJ, De Smedt SC, Braeckmans K. Cellular toxicity of inorganic nanoparticles: Common aspects and guidelines for improved nanotoxicity evaluation. Nano Today, 2011, 6, 446-465.

2) Soenen SJ, Himmelreich U, Nuytten N, De Cuyper M. Cytotoxic effects of iron oxide nanoparticles and implications for safety in cell labeling. Biomaterials, 2011, 32, 195-205.

3) Soenen SJ, Demeester J, De Smedt SC, Braeckmans K. The cytotoxic effects of polymer-coated quantum dots and restrictions for live cell applications. Biomaterials, 2012, 33, 4882-4888.

4) Soenen SJ, Manshian B, Montenegro JM, Amin F, Meermann B, Thiron T, Cornelissen M, Vanhaecke F, Doak S, Parak WJ, De Smedt S, Braeckmans K. Cytotoxic effects of gold nanoparticles: A multiparametric study. ACS Nano, 2012, 6, 5767-5783.

3

A new RNAi-based detection mechanism for imaging of tumors

Bernd Ebert

Physikalisch-Technische Bundesanstalt, Germany

Optical molecular imaging represents a modern field of research that allows the elucidation of molecular biological processes using appropriate probes and their visualization in vivo. This technique of imaging may - apart from the injection of contrast agents - referred to as non-invasive. To obtain the most accurate representation of the processes at the cellular level in vivo, the probes used must be small enough to obtain undisturbed information. As an essential part of molecular imaging contrast agents may contribute to the understanding of molecular processes and the development of customized diagnostics and therapeutics. Fluorescent dyes can be used as both nonspecific and specific contrast agents, the latter promise a higher detection sensitivity and use of lower doses. A new approach for imaging of tumors and inflammation is presented using small interfering (si)RNA-based probes which are qualified to enhance the concentration of endogenous Protoporphyrin IX (PpIX), thereby amplifying the fluorescence emission within cancerous and inflamed tissues. In earlier studies we demonstrated that the concentration of PpIX can be enhanced by down-regulation of the activity of the ferrochelatase (FECH) enzyme (Kemmner W. et al., FASEB J, 2008). A prerequisite for the accumulation of PpIX is a sufficient intracellular concentration of δ-amino levulinic acid (δ-ALA), which requires the exogenous supply of this compound. It will be demonstrated that PpIX fluorescence within tumor tissue in vivo can be stimulated by a low amount of δ-ALA and FECH-siRNA. To avoid the degradation of siRNA, folate-coupled liposomes or dendritic polyglycerolamine nanoparticles served as carriers (Wan K. et al. Nanomedicine:NBM, 2012). Small experimental tumors were easily demarcated by the detection of PpIX fluorescence.

The method shows a high specificity caused by the selective penetration of the carrier, leading to down-regulation of FECH in tumor cells only. It is worth to note, that the method presented here needs no exogenously labeled molecules. The fluorescence intensity depends only on the metabolic rate of FECH and on the concentration of δ-ALA. The targeted application of FECH-siRNA, due to the omnipresence of the hem synthesis pathway, may provide a general approach for molecular imaging. Nano-carriers containing FECH-siRNA could be valuable for sensitive detection of near surface tumors and for their selective photodynamic depletion.

4

Nanoparticle-based single cell detection in biological fluids - Approaches and challenges

Roland H. Stauber

University Hospital of Mainz, Germany

Spanning the needs for detecting and quantitating disseminated cancer (stem)cells to the assessment of specific blood cell types, the repertoire of potential applications for magnetic flow cytometry are multifaceted. Hence, the development of appropriate imaging techniques with optimized cell labeling conditions will have deep impact on multiple diagnostic applications for many diseases.

Here, we present our approaches to non-optical flow cytometry, based on magnetoresistive GMR sensors for the detection of superparamagnetic iron oxide nanoparticles (SPIONs)-labeled cells. First proof-of concept studies could be successfully performed under bio-fluid free conditions. However, employing nanoparticle-based detection systems in biological fluids appears to be a more challenging endeavor. In biological fluids, nanoparticles (NPs) bind proteins and other biomolecules forming the so called ‘corona’, potentially affecting the NPs’ ability to efficiently recognize their target cell populations. Employing snap-shot label-free quantitative proteomics, we show that coronas composed of >160 different proteins formed extremely rapidly (<0.5min) in human plasma, changed only quantitatively over time, and affected the NPs’ impact on cells, such as the activation of thrombocytes.

Collectively, we illustrate that our magnetic flow cytometry system addresses a number of key issues for a system solution. Also, we demonstrate that the protein corona evolution needs to be considered to optimizing operation of a magnetic flow cytometer in a clinical setting.

Emerging Technologies

Time: **Thursday, 11/Oct/2012: 3:00pm - 5:00pm**

Location: Lecture Hall
Session Chair: Wolfgang Beisker
Session Chair: Elmar Endl
Session Chair: Frank A. Schildberg

1

Droplet digital PCR: Molecular biology in high resolution

Daniel Burdziak
Bio-Rad Laboratories GmbH, Germany

Droplet digital™ PCR (ddPCR™) is the third generation of PCR technology. The QX100 ddPCR system from Bio-Rad Laboratories provides an absolute measure of target DNA molecules with unrivalled accuracy, precision, and sensitivity.

Droplet digital PCR provides researchers with a new tool for the precise measurement of copy number variation, low-abundance sequence detection, detection of rare mutations, including distinguishing rare sequences in tumors, and gene expression analysis.

In the QX100 ddPCR system, the target molecules in a DNA sample are partitioned into 20000 nanodroplets. The goal is to have either one or no molecules in each droplet, so that when we perform PCR we have a simple positive or negative amplification result. The initial amount of target molecules is then directly quantified by counting the number of droplets with a positive amplification.

2

Massively informative: Fundamentals and applications of mass cytometry

Ted George
DVS Sciences, Inc., United States of America

Mass cytometry uniquely resolves more than 30 probes simultaneously on a per-cell basis at high acquisition rates, thereby providing researchers with an unparalleled ability to phenotypically and functionally profile cell populations from normal and diseased states. We will describe the basic principles and workflow of the technology, and using data from published reports we will discuss the scientific questions that can be addressed with mass cytometry, the analysis methods used to explore the high-content data that is generated, and the unique insights and discoveries gained by utilizing this technology.

3

Apoptosis and associated assays for flow cytometry

Thomas M. Bauer
eBioscience, Germany

Apoptosis is a highly regulated and evolutionary conserved pathway of cell death that plays a critical role in development and maintenance of tissue homeostasis. Apoptosis progression in mammals can be broken down into a number of key steps: induction, activation, and execution. We will have a look at a cellular example and detect the different stages of apoptosis on a time depended manner by demonstrating the different assays associated beginning with mitochondrial changes and to end with controlled cellular disintegration.

Furthermore we will have a look at cellular processes like proliferation and conclude with live/dead discrimination options.

4

Simultaneous analysis of cell death mechanisms and oxidative stress using Molecular Probes® next generation reagents for imaging and flow cytometry

Bjoern Biedermann
Life Technologies, Switzerland

Cell death can occur through multiple pathways, such as apoptosis, autophagy, and necrosis. Although necessary for proper growth and development, dysregulation of apoptosis has been associated with a variety of diseases including cancer and neurodegenerative disorders. Increased oxidative stress has also been associated with these diseases and has been shown to lead to apoptosis and autophagy. Importantly, cell death can occur through a single pathway, or in concert with multiple pathways. Staurosporine has been shown to induce apoptosis; chloroquine is known to promote autophagy; and nefazodone results in both apoptosis and autophagy. In this study we utilized multi-parametric high content imaging and flow cytometry to differentiate between apoptotic and autophagic cell death after induction by different agonists. In addition, we simultaneously examined levels of oxidative stress to determine the relationship between oxidative stress and cell death. We used the fluorogenic CellEvent™ Caspase 3/7 Green Detection Reagent as an indicator of apoptosis, LC3B RFP and an antibody specific for LC3B to measure autophagy, and CellROX™ Deep Red Reagent, a near infrared fluorescent ROS probe to evaluate oxidative stress. Furthermore, loss of mitochondrial membrane potential was also observed in apoptotic cells. By using a multi-parametric approach to high content imaging or flow cytometry we were able to characterize the mechanism of cell death by discriminating between cells which were apoptotic (active caspase-3/7), autophagic (LC3Bpostive autophagosomes), or both. This multi-parametric approach provided detailed information at both the cellular and population level enabling correlation between oxidative stress and different mechanisms of cell death.

5

Standardized enumeration of human endothelial progenitor cells (EPCs) based on flow cytometry with the MACSQuant Analyzer

Andrea Völkel, Kathrin Pütsch, Leonie Wegener, Jürgen Schmitz
Miltenyi Biotec GmbH, Germany

Purpose: In the last decade, a number of experimental data and clinical observations have suggested that bone marrow represents a reservoir of endothelial progenitor cells (EPCs) that participate in regeneration and repair of many peripheral tissues. These EPCs are activated and mobilized to the blood stream by environmental stimuli for physiological and pathological tissue regeneration. Many attempts have been made to quantify EPCs in human whole blood or bone marrow but currently different protocols for isolation and quantification are used which complicates the comparison and interpretation of the results. In this study we introduce a fast and reliable flow cytometric assay to enumerate EPCs from human whole blood samples.

Method: To isolate and quantify EPCs, the EPC Enrichment & Enumeration Kit was used, which includes the EPC Enrichment Cocktail, EPC Staining Cocktail, Control Cocktail CD309, Control Cocktail CD133, FcR Blocking Reagent, Red Blood Cell Lysis Solution and propidium iodide (PI). For one test, two samples of 10 mL each (EPC sample and control sample CD309) and one 300 µl sample (control sample CD133) were used. Erythrocytes in all samples were lysed and EPCs were stained and enriched in the EPC and control sample CD309. Enrichment of EPCs was performed by incubating the EPC Enrichment Cocktail and

FcR Blocking Reagent followed by, the EPC sample, with EPC Staining Cocktail (CD14-PECy5, CD34-FITC, CD133-PE, CD309 (VEGFR-2/KDR)-APC) and the control sample CD309 with Control Cocktail CD309 (CD14-PECy5, CD34-FITC, CD133-PE, Mouse anti-IgG1-APC). After incubation, cells were separated with MACS Technology. Control sample CD133 was incubated with FcR Blocking Reagent and the Control Cocktail CD133. Finally, the complete enriched positive fraction of EPC and control sample CD309 and partly the control sample CD133 were analyzed by four-color flow cytometry for enumeration of EPCs using the MACSQuant Analyzer.

Results: With an optimized fluorochrome cocktail and four colour gating strategy it is possible to enumerate EPCs with a low coefficient of variation in whole blood, bone marrow and mobilized leucapheresis samples. Furthermore EPC frequencies in normal blood samples from male and female donors were analysed (n=14).

Conclusion: The assay is fast, reproducible and copes with the high requirements of rare cell analysis using flow cytometry.

6

Put a handle to the individual cell - a new way of sorting with the Deparray system

Nicolò Manaresi

Silicon Biosystems SpA, Italy

Isolating a specific rare cell from a given sample can be a critical excercize. Traditional techniques do not always guarantee the specificity, the monocellularity, and the survival of the selected cells, which provides challenges for subsequent molecular analyses or other downstream use of these cells. The novel DEPArray technology combines dielectric cage cell handling and multichannel identification and provides new ways to isolate individual and healthy cells for a number of applications. Basic principles, new possibilities, and analytical data for a variety of examples will be presented in this talk.

Innate Immunity

Time: **Friday, 12/Oct/2012: 9:00am - 10:15am**

Location: Lecture Hall
Session Chair: Eicke Latz
Session Chair: Frank A. Schildberg

1

Assessing innate immune activation with fluorescence techniques

Eicke Latz
Institute of Innate Immunity, University of Bonn, Germany & Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, USA

Innate immunity evolved to recognize microbial infection and to respond to danger signals that appear under disease conditions. The most recently described innate immune receptor family is the Nod-like receptor (NLR) family. The NLR member NLRP3 and the adapter protein ASC form a multi-molecular complex termed the NLRP3 inflammasome. Inflammasomes control the activity of caspase-1, which cleaves and activates the pro-form of the inflammatory cytokines IL-1b and IL-18. The NLRP3 inflammasome can be activated by various membrane active bacterial toxins or after phagocytosis of crystalline and aggregated materials.

We demonstrated that crystals and aggregated peptides activate the NLRP3 inflammasome in macrophages and microglial cells and contribute to disease processes in murine models of atherosclerosis and Alzheimer's disease. These novel insides into disease mechanisms open novel treatment approaches that are currently tested in preclinical models.

2

NLRP3 inflammasome activity is negatively controlled by miR-223

Anna Rieger[1], Franz Bauernfeind[1,2], Frank A. Schildberg[3], Percy A. Knolle[3], Jonathan L. Schmid-Burgk[1], Veit Hornung[1]
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The innate immune system provides the initial response against invading pathogens. Hereby, several germline-encoded pattern recognition receptors (PRRs) act as primary sensors that detect conserved microbial molecules or endogenous substances that arise during tissue or cell damage. The cytosolic sensor NLRP3 forms an inflammasome and is of particular relevance due to its diverse and important involvement in sterile inflammation, antimicrobial responses and hereditary autoinflammatory syndromes. NLRP3 activation leads to the cleavage of caspase-1, release of pro-inflammatory cytokines and also the initiation of a special type of cell death, known as pyroptosis. NLRP3 further seems to take on a special position, as it appears to be a general sensor of cell stress and, unlike other inflammasome sensors, NLRP3 is also under transcriptional control.

In this work we identify the myeloid specific miRNA miR-223 as another critical posttranscriptional regulator of NLRP3 inflammasome activity. miR-223 negatively regulates NLRP3 expression through a conserved binding site within the 3' UTR of NLRP3, leading to reduced NLRP3 inflammasome activity. While we could not observe that miR-223 itself is regulated by pro-inflammatory signals, its expression varies among different myeloid cell

types and inversely correlates with inflammasome activity. Together, given the tight transcriptional control of NLRP3, miR-223 functions as an important fine tuning mechanism that controls NLRP3 inflammasome activity.

3

Influence of RNA secondary structure on the activation of Toll-like receptors 7 and 8

Thomas Zillinger[1], Vera Wimmenauer[1], Eicke Latz[2], Gunther Hartmann[1], Winfried Barchet[1]

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Immunorecognition of viruses hinges on the detection of viral nucleic acids by germline encoded receptors at specific cellular locations. Human Toll like receptors (TLR) 7 and 8 are able to detect single stranded RNA in the endosome of distinct immune cells.While most stimulatory RNAs activate both TLR7 and TLR8, short Oligoribonucleotides (ORN) and small molecule agonists can show preferential engagement of either TLR7 or TLR8. A structural basis for this distinction is unknown. Here we identify RNA stem structures in the secondary structures commonly assumed by single stranded RNA, as a potent and highly selective activators of TLR7. Such stem structures induced type I IFN in plasmacytoid dendritic cells (PDC), but did not activate TLR8 in monocytes. TLR7 selectivity was maintained even when TLR7 and TLR8 were expressed in the same cell type. We further provide evidence that recognition of viral and bacterial RNAs via TLR7 is mediated by RNA secondary structure, rather than the linear sequence motifs required for the activation of TLR8. We idenfied and developed TLR7 agonistic RNA stems that show activity without a requirement for accessory components such as cationic polymers.

Microbiology

Time: **Friday, 12/Oct/2012: 10:30am - 12:00pm**

Location: Lecture Hall
Session Chair: Frank Schmidt
Session Chair: Susann Müller

1

Flow cytometry community fingerprinting to detect quickly stress in drinking water systems

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A follow up of water quality is important in different areas, ranging from food and beverage production to waste water treatment. Conventional methods for the determination of water quality, like heterotrophic plate count and molecular techniques, are time consuming and prevent an immediate intervention in case of problems. Flow cytometry is explored as a fast methodology for investigating the microbial community of water, but the data interpretation is until now subjective. During the presentation, the development of a rapid objective method for the assessment of water quality will be discussed. This approach is using the physiological status of single cells within a microbial community using flow cytometry with tailored statistical tools. The method consists of two main parts, first the generation of fingerprint data by flow cytometric analysis and second the analysis of the data by the new statistical tool. The combined method was proven useful for the discrimination and classification of drinking water. The classification rule was evaluated by leave-one-out cross validation, the estimated misclassification rate was 8%.

Next to differentiation of microbial communities of different origin, the method was tested to detect changes within a community due to different environmental stress factors. This would allow us to use the method to detect quickly subtle differences in microbial community composition, which would be a reflection of the water quality. The impact of four different treatments on the microbial communities in bottled water were both assessed by flow cytometry and validated by a commonly used fingerprinting technique denaturing gradient gel electrophoresis (DGGE). Significant changes in community composition could be detected in three out of four treatments by the new method, the same could be concluded out of the DGGE.

Generally, the method can be used as a fast fingerprinting method of microbial communities in aquatic samples. The system can give an indication of the microbial quality within one hour and can as such be used as an early warning system.

2

Monitoring microbial population dynamics using community flow cytometry

Christin Koch, Ingo Fetzer, Thomas Hübschmann, Hauke Harms, Susann Müller
Helmholtz Centre for Environmental Research - UFZ, Germany

Using a MoFlo cell sorter morphological cell features can be analyzed by forward scattering behaviour (FSC) and structural cell features by the DNA content, measured using the AT specific fluorescent dye DAPI. Cytometric analyses of these parameters result in distinct, fingerprint like, patterns. These patterns are highly reproducible and represent the composition of the microbial community at a certain point of time. Methods to define and evaluate differences between these patterns were established and tested for their sensitivity. Finally, their applicability was demonstrated for a long term experiment.

The microbial community of a labscale biogas reactor run with DDGS (dried distillers grains with solubles) was investigated over a period of nine months including phases of stability and disturbance. Biogas reactors contained a highly divers microbial community comprising of bacteria and archaea. They were able to degrade complex substrates to biogas mainly composed of methane and carbon dioxide. Substrate choice, temperature, retention time, pH and the presence of trace metals or noxious compounds were just some factors influencing the community composition and the total reactor performance. So far, most studies only focused on the microbial composition at certain time points using molecular fingerprinting techniques. Differences were found but distinct correlations to total reactor performance could hardly be made.

The combined approach of community flow cytometry and functional driven correlation analysis revealed key player within the complex community. Subsets of cells with relevancy within acidogenesis or methanogenesis as well as differential sensitivity to trace element supply were identified.

3

3D visualization of bacterial uptake by human platelets using fluorescence microscopy and electron tomography

Josef Neumüller[1], **Christof Jungbauer**[2], **Renate Renz**[2], **Adolf Ellinger**[1], **Margit Pavelka**[1]
[1]Medical University of Vienna, Center for Anatomy and Cell Biology, Department of Cell Biology and Ultrastructure Research, Vienna, Austria; [2]Blood donation Center of the Austrian Red Cross for Vienna, Lower Austria and Burgenland, Vienna, Austria

Objective: Platelets (PLT) obtained by thrombapheresis or by pooling of buffycoats were spiked with bacteria such as Escherichia coli (E.c.) and Staphylococcus aureus (S.a.) to see whether the PLT were able to phagocytose them. We wanted also to find out whether the direct contact of PLT with bacteria would induce PLT activation. Since bacteria can be covered by the open canalicular system (OCS) we used ruthenium red (RR) as tracer for the OCS which enabled discriminating completely engulfed bacteria from those that are only partially covered by the OCS.

Material and Methods: PLT were spiked with commercially available preparations of S.a. and E.c. (pHrodoTM, Invitrogen) in a ratio of approximately 1:10. The pH sensitive dye pHrodo covers the bacterial wall and indicates an acidic environment induced by phagocytosis. After 30 minutes of coincubation with bacteria, PLT were allowed to adhere to glass coverslips and investigated by fluorescence microscopy or were fixed with 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4, for 1 hour and with 2% OsO_4 and 3% RR in 0.1M cacodylate buffer, for 90 min. PLT and bacteria were cut parallel to the adherence plane using a diamond knife. 70 nm ultrathin sections were performed for conventional ultrastructural examination, 250 nm semithin sections for electron tomography (ET). Routine transmission electron microscopy (TEM) and electron tomography (ET) were carried out using a Tecnai 20 (FEI). For ET, tilting series from -65° to +65° with an increment of 1° were performed, which were reconstructed by the weighted back projection method. **Results and Conclusion:** PLT adherent to glass surfaces, form a granulomer and a hyalomer), already visible under the light microscope. In TEM, different stages of formation of these compartments were visible. In slightly adherent PLT the circumferential microtubular coil could be clearly demonstrated with adjacent lacunae of the dense tubular system (DTS) at the inner edge of this coil. In firmly adherent PLT, the microtubular ring was centralized lining the granulomer containing organelles.

Both species of bacteria formed cell contacts with PLT. The activation of PLT due to glass adherence was accelerated by the interaction with bacteria. The activated PLT extended filopodia forming aggregates by free cell contacts sequestering bacteria. By the use of RR,

particular OCS compartments did not show any tracing while other OCS parts were distinctly decorated by the tracer. Sequestration or engulfment of bacteria by PLT could be convincingly underlined by ET. Using ET, we could also demonstrate the interconnections of significantly enlarged areas of the OCS and also the rearrangement of cytoskeleton due to the bacterial-induced activation of PLT. The enhanced fluorescence of the dye pHrodo indicated an acidic milieu at sites of bacterial ingestion.

4

Preoteomic characterisation of host pathogen interactions during internalisation of Staphylococcus Aureus HG001 by A549 cells

Kristin Surmann

University of Greifswald, Germany

S. aureus, a Gram-positive coccus can cause a broad range of infection related diseases in humans ranging from milder skin infections to severe diseases such as the toxic shock syndrome or systemic infections like sepsis [1]. Its virulence is predominantly caused by various secreted virulence factors which interfere with host cell signaling or survival. Though widely considered an extracellular pathogen, it could be recently shown that S. aureus is able to invade and persist in non-professional phagocytic cells [2].

Therefore, it is of interest to understand the adaptive mechanism of the pathogen upon internalization as well as the response of the host.

We developed a protocol which allows identification and quantification of changes in the protein levels of bacteria and host cells by combining a pulse-chase SILAC approach, GFP supported enrichment of internalized bacteria and infected cells by cell sorting and gel-free HPLC-MS [3].The host model in this study is the A549 cell line isolated from a human lung cancer. Those cells belong to the alveoli of human lungs and as type II pneumocytes they secrete certain cytokines and have therefore an impact on the innate immune system [4]. One-D gel analysis of fractions of infected A549 cells (cytosol, membrane, nucleus and cytoskeleton)showed a distinct pattern of human proteins and subsequent Western Blot analysis revealed an enrichment of staphylococcal lipase in the cytoplasmic fraction.

Furthermore, proteins belonging to biosynthesis of secondary metabolites, microbial metabolism in diverse environments, Peptidoglycane biosynthesis, pentose phosphate pathway, lycolysis/gluconeogenesis showed a higher level during infection while ribosomal proteins showed reduced levels in comparison to non-internalized bacteria.

This study, which includes the visualization of intracellular bacteria and proteomic analysis of the adaptation of the pathogen as well as the response of the host with the help of high precision HPLC-MS approaches, provides new insights into host pathogen interactions during infection.

[1] Lowy, N. Engl. J. Med., 1998; 339: 520-532; [2] Garzoni et al., Microbiol., 2009; 17: 59-65; [3] Schmidt et al., Proteomics, 2010; 10: 2801-11; [4] Cheon et al., Mol Immunol., 2008; 45: 1665-73

Immunology

Time: Friday, **12/Oct/2012: 1:00pm - 2:30pm**

Location: Lecture Hall
Session Chair: Hyun-Dong Chang
Session Chair: Frank A. Schildberg

1

How TH17 cells are controlled in the gut

Enric Esplugues
Deutsches Rheuma-Forschungszentrum (DRFZ), Germany

Interleukin (IL)-17-producing T cells (TH17) have been identified recently as a subpopulation of CD4+ T cells. Besides having a protective function in the defense against extracellular bacteria and fungi, they also play an important role in autoimmunity and chronic inflammation. They form the major fraction of pathogenic T cells in experimental autoimmune encephalitis (EAE), a mouse model of multiple sclerosis, and they have also been shown to play a role in the pathogenesis of inflammatory bowel disease (IBD) and rheumatoid arthritis.

A series of signals controlling the differentiation of this subset in vitro have already been identified. However, it remains enigmatic how the control of this pro-inflammatory cell population occurs.

Our work identifies the gut as a crucial organ for homeostatic regulation of this subset. TH17 cells are controlled by two different ways in the small intestine: first, pro-inflammatory T cells are eliminated via the intestinal lumen. Simultaneously, TH17 cells can be reprogrammed and converted into regulatory CD4+IL-17A+FoxP3- T cells with immuno-suppressive and regulatory properties (rTH17).

These findings may have impact for future therapeutic manipulation of the pro-inflammatory TH17 cell population in different autoimmune diseases.

2

Antigen-reactive T cell enrichment for direct high resolution analysis of the human naïve and memory T helper cell repertoire

Petra Bacher[1], Janka Teutschbein[2], Olaf Kniemeyer[2], Mario Assenmacher[1], Axel A. Brakhage[2], Alexander Scheffold[3]
[1]Miltenyi Biotec GmbH, Germany; [2]Institute for Natural Product Research and Infection Biology - Hans Knoell Institute; [3]Clinic for Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin

CD4+ T cells play a central role in the immune defense against pathogens or tumors but also in immunopathology, such as autoimmunity or allergy. However, due to technical limitations, comprehensive information about the frequency and phenotype of antigen-specific CD4+ T cells against many disease-relevant antigens is missing. In particular the nature of the human naïve repertoire is elusive. We have developed a new, sensitive detection system for antigen-specific T helper cells, based on a combination of magnetic pre-enrichment and multi-parametric flow cytometric analysis of CD154 (CD40L)-expressing CD4+ T cells upon *in vitro* activation with antigens. The high sensitivity of 1 cell out of 10e5 to 10e6 enables the direct enumeration of memory and naïve CD4+ T cells reactive against basically any recall or primary antigen. Importantly, our technology allowed us to rapidly analyze sufficient numbers of target cells to discriminate even small functional or phenotypic subpopulations within the total antigen-specific T cell pool, undetectable by conventional methods. We used this assay for a first characterization of tumor- and auto-antigen reactive CD4+ T cells from healthy subjects as well as for the in depth characterization of rare pathogen specific T cells.

The possibility to visualize T cells reactive against any antigen of interest even in the naïve repertoire will open up new possibilities to advance human T cell research and to improve clinical diagnostics and prognosis.

3

Differentiation of human leukocyte subsets by flow cytometry

Dorothee Köhler, Manuela Herber, Petra Bacher, Martin Büscher, Mario Assenmacher
Miltenyi Biotec, Germany

Determination of white blood cell count is often used as a diagnostic tool for a patient's immune status. Traditional methods for determining white blood cell populations have several limitations. The counting of leukocyte subsets via visual inspection of blood smears is often time consuming and labor intensive. Furthermore, proper analysis requires well-trained technicians and even with experienced technicians, inter-operator variation cannot be excluded. Hematology analyzers are another standard method for counting and identification of white blood cells. However, this method only allows discrimination of granulocytes, monocytes and lymphocytes, but further dissection of lymphocyte subsets is often not possible.

In contrast flow cytometry offers the opportunity to detect multiple parameters simultaneously and can be used for the differentiation of white blood cells by identification of specific surface antigens on cell populations. Additionally, this technique enables the rapid analysis of high cell numbers, which allows the characterization of rare cell subsets.

Therefore, we present here a 7-color Immunophenotyping Kit for flow cytometry to differentiate nine different subsets of leukocytes using the following antibody cocktail: CD45-VioBlue/CD14-FITC/CD56-PE+CD16-PE/CD4-PerCP/CD19-PE-Vio770/CD3-APC/CD8-APC-Vio770. Using this cocktail the detection of Monocytes, B cells, T cells, T helper cells, cytotoxic T cells, NK cells, NKT cells, and Granulocytes (eosinophils and neutrophils) becomes possible. In combination with the MACSQuant Analyzer the 7-color Immunophenotyping Kit additionally provides absolute cell numbers of distinct leukocyte based on volumetric cell count in a single platform setting. Furthermore, automation of the staining procedure via the autolabeling function of the MACSQuant Analyzer and subsequent automated acquisition aids in reducing inter-operator variability. In a next step towards complete automation of the process, automated gating is required, which has already been implemented on the MACSQuant Analyzer for distinct applications via the Express Modes. Here we investigated a novel tool to analyze flow data by unsupervised automated gating: UNCLE DOC (UNsupervised CLustering Engine). It allows fast analysis of high-dimensional flow cytometric data using a standardized process, which minimizes gating variability.

Taken together, the combined use of the MACSQuant Analyzer and the 7-color Immunophenotyping Kit provides a standardized, reproducible system for sensitive analysis of blood cell subsets.

4

Identification of cell type-specific type I interferon signatures as biomarkers in viral infection and autoimmunity

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Purpose: In peripheral blood mononuclear cells (PBMCs) of patients with systemic lupus erythematosus (SLE), a strong activation of the type I interferon system has been reported that is detectable at the transcriptional level by a prominent pattern of differentially expressed genes, that is known as “type I IFN signature”. However, it is unknown how particular leukocyte subsets contribute to the overall type I IFN signature described for PBMCs. In addition, a detailed analysis of how IFN signatures differ in autoimmune disease from that observed after viral infection is missing so far. The knowledge about cell- and disease-specific IFN signatures is of major interest for diagnostic purposes to monitor disease activity and success of therapies. Thus, we aimed to use IFN signatures to follow the resetting of cellular immune system of therapy-refractory SLE patients after autologous stem cell transplantation (ASCT).

Methods: We compared expression levels of 2442 IFN-related genes cited from recent literatures, in CD4+ T cells and monocyte (Mo) subsets from SLE patients with active and inactive disease treated by standard drug treatment or ASCT. These date were compared to expression profiles of healthy donors (ND) and ND vaccinated against yellow fever. Leukocytes from peripheral blood of patients and ND were stained for CD14, CD16, CD3 and CD4 antibodies to isolate CD4+ T cells, CD16-negative inflammatory Mo and CD16-positive resident Mo in parallel. After sorting, total-RNA was isolated and processed for hybridization on Affymetrix microarrays. Data analysis was done using BioRetis database, Genesis software and Ingenuity Pathway Analysis (IPA).

Results: Mo showed a more complex IFN response than CD4+ T cells. Comparing cellular responses in SLE and in viral vaccinated patients allowed us to identify a “common” IFN signature detectable in autoimmunity and in viral infection and an “autoimmune-specific” IFN signature that was exclusively detectable in SLE. Expression pattern of “common” and “autoimmune-specific” IFN signature genes clearly distinguished SLE patients from immunized ND by hierarchical cluster analyses. Although major IFN signature genes were commonly expressed, their absolute magnitudes of expression were significantly increased in SLE patients compared to immunized ND. In SLE, more genes were identified, for example that are involved in JAK/Stat signaling pathways.

In Mo of inactive SLE under successful treatment, the IFN signature was almost deleted. Surprisingly, in CD4+ T cells from two out of four ASCT- treated patients, a clear IFN imprint was detectable, while it was deleted in inactive SLE under standard immunosuppressive drug treatment.

Conclusion: We demonstrated that the IFN signatures observed in autoimmunity and in viral infection are quantitatively and qualitatively quite different. Autoimmunity is characterized by a much stronger expression of IFN signature genes and is obviously modulated by a separate set of co-regulated genes. “Common” and “autoimmune-specific” IFN signature genes can be applied as a clinical biomarker to diagnose SLE flare discriminating from viral infection. Recently, we could validate the monocyte-specific adhesion molecule Siglec-1 (CD169) by flow cytometry as a type I IFN surrogate biomarker that can be used to monitor disease activity. Furthermore, our results indicated for a cell type-specific pro-inflammatory cytokine memory in CD4+ T helper lymphocytes even after ASCT-therapy. Most interestingly, CD4+ T

cells of ASCT-treated patients, but not Mo, are characterized by an apparent IFN imprint although they are under long-term remission. Therefore, monitoring of this ASCT-associated IFN signature in CD4+ T cells may be important for the follow-up of ASCT-treated patients. In summary, our data are of importance to identify and establish biomarker and biomarker signatures for diagnostic applications to bring up the principle of individualized medicine one step closer to daily practice.

5

Blocking type I IFN receptor signaling pathway during IDDM clearly restored the perturbation in the B and T lymphocyte, antigen-presenting cells (APCs) through the pro-inflammatory cytokines (IL-1α, IL-1β and TNFα and IL-6)

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Autoimmune type 1 diabetes (IDDM) is a chronic pro inflammatory condition characterized by both B and T lymphopenia as well as impairment in the ability of lymphocytes to respond to immune stimuli. Increasing levels of IFN-α during TID might act as pro-apoptotic factor for many cell types such as activated lymphocytes, blocking type I IFN receptor represents a novel immunotherapy for treatment of such chronic autoimmune diseases.

Immunohistochemical study of diabetic group demonstrated reduced number of CD20+ B cells, CD3+ T cells, CD8+ T cells and MHC class II in the spleen associated with increased apoptotic changes in the immune cells. Moreover, blocking type I IFN receptor restored number of these cells to the control level ,and reduced apoptosis as well, these changes were associated with significant increase in the level of IFN-α, pro inflammatory cytokines as IL-1α,IL-1β, IL-6, TNF-α and CXCL10 (IP-10) levels in the blood of diabetic group as compared to control. While blocking type I IFN receptor in diabetic group partially and significantly restored the altered levels of IFN-α as well as of IL-1α, IL-1β, TNF-α and IL-6, TNF-α and CXCL10 (IP-10) levels as compared to diabetic non-treated group, these data further indicated that IFN-α induced the production of pro inflammatory cytokines during diabetes. Furthermore we explore effects of IFN-α on cellular proliferation in response to mitogen and using PCNA marker. It was observed that the proliferative capacity of peripheral blood mononuclear cells and immune cells respectively were significantly decreased in diabetic group as compared with the control group and anti-IFN-treated diabetic group. Thus, the present study revealed that IFN-α activates the peripheral immune system and induces the production of pro inflammatory cytokine during type I diabetes mellitus. So the pro-inflammatory cytokines (IL-1α, IL-1β, and TNF-α and IL-6) represent a new target for the immune regulatory effect of blocking type I IFN receptor signaling pathway during IDDM and provide a possible mechanism that might be considered a novel therapy in the treatment of IDDM.

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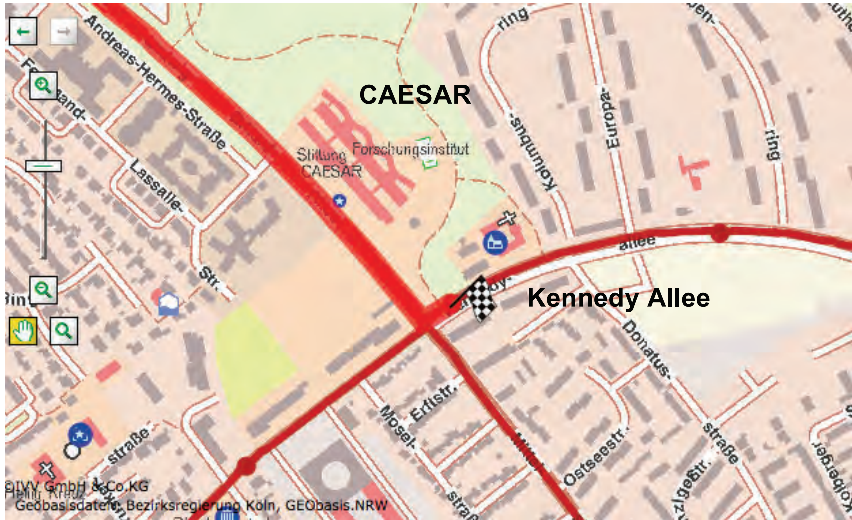
Public Transport

From Bonn Central Station, bus stop section C4, take Bus **610/611** (Pappelallee) and get off at bus stop **Kennedyallee**.



By train:
ICE Station Siegburg/Bonn
The taxi from Siegburg to caesar (17 km/15 minutes) costs about 20 Euros. You can also take public transportation (tram SB 60) to Bonn Central Station, from there take Bus 610/611 to Kennedyallee.

By car:
From the south: Take highway A 565 to *Dreieck Beuel* and follow on to A 59 Königswinter.
From the north: Take highway A 59 to Bonn/Königswinter.
At *Autobahnkreuz Bonn-Ost* (exit 42) continue to Bonn-Bad Godesberg on the A 562. After crossing the Rhine take the exit *Bonn Rheinaue (*exit 2*).*
Stay left and follow the caesar signs about 1.5 km to caesar. caesar is located on the left side.
There are various parking spaces associated with the "Rheinaue" park next to the CAESAR building.

By taxi:
Bonn Central Station
The taxi to caesar (6 km/10 minutes) costs about 12 Euros.

Travel Information

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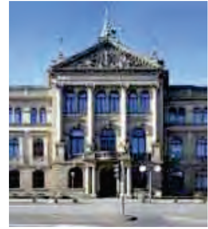
Ludwig-Erhard-Allee 2
D-53175 Bonn

Telefon +49-228-9656-0
Telefax +49-228-9656-111
E-Mail office(at)caesar.de

GPS-coordinates:
N 50 42.233
E 7 09.03

Conference Dinner

The **Conference Dinner** is at the **Museum König**.

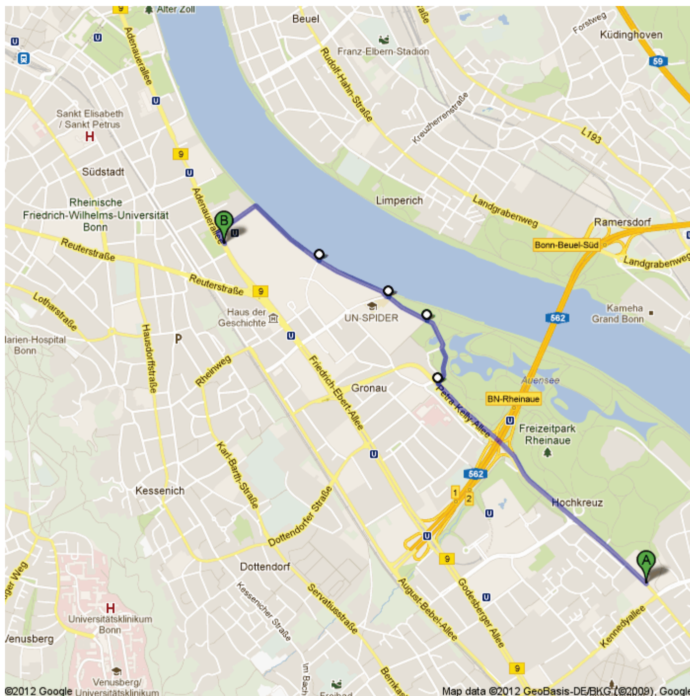


From Bonn Central Station, take U16, U63 (Bad Godesberg Stadthalle), U66 (Ramersdorf, Bad Honnef) and leave at "Museum König". Exit too Adenauer Allee, the Museum is located in your back.

For those who prefer to walk, its 50 minutes from the conference site and you can walk along the scenic Rhine river.

The museum will open at 19:00 and participants can attend a guided tour through the exhibition, which will start at the same time. The buffet will open at 20:00.

Below you find a map and the description of the route to the Museum König, if you decide to take a walk.



Conference Dinner

center of advanced european studies and research - Forschungszentrum caesar
Ludwig-Erhard-Allee 2, 53175 Bonn

1.	Auf **Ludwig-Erhard-Allee** nach **Nordwesten** Richtung **Johanna-Kinkel-Straße** Ca. 5 Minuten	400 m weiter gesamt 400 m
2.	Rechts halten, um auf **Ludwig-Erhard-Allee** zu bleiben Ca. 11 Minuten	850 m weiter gesamt 1,3 km
3.	Weiter auf **Petra-Kelly-Allee** Ca. 7 Minuten	500 m weiter gesamt 1,8 km
4.	Rechts Richtung **Charles-de-Gaulle-Straße** abbiegen	58 m weiter gesamt 1,8 km
5.	Rechts Richtung **Charles-de-Gaulle-Straße** abbiegen	12 m weiter gesamt 1,9 km
6.	Links Richtung **Charles-de-Gaulle-Straße** abbiegen Ca. 2 Minuten	180 m weiter gesamt 2,0 km
7.	Rechts abbiegen auf **Charles-de-Gaulle-Straße** Ca. 6 Minuten	500 m weiter gesamt 2,5 km
8.	Links halten auf **Hermann-Ehlers-Straße**	17 m weiter gesamt 2,5 km
9.	Rechts halten auf **Stresemannufer**	16 m weiter gesamt 2,5 km
10.	Links halten, um auf **Stresemannufer** zu bleiben Ca. 7 Minuten	550 m weiter gesamt 3,1 km
11.	Weiter auf **Wilhelm-Spiritus-Ufer** Ca. 5 Minuten	400 m weiter gesamt 3,5 km
12.	Links abbiegen auf **Kaiser-Friedrich-Straße** Ca. 4 Minuten	280 m weiter gesamt 3,8 km
13.	Links abbiegen auf **Adenauerallee/B9** Das Ziel befindet sich rechts Ca. 56 Sekunden	80 m weiter gesamt 3,9 km

museum König

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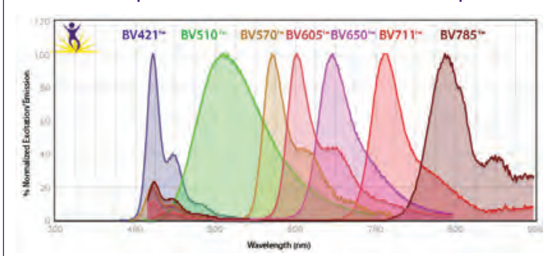

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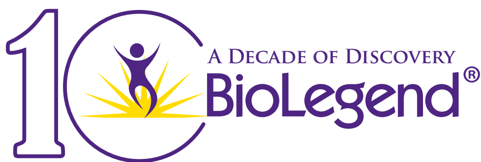

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Compact Time Schedule

Date: Wednesday, 10/Oct/2012	
12:15pm - 12:30pm	**Welcome**
12:30pm - 2:00pm	**Advanced Microscopy**
2:00pm - 2:30pm	Coffee Break
2:30pm - 4:30pm	**Cancer**
4:30pm - 5:00pm	Coffee Break
5:00pm - 6:30pm	**Rare Cells**
6:30pm - 7:00pm	Coffee Break, Get Together
7:00pm - 10:00pm	**Core Managers Workshop**

Date: Thursday, 11/Oct/2012	
9:00am - 10:30am	**Biotechnology**
10:30am - 11:00am	Coffee Break
11:00am - 12:00pm	**Meet the Expert Lecture**
12:00pm - 1:00pm	Lunch
1:00pm - 2:30pm	**New Materials and Methods**
2:30pm - 3:00pm	Coffee Break
3:00pm - 4:30pm	**Emerging Technologies**
4:30pm - 5:00pm	Coffee Break
5:00pm - 6:30pm	**Meeting of DGfZ Members**
6:30pm - 8:00pm	Break and Departure for the Dinner
8:00pm	**Conference Dinner**

Date: Friday, 12/Oct/2012	
9:00am - 10:15am	**Innate Immunity**
10:15am - 10:30am	Coffee Break
10:30am - 12:00pm	**Microbiology**
12:00pm - 1:00pm	Lunch
1:00pm - 2:30pm	**Immunology**
2:30pm - 3:00pm	Farewell Coffee

We would like to say thank you to