30th International Conference

Advances in the Applications of Monoclonal Antibodies in Clinical Oncology and Symposium on Cancer Stem Cells

Santa Marina Hotel, Mykonos, Greece

24th – 26th June 2013

In Memory: Philip E. Thorpe, Ph.D., 1951 – 2013

It is with deep regret that we heard the sad news of the passing of Philip E Thorpe. Philip was Professor of Pharmacology, who also was associated with the Harold C. Simmons Comprehensive Cancer Center and held The Serena S. Simmons Distinguished Chair in Cancer Immunopharmacology at the UT Southwestern Medical Center in Dallas,Texas.
His achievements in drug targeting, angiogenesis, and antibody-based therapeutics had global impact, and his loss will be felt deeply on the UT Southwestern Medical Center campus and well beyond.

Philip's research focused on the development of novel drugs targeting tumor blood vessels. His laboratory made the remarkable discovery that a fatty lipid molecule, phosphatidylserine, is preferentially expressed on cancer blood vessels, where it can serve as a target to increase the specificity of drugs to the tumor.

He was firmly dedicated to the translation of his novel concepts in drug design into practical drug therapies for cancer, imaging agents, and anti-virals. His expertise covered a wide range of fields including protein engineering, synthetic chemistry, pharmacological testing, cell biology, and immunology. Five drugs developed wholly or partially in Philip's laboratory have entered clinical trials.

Philip also worked as a scientific advisor at Peregrine Pharmaceuticals, Inc., based in Tustin, Calif., and had worked with the company for 15 years to develop novel therapeutics. He was included in 252 issued and pending worldwide patents, including 74 in the U.S. He was the author of more than 200 publications in the fields of drug targeting, angiogenesis, and antibody-based therapeutics.

Among other honors, he was one of the first recipients of the Pierce Immunotoxin Award, presented every two years for outstanding contributions to immunotoxin research, in 1998, and he received the Texas State Legislature Award for Research Excellence in 1997 and the American Cancer Society Award of Excellence in 1999.

Philip graduated summa cum laude with a Bachelor of Science in Pharmacology from the University of Liverpool in 1972, and he received a Ph.D. in Immunology from the Clinical Research Centre in London in 1976. He served as a Medical Research Council Fellow at Chester Beatty Research Institute in London (now The Institute of Cancer Research) until 1981, then as Director of the Drug Targeting Laboratory, Imperial Cancer Research Fund in London until 1991, when he first joined UT Southwestern as Professor of Pharmacology. Philip served as Associate Director of the Center for Molecular Medicine at Maine Medical Center Research Institute from 1998-1999.

On hearing the news, Dr. Alfred Gilman, Nobel Laureate and Regental Professor Emeritus of Pharmacology, said: "Phil Thorpe was a marvelous combination of immunologist and pharmacologist. His insights into biological problems were keen, and his imagination and sense of daring were inspiring. We were privileged and honored that he was our colleague and friend for more than two decades."

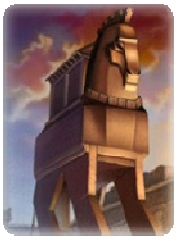
"Dr. Thorpe was a valued colleague on every level," said Dr. David Mangelsdorf, Chairman of Pharmacology. "As a brilliant translational scientist, he saw his work move from the inception of an idea to experiments at the bench, and on to the clinic. As an educator, he was one of the best, and took his job teaching graduate and medical students seriously. As a mentor, he trained some of the finest new scientific minds. He will be sorely missed."

Dr. Ellen Vitetta, Director of the Cancer Immunobiology Center, Professor of Immunology and Microbiology, commented that "Phil was one of a kind … a Renaissance man who was at once a Brit and a Texan, a scientist and a poet, an amazingly creative out-of-the box thinker who was also his own toughest critic. He loved structures and chemistry but thrived on art and music. He was a complex man with many facets. We lost him far too soon and we will all miss him. We take some solace in the fact that the world is a better place because of him."

We dedicate this Conference to Philip Thorpe.

WE ARE GRATEFUL TO OUR SPONSORS FOR THEIR SUPPORT

Roche



Trojantec

PROGRAMME

Monday 24th June 2013

8.00-9.00 **Registration**

9.00-9.05 **Welcome**: Agamemnon Epenetos

SESSION 1 Chairman: Sir Walter Bodmer

9.05-9.20 **Agamemnon Epenetos PhD ,FRCP, Visiting Professor Imperial College London ,Honorary Consultant in Medical Oncology , St Bartholomew's Hospital and the Harley Street Oncology Clinic, London UK**
Chairman , Trojantec Ltd, Lifeline Biotech Ltd, Alexis Biotech Ltd, London UK and Nicosia, Cyprus

THE BEGINNING OF THE APPLICATIONS OF MONOCLONAL ANTIBODIES IN CLINICAL ONCOLOGY

Production of monoclonal antibodies involving human–mouse hybrid cells was described by Jerrold Schwaber in 1973 and remains widely cited among those using human-derived hybridomas but claims of priority have been controversial. A science history paper on the subject gave some credit to Schwaber for inventing a technique that was widely cited, but stopped short of suggesting that he had been cheated. The invention was conceived by George Pieczenik, with John Sedat, Elizabeth Blackburn's husband, as a witness and reduced to practice by Cotton and Milstein, and then by Kohler and Milstein. Georges Köhler, César Milstein, and Niels Kaj Jerne in 1975 shared the Nobel Prize in Physiology or Medicine in 1984 for the discovery. The key idea was to use a line of myeloma cells that had lost their ability to secrete antibodies, come up with a technique to fuse these cells with healthy antibody-producing B-cells, and be able to select for the successfully fused cells. This was put into practice by Milstein and Köhler in their search for a laboratory tool to investigate antibody diversity.

Although initially monoclonal antibodies were thought to be primarily useful as laboratory tools to investigate antibody diversity, there was uncertainty and debate about their potential as in vivo clinical diagnostic agents and therapeutic drugs.

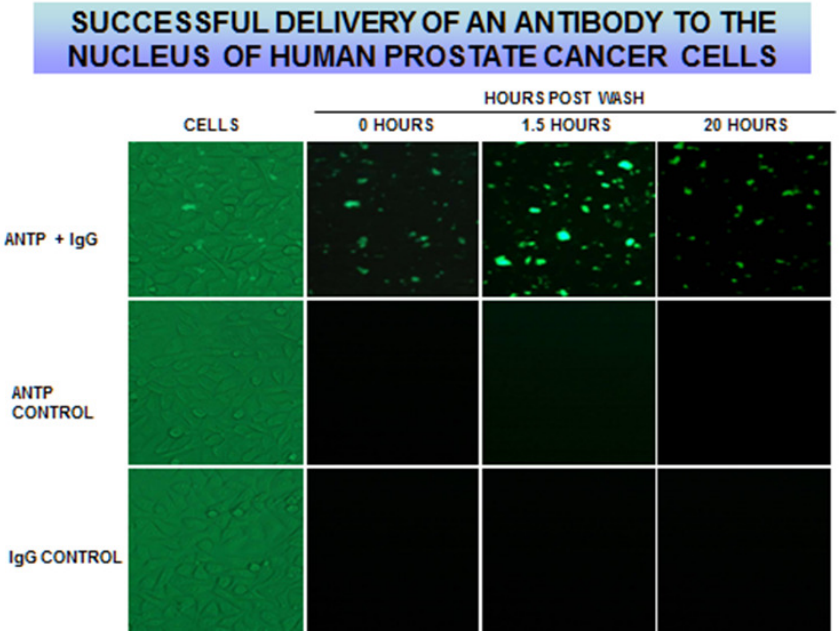
In 1979 we begun to explore this area and demonstrated in:

- 1982 - in a preclinical xenograft model that radiolabelled monoclonal antibodies could selectively localise in vivo to tumours.
- 1982 - in patients with various types of cancer that monoclonal antibodies could detect single tumour cells among normal tissues and cells
- 1982 - that radiolabelled monoclonal antibodies could selectively localise in vivo to primary and metastatic lesions in patients with cancer
- 1984 - that radiolabelled monoclonal antibodies could be used therapeutically in patients with ovarian cancer.
- 1985 - that antibodies directed against epidermal growth factor receptor, EGFR, could produce a therapeutic benefit in patients with glioblastoma.

Thus the huge potential of monoclonal antibodies in Clinical Oncology (and by extrapolation , in other diseases) became plain to see.

In 1988, Greg Winter and his team pioneered the techniques to humanize monoclonal antibodies, removing the reactions that many monoclonal antibodies caused in some patients.

Our current focus, since the beginning of the 21st century has been the research and development of monoclonal antibodies targeting intracellular, and intranuclear antigens including those inside the blood brain barrier by the use of non-specific, non-traumatic, non-receptor mediated transporter proteins (**transporter**) that can penetrate all cells in the body, and transport highly specific monoclonal antibodies(**transported**) .



Delivering functional antibodies intracellularly, intranuclearly and through the blood brain barrier expands substantially the universe of desirable targets .

9.20-9.40 Robert C. Bast, Jr., M.D. ,University of Texas M.D. Anderson Cancer Center,Houston, Texas, USA

BIOMARKERS FOR OVARIAN CANCER: THREE DECADES OF PROGRESS

Biomarkers for ovarian cancer can address several clinical needs including early detection, referral to surgeons with specialized training, monitoring therapy, identifying persistent disease, detecting recurrence and predicting response to targeted therapy. Among the ovarian cancer biomarkers, CA125 has been evaluated most thoroughly. The CA125 assay was originally developed three decades ago to monitor response to chemotherapy. When the biomarker is elevated, CA125 tracks progression or regression of ovarian cancer with >90% accuracy. At the conclusion of conventional treatment, elevated CA125 indicates persistent disease in greater than 90% of patients. Normal levels of CA125 can, however, be associated with residual disease in half of the cases. Sequential monitoring of CA125 can detect disease recurrence with a lead time of 3 to 4.8 months in 70% of patients. Whether monitoring recurrence actually benefits patients has been debated. Only one study has evaluated this question directly and this trial has significant limitations. While each patient must decide whether she wants to be monitored, earlier detection of disease does provides additional time for participation in clinical trials and for administration of the several drugs known to have activity against the disease.

Several studies have documented improved outcomes when patients are referred to specially trained gynecologic oncologists for their primary operations. In the United States less than half of women with ovarian cancer undergo surgery in the hands of such specialists. Preoperative diagnosis has depended upon age, physical examination and imaging with ultrasonography, MRI or computerized tomography. Elevation of serum biomarkers has also been utilized to increase the accuracy of differential diagnosis. Integrating biomarker, clinical and imaging data has required mathematical analysis. The Risk of Malignancy Index (RMI) has been developed in the United Kingdom and includes menopausal status, CA125 and imaging. The OVA1 algorithm includes five serum biomarkers (CA125, $\beta_2$microglobulin, transferrin, apoplipoprotein A1, and transthyretin) that are used in combination with imaging data. A Risk of Malignancy Algorithm (ROMA) utilizes CA125 and HE4 to triage patients for operation with a specially trained surgeon. The ROMA has proven more sensitive than the RMI in a direct comparison. While the ROMA and OVA1 have not been compared directly, they exhibit similar sensitivity, but the ROMA is somewhat more specific.

Perhaps the most promising application of serum biomarkers is for early detection of ovarian cancer in combination with transvaginal sonography (TVS). Two stage strategies appear most promising, where rising levels of biomarkers trigger TVS in a small fraction of healthy postmenopausal women. The UKCTOCS trial in the United Kingdom is screening approximately 200,000 postmenopausal women at average risk. Some 101,359 women receive conventional care as controls, 50,639 receive annual TVS and 50,640 have CA125 determined each year and the trend of values analyzed with a Risk of Ovarian Cancer Algorithm (ROCA). A report from the prevalence phase of the study suggests that CA125 followed by TVS will detect 89% of ovarian cancers with a specificity of 99.8% and a positive predictive value of 35%, i.e. only three operations for each case of ovarian cancer detected. Some 48% of cancers detected were in early stage, doubling the expected prevalence. The UKCTOCS trial will conclude in 2015 and is powered to detect a survival advantage. A smaller study of 4,675 women, coordinated by the UT M.D. Anderson SPORE in Ovarian Cancer over the last 12 years, has also utilized the ROCA algorithm and found a specificity of 99.9% and a positive predictive value of 42%. In the US study, twelve operations were prompted by the algorithm and seven cases of ovarian cancer were detected (two borderline and five invasive high grade) all in early stage (IA-IIC).

Use of CA125 as an initial step in a two stage screening strategy will miss at least 20% of cancers. Multiple biomarkers have been evaluated to identify a panel that would improve upon the sensitivity provided by CA125. A four biomarker panel has been defined (CA125, HE4, CA72.4 and MMP-7) that detects early stage disease with 86% sensitivity at 98% specificity, improving on CA125 alone. A new algorithm is being developed and will be tested for specificity and positive predictive value in a prospective trial. Assays are being adapted to a nano-bio-platform that will permit immediate assay at point of service from a fingerstick in a screening clinic. If the UKCTOCS trial is positive, screening could be made substantially more convenient. Autoantibodies against human proteins are also being evaluated to increase detection of preclinical disease prior to conventional diagnosis.

To individualize therapy, predictive tissue biomarkers will be required. Low grade (Type I) ovarian cancers respond poorly to conventional chemotherapy, but have mutations of Ras and express IGF receptors that can be targeted. High grade cancers (Type II) have mutations of p53, mutations of BRCA 1/2 and amplification of genes that activate the PI3K pathway. Strategies to target p53 mutations have not yet succeeded, but PARP inhibitors have produced a 40-50% response rate in patients with germ line BRCA 1/2 mutations. While 10-15% of ovarian cancers have germ line mutations, approximately 40% have deficiency of BRCA function or BRCAness.

Consequently, a fraction of sporadic ovarian cancers might benefit from PARP treatment. Signatures for PI3K activation or PI3Kness are being developed to identify cancers with "PI3Kness" which might respond to inhibitors of mTOR, AKT and PI3K.

REFERENCES:

Bast RC Jr. CA 125 and the detection of recurrent ovarian cancer: a reasonably accurate biomarker for a difficult disease. Cancer 2010; 116:2850-2853.

Bast RC Jr, Hennessy B, Mills GB. The biology of ovarian cancer: New opportunities for translation. Nat Rev Cancer 2009; 9:415-428.

Bast RC Jr, Klug TL, St. John E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. New Engl J Med 1983; 309:883-887.

Bast RC Jr, Mills GB. Personalizing therapy for ovarian cancer: BRCAness and beyond. J Clin Oncol. 2010; 28:3545-8.

Bast RC Jr, Mills GB. Dissecting PI3Kness: The complexity of personalized therapy for ovarian cancer. Cancer Discov 2012; 2:16-18.

Bast RC Jr, Skates S, Lokshin A, et al. Differential diagnosis of a pelvic mass: Improved algorithms and novel biomarkers. Int J Gynecol Cancer 2012; 10 (suppl 1):S5-8.

Diamandis EP, Bast RC, Gold P, et al. Reflection on the discovery of carcinoembryonic antigen, Prostate-Specific Antigen, and Cancer Antigens CA125 and CA19-9. Clin Chem 2013; 59:22-31.

Jacobs I, Oram D, Fairbanks J, et al., A risk of malignancy index incorporating CA 125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer. Br J Obstet Gynaecol. 1990; 97(10):922-9.

Lu KH, Skates S, Hernandez MA, et al. A two-stage ovarian cancer screening strategy using the risk of ovarian cancer algorithm (ROCA) identifies early stage incident cancers and demonstrates high positive predictive value. Cancer, In press.

Menon U, Gentry-Maharaj A, Hallett R, et al. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). Lancet Oncol 2009; 10:327-40.

Moore RG, MacLaughlan S, Bast RC. Current state of biomarker development for clinical application in epithelial ovarian cancer. Gynecol Oncol 2010; 116:240-245.

Moore RG, McMeekin DC, Brown AK, et al. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. Gynecologic Oncology 2009; 112:40–46.

Simmons AR, Baggerly K, Bast RC Jr. The emerging role of HE4 in the evaluation of ovarian and endometrial cancer. Oncology 2013; 85:1-10.

9.40-10.00 **Sir Greg Winter LMB, Cambridge , UK**
Enabling Antibodies to work in humans

10.00-10.20 **Ivan D Horak, Helle J. Jacobsen, Mikkel W Pedersen, Thomas T Poulsen, Ida Kjær, Klaus Koefoed, Jette W. Sen, Dietmar Weilguny, Bolette Bjerregaard, Christina R. Andersen Michael Kragh, and Johan Lantto. Symphogen, Elektrovej Building 375, Lyngby, Denmark.**

Antibody mixtures: A novel strategy to target tumor heterogeneity and tumor plasticity Pan-HER; A multi-targeting strategy to address tumor heterogeneity and tumor plasticity.

Recently developed technologies allow to document a remarkable tumor heterogeneity which will likely represent a major challenge to targeted therapy. In the clinical scenario, tumor heterogeneity as well as tumor plasticity could explain the mixed responses or short responses to individual targeted therapies. Symphogen has generated Pan-HER, a mixture of six mAbs consisting of pairs of synergistic mAb targeting EGFR, HER2 and HER3 respectively, The HER family of RTKs all play an important role in the development and progression of human epithelial tumors and show high level of plasticity why pan targeting could be advantageous. Pan-HER displays broad and potent receptor degradation and tumor growth inhibition in preclinical models and prevents compensatory receptor up-regulation/activation. The presented data indicate that Pan-HER is superior to existing targeted therapies in dealing with both primary and acquired resistance due to tumor heterogeneity and plasticity.

10.20-11.00 **Coffee break /poster review**

SESSION 2 Chairman: Kerry Chester

11.00-11.20 Klaus Bosslet, PhD, Head Discovery Oncology pharmaceutical Research and Early Development (pRED), Penzberg, Germany

Overview on antibody mediated treatment modalities in cancer therapy

Since the early description of monoclonal antibodies in 1975 by Köhler and Milstein, great efforts were made to develop these large molecules to their full potential for both diagnostic and therapeutic purposes.

Signal transduction inhibitory antibodies:
In the mid-nineties, the approval of MabThera/Rituxan, the first recombinant antibody directed against CD20 on human malignant B-cells, paved the path for successful development of the so called “naked antibodies” for cancer therapy. The approval of Herceptin, an antibody targeting the Her2 receptors, as well as CetuxiMab, which inhibits Her1 (EGFR1) signalling, underscores the significant therapeutic efficacy these signal transduction inhibitory antibodies have in various solid tumours. Since the Fc part of these antibodies is of IgG1 isotype, the contribution of innate immune effector functions (NK-cells) to therapeutic efficacy cannot be ruled out.

Antibody Drug Conjugates:
More recently, antibody drug conjugates (ADC) have shown promising therapeutic improvements in a few indications. Adcetris, an ADC targeting CD30 while harnessed with monomethyl auristatin, was approved for the treatment of anaplastic large cell leukemia and hodgkin’s lymphoma. In February 2013, Kadcyla, the first ADC for the treatment of Her2+ breast cancer, was approved by the FDA. Kadcyla consists of the Her2 antibody Herceptin covalently linked to mertansin. ADC works preferentially via the targeted delivery of small molecule cytotoxic component to cancer cells. However, contributions of the variable region of the antibody to the efficacy signal cannot be excluded.

Immune check point inhibitors:
With the approval of Ipilimumab, a humanized antibody blocking the CTLA4 inhibitory signal, for the treatment of metastatic melanoma, a new class of immunotherapeutic agent emerged whose mode of action might be the activation of a CTL response towards tumor cells. Since Ipilimumab is of IgG1 isotype, an alternative mode of action may be the elimination of T regulatory cells, which in turn allows immune-activating mechanisms to destroy cancer cells.

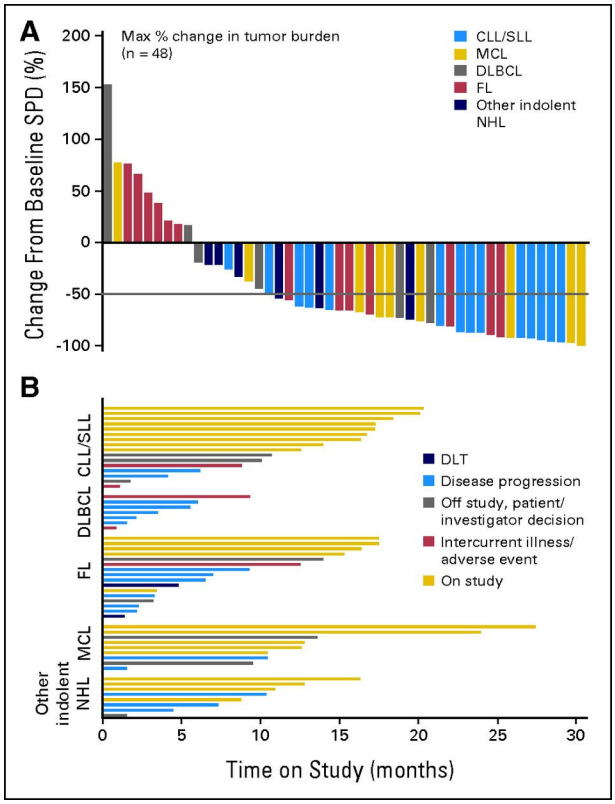
Modulation of the tumour micro environment:
The presentation will focus on the modulation of tumour microenvironment using a humanized antibody targeting CSF1R. RG7155, a novel anti CSF1R antibody, has been shown to eliminate M2 macrophages which are considered to be responsible for the immunosuppressive phenotype in a variety of cancers. Preclinical and preliminary clinical data will be presented.
In addition, advances in the characterization of the HumISmouse, a Rag2-/- yc-/- mouse reconstituted with human cord blood cells, will be presented.

11.20-11.40 **Ronald Levy, Professor of Medicine, Stanford University School of Medicine, Palo Alto, CA, USA**

Taking Therapeutic Antibodies to a New Level by a Second Antibody that Stimulates the ADCC Effector Cell

Many of the current approaches to immunotherapy require the production of a protein, gene or cell that is customized for each patient. While scientifically exciting, these approaches strain the limits of available resources and are not ready for widespread application.

A great example was the use of antibodies to target the unique idiotype protein on the surface of B cell lymphomas. Customized monoclonal antibodies, made from each patient, were extremely effective therapies but they proved impractical in the market place. Instead, Rituximab, a generic antibody against the CD20 molecule, present on all normal and malignant B lymphocytes became a blockbuster drug and is now used to treat every patient with B cell lymphoma. Rituximab improves patient survival and has changed the standard of care for lymphoma. Despite this success, most lymphoma patients still eventually die of their disease. Rituximab and many other monoclonal antibodies work by antibody dependent cytotoxicity (ADCC) mediated by NK cells.



Now second generation antibodies, engineered to interact better with Fc receptors on NK cells are being tested in the clinic. However, as yet, these re-engineered antibodies have not proven superior to the original version. Evidently, interaction with the Fc receptor is not the rate-limiting step. Instead, the killing activity of effector cells seems to be the limiting factor and new antibodies against targets that enhance the activity of the killer cells, such as CD47 and CD137 are likely to boost the effectiveness of monoclonal antibody therapy to new levels. Hopefully, by targeting the host as well as the tumor monoclonal antibodies will move from palliative to curative therapies.

PCI-32765 (Ibrutinib) a selective and irreversible inhibitor of Bruton's tyrosine kinase (Btk) has now demonstrated impressive clinical activity in lymphoma and chronic lymphocytic leukemia.

Antitumor response in all evaluable patients treated by Ibrutinib. (A) Best responses in the 48 patients evaluated by computed tomography scan for change from baseline in sum of product of greatest diameters (SPM); negative values indicate tumor shrinkage. (B) Time on study for all 56 patients grouped by histology; bars show patients on study and describe the reason for patient discontinuation. DLT, dose-limiting toxicity; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; NHL, non-Hodgkin lymphoma; MCL, mantle-cell lymphoma; SLL, small lymphocytic lymphoma (1).

A great opportunity now exists to combine immune therapy with small molecule targeted therapies, such as tyrosine kinase inhibitors.

At the current time little is known about the mechanisms of clinical resistance to Ibrutinib. But we can anticipate that tumor cells will eventually arise with mutation in their BTK enzyme sequence or with signaling pathways that bypass BTK.

It should be possible to reduce the emergence of tumor resistance by combining immunotherapy with Btk inhibition.

We have been able to enhance the therapeutic effects of many monoclonal antibodies, including Rituximab, Traztumumab and Cetuximab, by administering a single second antibody against CD137, and activation molecule on NK cells (2,3). We are now exploring this dual antibody approach in human clinical trials (NCT01471210, NCT01307267).

The easiest way to combine different therapies is to administer them together. Indeed, several international clinical trials are about to begin that simply add Btk inhibitors to Rituximab. However, we have found that Ibrutinib interferes with NK mediated ADCC and can completely abrogate the therapeutic effect of Rituximab. Therefore we needto find the most effective way of combining Btk inhibition with monoclonal antibody therapy and to avoid any interference by the Btk inhibitor of ADCC action by NK cells.

It should also be possible to combine active immunotherapy with the Btk inhibitor. Injection of CpG oligonucleotide, a TLR9 agonist, directly into a single site of tumor triggers a systemic anti-tumor T cell immune response that can sause regression of tumors at un-injected sites (4). This immunotherapy has several practical features. No antigen target needs to be pre-identified or manufactured. The therapy is completely “off the shelf” and the personalization occurs at the point of administration. Such active immunotherapy can prevent the emergence of mutant cells that can occur during therapy directed against the B cell receptor (5). Therefore it should be possible to combine this form of active immunotherapy with the Btk inhibitor therapy. We have already observed impressive synergy between these two treatment modalities.

References:

1. Advani RH, Buggy JJ, Sharman JP, Smith SM, Boyd TE, Grant B, Kolibaba, KS, Furman, RR, Rodriguez S, Chang BY, Sukbuntherng J, Izumi R, Hamdy A, Hedrick E, Fowler NH. Bruton Tyrosine Kinase Inhibitor Ibrutinib (PCI-32765) Has Significant Activity in Patients with Relapsed/Refractory B-Cell Malignancies. J Clin Oncol. 31(1): 88-94

2. Kohrt HE, Houot R, Goldstein MJ, Weiskopf K, Alizadeh AA, Brody J, Muller A, Pachynski R, Czerwinski D, Coutre S, Chao MP, Chen L, Tedder TF, Levy R. (2011) CD137 Stimulation Enhance the Antilymphoma Activity of anit-CD20 Antibodies. Blood 117(8): 2423-32

3. Kohrt HE, Houot R, Weiskopf K, Goldstein MJ, Scheeren F, Czerwinski D, Colevas AD, Weng WK, Clarke MF, Carlson RW, Stockdale FE, Mollick JA, Chen L, Levy R. (2012) Stimulation of Natural Killer Cells with a CD127-Specific Antibody Enhances Trastuzumab Efficacy in Xenotransplant Models of Breast Cancer. J Clin Invest 122(3): 1066-75

4. Brody JD, Ai WZ, Czerwinski DK, Torchia JA, Levy M, Advani RH, Kim YH, Hoppe RT, Knox SJ, Shin LK, Wapnir I, Tibshirani RJ, Levy R. (2010) In Situ Vaccination with a TLR9 Agonist Induces Systemic Lymphoma Regression: A Phase I/II Study. J Clin Oncol 28(8): 4324-32

5. Varghese B, Widman A, Do J, Taidi B, Czerwinski DK, Timmerman J, Levy S, Levy R. (2009) Generation of CD8+ T Cell-Mediated Immunity Against Idiotype-Negative Lymphoma Escapees. Blood 114(20): 4477-85. PMCID: PMC2777127

Ron Levy Stanford University , Palo Alto, USA

11.40-12.00 **Rob C. Roovers Merus BV, Padualaan 8, 3584CH Utrecht, the Netherlands**

Fully human, highly potent bispecific antibodies using MeMo®, a novel transgenic mouse antibody generation platform

As cancer therapeutics, bispecific antibodies allow the simultaneous targeting of different growth factor receptors involved in tumorigenesis, providing more efficacious treatment modalities. This approach benefits from bispecific human antibodies in a full-length 'natural' IgG format regarding clinical manufacturing and application in humans. A novel transgenic mouse harbouring a single rearranged human light chain, MeMo®, has been created to efficiently generate bispecific human antibodies. The MeMo® transgenic mouse is engineered to generate antibody diversity based on this common light chain (cLC) and diversified heavy chains. The cLC, selected for stability and high frequency in natural repertoires, facilitates rapid and comprehensive mining of antibody repertoires by simple sequencing. Using small cohorts of MeMo® mice and straightforward immunizations with several receptor tyrosine kinases, large and diverse panels of cLC antibodies against all targets were obtained. E.g. immunization with EGFR resulted in antibodies against all four sub-domains of the receptor and many of them functionally inhibited the receptor. These antibodies serve as building blocks for the quick and efficient generation of bispecific therapeutic IgG. Employing a proprietary CH3 dimerisation technology, two different heavy chains and the cLC are expressed in a single cell and efficiently assemble into highly pure, full length bispecific antibodies: Biclonics™, allowing direct screening for functional activity. Finally, hundreds of EGFR x HER3 bispecific antibodies were prepared and screened in this manner and several of these showed great activity in an orthotopic mouse model for pancreatic cancer.

12.00-12.20 **Janine Schuurman, Aran F. Labrijn, Kristin Strumane and Paul W.H.I. Parren, Genmab, Yalelaan 60, 3584 CX Utrecht, The Netherlands**

Efficient generation of stable bispecific IgG1 by controlled Fab-arm exchange

The prototypic monoclonal antibody (mAb) used in human therapy contains two identical antigen-combining sites and thus binds monospecifically and bivalently. Due to the multifactorial nature of certain diseases, however, not all patients respond adequately to monospecific antibody therapy. Antibodies with dual-targeting properties, i.e. bispecific antibodies, are thought to have great potential

because it has been demonstrated that synergizing binding pairs, resulting in antibodies with increased potency, can be identified. Most strategies to create bispecific antibodies by design are based on genetic fusion or co-expression of two antigen binding moieties. However, their application for therapeutic use has generally been hampered due to shortcomings with respect to manufacturability, purification, stability, pharmacokinetics as well as immunogenicity. Here we present a novel and elegant post-production technology for the generation of bispecific antibodies. This bispecific antibody platform is built on the process of Fab-arm exchange which is leveraged to generate stable bispecific human IgG1 antibodies. Our controlled Fab arm exchange process requires a single point mutation in the CH3 domain interface of each of the two parental IgG1 antibodies in combination with reducing conditions permitting in vitro exchange. Our data show that our platform ensures highly efficient generation of bispecific antibodies with a regular IgG1 structure, Fc-mediated effector functions and *in vivo* half life, next to fully scalable manufacturing using standard unit operations. Proof-of-concept studies using demonstrate that bispecific antibodies with increased in vivo activity compared to parental antibody combinations can be obtained. The mechanism of controlled Fab-arm exchange represents an attractive method for bispecific antibody discovery and development overcoming limitations encountered by other strategies.

12.20-12.40 Eugene Zhukovsky Affimed Therapeutics AG, Germany

Bispecific TandAbs recruit NK or T cells for the treatment of cancer

The TandAb technology utilizes the CD3 RECRUIT and CD16 RECRUIT effector modules for the recruitment of T and NK cells respectively, and a tumor-specific antigen module that targets the TandAb to cancer cells, leading to their lysis. *In vitro* and *in vivo* assays have demonstrated potent anti-tumor activity of these bi-functional tetravalent antibodies. Advantages of TandAbs, relative to the other bi-functional antibody scaffolds, include improved PK, drug-like properties, and specificity and efficacy of targeting tumor cells.
AFM13 is a TandAb antibody construct designed for the treatment of Hodgkin Lymphoma (HL) and other CD30+ malignancies. AFM13 targets CD30 on HL tumor cells and recruits NK cells via CD16A. Preclinical data demonstrate that this targeted immunotherapeutic specifically and efficiently displayed anti-tumor activity by selectively recruiting NK cells and killing target CD30+ cancer cells. AFM13 was investigated in an open-label single-arm phase I dose escalation trial in heavily pre-treated patients with relapsed/refractory HL. The overall objective of this study was to evaluate the safety, tolerability, pharmacokinetics, antitumor

activity, and the maximum tolerated (MTD). Seven dose levels (0.01 - 7.0 mg/kg) were escalated in cohorts of 3 patients. Each patient received a cycle of 4 weekly doses and responders received a second cycle. All dose levels of AFM13 were well tolerated and safe, with Grade 1-2 infusion reactions being the most frequent adverse event. AFM13 induced objective responses and a reduction in the Standardized Uptake Value (from PET imaging) in a dose-response manner, demonstrating clinical activity of the drug. A significant dose-dependent increase in the activation of NK cells and a reduction in soluble CD30 levels demonstrated a correlation between anti-tumor activity and response biomarkers. Furthermore, AFM13 exhibited a half-life of 1 day, which represented a substantial increase relative to that of alternative diabody-like formats currently being evaluated in the clinic for hematological malignancies. Overall, AFM13 has demonstrated encouraging biological activity and has a potential to become a new targeted therapy for heavily pre-treated patients with HL.
T cells are potent tumor-killing effectors that cannot be recruited by full length antibodies, however, TandAb technology harnesses their cytotoxic capacity for oncology indications. The T cell-recruiting TandAb AFM11 is a bispecific tetravalent antibody with two binding sites for both CD3 and CD19, enables T-cells to potently and specifically kill CD19+ tumors. In vitro assays demonstrate higher AFM11 target cytotoxicity relative to a bispecific tandem-scFv. CD8+ T-cells dominate early cytotoxicity (4 hrs) while after 24 hrs both CD4+ and CD8+ T-cells equally contribute to tumor lysis with EC50 of 0.5–5 pM. AFM11 exhibits similar cytotoxicity at a broad range of Effector:Target ratios and facilitates T-cell serial target killing. AFM11 activates T-cells only in the presence of CD19+ cells. In PBMC cultures AFM11 induces CD69 and CD25 expression, T-cell proliferation, production of IFN-γ, TNF-α, IL-2, IL-6, and IL-10. Depletion of CD19+ cells from PBMC abrogates these effects indicating strict CD19+ target-dependent T-cell activation. In a NOD/scid xenograft model reconstituted with human PBMC AFM11 exhibits a dose-dependent growth inhibition of Raji tumors; a single dose of AFM11 exhibits similar efficacy as five daily injections. Overall, AFM11 is an efficacious drug candidate for the treatment of CD19+ malignancies with an advantageous safety profile and anticipated dosing regimen.

12.20-2.00 ***Lunch break***

2.00-3.00 ***Open Air Workshops*:**

- Antibody engineering: Greg Winter, Klaus Bosslet
- Activating the Immune system: Ron Levy , Steffen Goletz

SESSION 3 Chairman: Robert Bast

5.00-5.20 K.A. Vallis, H.L. Chu, CR-UK/MRC Gray Institute for Radiation Oncology and Biology, Oxford University

Early Development of Radioimmunotherapeutics that Target Cancer Testis Antigens

Background: Cancer testis antigens (CTA), including NY-ESO-1, represent attractive targets for in situ radiotherapy as their expression is restricted to cancer cells and germ cells.
Methods: Anti-NY-ESO-1 antibodies were modified by addition of the cell-penetrating peptide, TAT, and DTPA for ^{111}In-labelling. A protein transfection reagent, SAINT-PhD (PT), was used to promote intracellular delivery of radioimmunoconjugate (RIC) giving the final product, ^{111}In-DTPA-aNY-ESO-1-TAT-PT (^{111}In-aNYE1-TP). SK-MEL-37 and SK-MEL-23 melanoma cell lines, with high and low NY-ESO-1 expression respectively, were incubated with ^{111}In-aNYE1-TP (3MBq, 6MBq/μg) or a control RIC, ^{111}In-DTPA-mIgG-TAT-PT (^{111}In-mIgG-TP) in cell fractionation and retention assays. DNA damage and cytotoxicity were evaluated using □H2AX and clonogenic assays, respectively. RICs were administered intravenously to SK-MEL-37 and SK-MEL-23 xenograft-bearing Balb/c nu/nu mice in biodistribution studies.

Results: ^{111}In-aNYE1-TP was retained longer in SK-MEL-37 cells than ^{111}In-mIgG-TP, with half-life times of 43.4 and 4.8 h, respectively (P=0.001). In contrast retention of the RICs in SK-MEL-23 cells was the same, with half-life of 3.3±0.1 h. The number of □H2AX foci was greater after exposure of SK-MEL-37 cells to ^{111}In-aNYE1-TP (3 MBq/□g) compared to ^{111}In-mIgG-TP (11.7±0.6 versus 5.0±0.5 foci/cell; P<0.005 one way anova test). In clonogenic assays ^{111}In-aNYE1-TP (3 MBq/ug) resulted in a statistically significant lower surviving fraction (SF) in SK-MEL-37 cells compared to ^{111}In-mIgG-TP (22% versus 80%;P<0.01; one way anova test). There was an inverse relationship between SF and concentration (0-500 nM) and specific activity (0.2-3 MBq/μg) of ^{111}In-aNYE1-TP. siRNA knock down of NY-ESO-1 resulted in partial reversal of ^{111}In-aNYE1-TP-associated cytotoxicity. In preliminary biodistribution studies in SK-MEL-37 xenograft-bearing mice, the tumour to blood ratio for ^{111}In-aNYE1-TP and ^{111}In-mIgG-TP was 5.3 and 2.6 respectively.

Conclusion: NY-ESO-1 presents an interesting target for radioimmunotherapy.

5.20-5.40 **Markus Thomas, Pharma Research and Early Development (pRED), Discovery Oncology, Roche Diagnostics GmbH, Penzberg, Germany**

CrossMab (Neutralizing VEGF-A and Ang-2) - From Bench to Clinic

VEGF-A blockade has been validated clinically as a treatment for human cancers. Angiopoietin-2 (Ang-2) expression has been shown to function as a key regulator of tumor angiogenesis. We have generated a bispecific human IgG1 antibody (CrossMab) blocking VEGF-A and Ang-2 function simultaneously. Our data show that the CrossMab has very good stability, an IgG like half-life in cynomolgus monkey and a favorable safety profile. Additionally the data establish Ang-2-VEGF-A CrossMab as a potent anti-tumor, anti-angiogenic, and anti-metastatic compound, which represents a promising therapeutic agent for the treatment of cancer.

5.40-6.00 **John McCafferty , Cambridge University, Cambridge, UK**

Blocking Mediators of the Tumor-Supportive Microenvironment with Antibodies

Primary and metastatic cancers comprise not only transformed cells but also host cells such as macrophages, fibroblasts and endothelial cells which are recruited to support tumour growth and invasion. Extra-cellular proteins which contribute to this supportive microenvironment provide exciting targets for therapeutic antibody discovery. This presentation will describe the development of blocking antibodies to targets in this class including c-Met and the metalloprotease ADAM17.

8.00-10.00 ***Welcome Reception Cocktail***

Tuesday 25th June 2013

SESSION 4 Chairman: Mark Greene

9.00-9.20

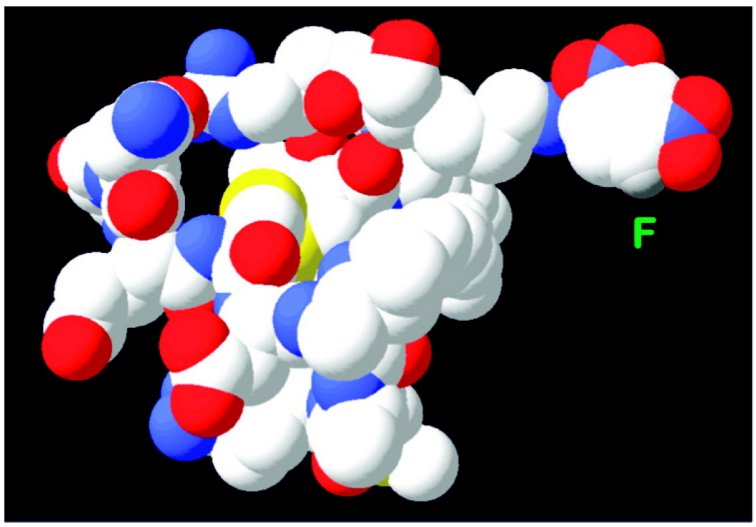
Authors	Heather E. Beck,* Bernadette V. Marquez,** Tololupe Aweda,** and **Claude F. Meares***
Affiliations	*Chemistry Department, University of California, Davis, California USA ***Current address: Mallinckrodt Institute of Radiology, Washington University, St. Louis, Missouri USA*
Title	**Covalent approaches to targeting cancer**

Abstract

Vascular endothelial growth factor (VEGF) is a small, dimeric protein important for angiogenesis, the formation of new blood vessels. The elegant phage-display studies of Fairbrother and co-workers led to peptide ligands that blocked the receptor binding of VEGF, but with approximately micromolar dissociation constants, which are much weaker than the nano- to picomolar dissociation constants of acceptable drugs. While not a widely used design concept, a ligand that binds *irreversibly* to its target has several potential advantages over such weakly binding reversible ligands. For reversible binding, the concentration of free ligand must be larger than the dissociation constant in order for the target to be >50% occupied; for a covalent ligand, under ideal conditions the target could be titrated with equimolar ligand. Once tagged, the target is permanently inactivated, an outcome qualitatively different from reversible binding. To create a site on a weakly binding peptide ligand that would carry a covalent labeling reagent, we replaced a residue that does not change the peptide's affinity for VEGF, but is located within reach of a lysine side chain on the protein. We will describe the interaction of reactively tagged peptides with VEGF, including recent studies in vivo in animal models, with particular focus on a nitroaryl fluoride reagent (see figure).

Contacts	Tel.	+1 530 277 0202
	E-mail	cfmeares@ucdavis.edu

Figure



Reactive peptide for inhibiting VEGF.
Marquez et al.,
Bioconjugate Chem., 2012, 23 (5), pp 1080–1089

9.20-9.40 **Giovanni Paganelli, MD, Division of Nuclear Medicine, European Institute of Oncology - Milan, Italy**

Pretargeting without antibody: the IART® approach in breast and prostate cancer

Breast conserving surgery followed by post-operative external beam radiotherapy is the treatment of choice in patients with early breast cancer (Stage I-II). The possibility of irradiating the mammary gland using a nuclear medicine approach is being proposed. IART® is a simple technology based on two steps:
1) intra-operative “avidination” of the anatomical area of the tumour after its removal by the surgeon, who injects with a syringe a solution of avidin into the mammary gland;
2) post-operative (16-24 h after surgery) delivery of a radiation dose by targeting the avidinated area of the mammary gland with 90Y-labeled biotin, via a slow intravenous injection. The two steps are based on the incredible high affinity between avidin and biotin, the highest affinity known in nature. Phase I and II studies conducted at the European Institute of Oncology in Milan, Italy (Breast 2007, Clin Cancer Res 2007, Eur J Nucl Med Mol Imaging 2010) showed that IART® can safely deliver a dose of radiation (20 Gy), targeting the mammary region of interest, sparing the surrounding organs (lungs and heart), with minimal skin

damage. IART® peri-operative timing allows starting adjuvant treatment after conservative surgery immediately, thus minimizing the negative outcomes associated with delayed radiotherapy. This, in turn, permits abbreviated external beam radiotherapy, that can reduce the burden for patients from the usual 5-7 weeks of daily sessions to just 2 weeks. In low risk patients such as postmenopausal women IART® has the potential to replace EBRT after breast conserving surgery.
IART® offers several advantages over other irradiation methods. It is applicable to the majority of breast cancers, without limitation as to location, size or multi-focality; it can be carried out in any hospital, independently of the availability of radiotherapy, thus facilitating a wider adoption of conservative breast surgery. This standard of care is not practiced in many areas of the world because radiotherapy centers are unable to cope with the current demand (which is expected to further increase in the next 10 years), or are too distant from patients' homes.
IART® is a radio-metabolic platform for delivering an appropriate amount of radiation in a very selected anatomical area. As such, it can be used in a variety of solid tumors, such as those of bladder and prostate.
A Monte-Carlo dosimetric model has been developed. The methodology is theoretically feasible and can deliver an effective treatment in T1-T2 prostate cancer.

9.40-10.00 Teodor Aastrup, Attana, Stockholm, Sweden

Drugability of receptors – Evaluating accessibility of target receptors by means of label free kinetic cell based biosensor

Too low efficacy in clinical trials is one of the main reasons why new drug candidates fail in clinical trials. The reasons for the low efficacy may vary, but is clear that more biological relevant information prior to clinical trials is needed in order to increase the success rate. We believe that one important parameter to study early in the development process is the drugability of the target receptor by means of measuring the accessibility of the receptor.

Here we present a *in-vitro* method to obtain accessibility information of the receptor enabling evaluation the drugability of the receptors. The accessibility is measured using a continuous flow and label free cell based biosensor. Thereby the receptors remain unmodified in the cell membrane and the transport mechanism of the drug molecule is under *in-vivo* similar conditions.

By studying the differences of the association rate in a biochemical and a cell based assay can the therapeutically accessibility of the targeted epitope of the receptor be determined.

A therapeutic candidate molecule needs to reach its target without much hindrance to be successful. On the cell surfaces, there are *i.e*. carbohydrates which can sterically hinder the binding and thus make the receptor less drugable.

Accessibility experiments using Attana Cell 200 biosensor with continuous flow are compared with incubation flow cytometry and the results elucidates the impact that a continuous flow transport mechanism has on the binding of a drug molecule to a receptor and thus the importance of targeting a highly accessible receptor.

10.00-10.20 **Stefan Lohse, Stefanie Derer, Janne Böck, Thies Rösner, MatthiasPeipp, Thomas Valerius**
Division of Stem Cell Transplantation and Immunotherapy,II. Department of Internal Medicine ,Christian-Albrechts-University, Schittenhelmstraße 12, 24105 Kiel, Germany

Progress in the development of therapeutic antibodies of IgA isotype

Monoclonal antibodies against tumor-related antigens have significantly improved cancer therapy over the last decades. So far, all approved antibodies are of human IgG isotype. Antibodies of IgA isotype are key players of the mucosal immune system. In contrast to IgG, IgA antibodies form natural dimers, which are transported to serosal surfaces to form secretory IgA. Little is known about the immunotherapeutic potential of these three different IgA isoforms. Meanwhile, efficient production and purification protocols have been reported for the generation of recombinant monomeric and dimeric IgA antibodies. In vitro, monomeric and dimeric IgA antibodies trigger significant ADCC by myeloid effector cells (monocytes/macrophages and PMN), but do not recruit NK cells or complement for tumor cell lysis. In vitro, dimeric IgA antibodies were more effective than monomeric IgA, and the IgA2 isotype proved more effective than the IgA1 isotype. In vivo evaluation of IgA antibodies was complicated by the fact that mice do not express an orthologe of the human myeloid IgA receptor (Fc□RI; CD89); which is the predominant cytotoxic trigger molecule on effector cells. Here, generation of human Fc□RI transgenic mice provided novel opportunities. Recent studies in these mice demonstrated that monomeric IgA antibodies were also effective in vivo - inhibiting tumor growth in different xenogeneic and

syngeneic tumor models. However, also limitations of the currently available molecules became apparent during preclinical development.
For example, the most common IgA2m(1) allotype consists of heavy and light chain homodimers, respectively, which can be converted to the classical heavy-light chain heterodimers by introduction of a P221R mutation into the heavy chain. Furthermore, IgA antibodies carry an 18 amino acids C-terminal tailpiece extension with a cysteine at position 471, which is important for the formation of dimeric IgA antibodies. In the present work, we investigated the functional relevance of this tailpiece for monomeric IgA. Thus, we generated and characterized a d471-mutated IgA2m(1) antibody against the epidermal growth factor receptor (EGFR). The genetic deletion of the penultimate cysteine 471 improved biochemical as well as functional properties compared to wild type IgA2m(1). Thus, the deletion of the C-terminal cysteine is a further improvement of an IgA2m(1) antibody towards a therapeutic antibody. Together, these studies provide further insight into the immunotherapeutic potential of recombinant IgA antibodies and suggest additional approaches to further improve their characteristics as therapeutic drugs.

10.20-11.00 ***Coffee break*** **and poster review**

SESSION 5 Chairman: Katherine Vallis

11.00-11.20 **Enrique Miranda, Heide Kogelberg, Berend Tolner, Gareth Thomas, Tim Meyer, John Marshall, Stephen Mather, <u>Kerry Chester</u>**

Dual-Specific Antibodies for Cancer Targeting

The αvβ6 integrin is up-regulated in cancer and wound healing but is not generally expressed in healthy adult tissue. This integrin plays a variety of roles in the tumour - including activation of transforming growth factor (TGF)-β and the promotion of cell migration/tumour invasion by interaction with specific extracellular matrix proteins. There is increasing evidence that αvβ6 plays a role in cancer progression and will be a useful target for antibody-directed cancer therapies. A series of engineered αvβ6-reactive antibodies have been generated, either as αvβ6-specific agents or as dual-reactive antibodies that bind both to αvβ6 and carcinoembryonic antigen (CEA or CEACAM5). Pre-treatment of αvβ6-expressing cells with the recombinant antibodies resulted in a reduction of cell migration and a down-regulation of (TGF)-β-mediated effects, demonstrating biological function-blocking activity. The results will be discussed in relation to cell biology, tumour heterogeneity and therapeutic potential.

11.20-11.40 Steffen Goletz, CEO / CSO, Glycotope GmbH, Berlin, Germany

PankoMab-GEX targeting the tumor specific TA-MUC-1 – Results from a clinical Phase I study

Background: PankoMab-GEXTM is a potent glyco-designed and glyco-optimized humanized monoclonal antibody with fully human glycosylation recognizing the novel carbohydrate-induced conformational tumor epitope TA-MUC1 expressed on large fractions of tumor cells in a wide variety of cancers. It comprises a tumor specific carbohydrate antigen (TF or Tn) together with the immunodominant peptide region of MUC1 and is human-specific and virtually only expressed on malignant cells.

Results: Key modes of action: potent tumor cell killing via ADCC and phagocytosis, apoptosis induction and proliferation inhibition. Main target indications due to expression (up 90-100%) could be ovarian, breast and lung, but also cervix, endometrium, gastrointestinal, kidney, urothelial and other cancers. The Current clinical development is focused on ovarian cancer and non-small-cell lung cancer (NSCLC) due to clinical evidence in addition to expression patterns.

A first-in-man Phase I single agent dose escalation trial with late stage patients progressive upon inclusion was just finalized treating 74 patients at several schedules and escalating doses. PankoMab-GEX showed very good tolerability, long half-life following q3w dosing schedules as well as strong responses including a complete response and long lasting clinical benefit including disease stabilization and tumor reduction of up to 2 years. Especially a difficult to treat subgroup of ovarian cancer patients and lung cancer patients showed strong responses and clinical benefit. steffen.goletz@glycotope.com

11.40-12.00 Djamila Ouaret, John Radcliffe Hospital, Oxford, UK

Toward a Modulation of Antibody-Dependent Cellular Cytotoxicity using combination of glyco-engineering antibodies and HDAC inhibitors

Monoclonal antibodies target tumour cells by both immune and non-immune mechanisms. Antibody-based immune killing occurs largely through Fc gamma receptor (Fc□R) interactions with the Fc segments of IgG1. These receptors are expressed on immune effector cells, such as macrophages and NK cells, present in the tumour environment. Methods that increase immune mediated killing are therefore of interest in antibody-based therapeutics. One such technology

involves Fc segment glyco-engineering that increases the affinity between IgG antibodies and Fc□Rs. In addition to glyco-engineering the antibody, another potential strategy to increase antibody-dependent cellular cytotoxicity (ADCC) is to combine therapy with drugs that increase the susceptibility of tumour cells to immune attack. HDAC inhibitors are a class of drugs that have been shown to modulate MICA/B expression in HT29 and DLD1, two colorectal cancer (CRC) cell lines. These proteins act as ligands for NKG2D receptors, a class of natural cytotoxicity receptors that are found on NK cells.

Using flow cytometry, the influence of SAHA, an HDAC inhibitor, on expression of natural killer cell receptor ligands (NKG2DL) was assessed in a panel of CRC cell lines. Immune mediated killing was evaluated using fresh PBMC's from healthy donors and measured using a LDH assay. Using SAHA-treated cells, the contribution of NKG2DL up-regulation to background killing and ADCC was also investigated.

We showed that SAHA, an HDAC inhibitor, is able to increase surface MICA/B expression across a panel of CRC cell lines, including cell lines that do not express a basal level of surface MICA/B. These data encouraged us to study the effect of SAHA on non-specific natural killing as well as on ADCC. Preliminary results showed that the combination of SAHA and glyco-engineered antibodies increases the percentage of ADCC. This result will now be confirmed on a larger selected set of CRC cell lines.

Our data provide a rationale for studying further the combination of antibody-based therapies with other compounds that may modulate the expression of NKG2DL, which include 5-fluorouracil.

12.00-12.20 **Lars Stöckl/ Steffen Goletz, CEO / CSO Glycotope GmbH, Berlin**

Biosuperiors: TrasGEX and CetuGEX – Results from clinical Phase I studies

Background: Glycosylation is one of the major post-translational modifications of biotherapeutics important for bioactivity, bioavailability, immunogenicity and patient coverage.

Methods: By establishment of the GlycoExpress toolbox (GEX) we have generated a set of glycoengineered human cell lines for the high yield production of fully human glycoproteins to optimize the glycosylation of antibodies and non-antibody biotherapeutics for improvement of the clinical efficacy and side effects. The system is biotechnologically superior in quality,reproducibility and yield compared to other including conventional production systems.

Among other non-antibody molecules 3 glycooptimized antibodies are presently in clinical development. Two of these are the BioSuperior antibodies CetuGEX™ and TrasGEX™ based on the monoclonal antibodies Cetuximab and Trastuzumab and glycooptimized with respect to manifold improvement of anti-cancer activity, optimization of bioavailability, removal of immunogenic components and broadening of the patient and indication coverage.

Besides the improvement in ADCC function, in contrast to Cetuximab, CetuGEX™ does not contain any immunogenic non-human glycan structures such as NeuGc and Galili epitope (Gal-Gal carbohydrate structures), the latter have been shown to lead to severe hypersensitivity reactions including life threatening anaphylactic shocks based on preexisting IgE in some regions with high incidences of the latter.

Phase I clinical studies of CetuGEX and TrasGEX were just finalized or will be finalized soon, respectively and results for CetuGEX were just presented on ASCO 2013. Single agent dose escalation studies with late stage patients with progressive disease showed for both agents strong single agent activity including various complete and partial responders as well as long lasting clinical benefit, in case of CetuGEX a Clinical Benefit Rate of 76%.

Conclusions: The glycooptimization principle was clinically proven by the fact that strong responses and clinical benefit was seen in patients who showed no benefit or were progressive with the non-glycooptimized trastuzumab or cetuximab, especially in patients with F allotype ADCC receptors, at lower dosages, in new and known indications, and with better side effect profile. Phase II trials are in preparation.
steffen.goletz@glycotope.com

12.20-2.00 Lunch break

2.00-3.00 *Open Air workshop*s:

- Enpowered Antibodies - Kerry Chester, Steffen Goletz

- Cancer Stem Cells - Walter Bodmer, Bob Bast

SESSION 6 Chairman: Mahendra Deonarain

5.00-5.20 Mark I. Greene M.D.,Ph.D. ,John Eckman Professor of Medical Science, University of Pennsylvania, Philadelphia, USA

THE DEVELOPMENT OF TARGETED THERAPY TO ERBB2/NEU TRANSFORMED CELLS

The development of targeted therapy of erbB2/neu transformed cells arose because of study of the linkage of transforming gene encoded antigens and cell surface proteins. We studied a variety of transforming genes and developed monoclonal antibodies to proteins they encoded. The development of monoclonal antibodies to different domains of the p185 protein produce of the neu oncogene was followed by the realization that the protein was a cell surface molecule invested with tyrosine kinase activity in its endodomain. We solved how the protein led to transformation by forming active kinase dimers and made the discovery that disabling or down regulating the kinase dimer would reverse many features of the malignant phenotype of neu transformed cells in vitro and in vivo. Monoclonal antibodies to distinct domains when used together produced even more dramatic effects in vivo.
This approach was licensed and adopted by pharmaceutical companies and led to Herceptin and Pertuzumab now in the clinic.
The actual mechanisms of phenotype reversal and down regulation were resolved by detailed biochemical and structural studies which will be reviewed in the presentation.

5.20-5.40 Birgit Bossenmaier , Roche Germany

RG7116, a novel anti-HER3 antibody with a dual mode of action

HER3 has emerged as an attractive therapeutic target in oncology due to its central position in the HER signaling network. RG7116 is a novel anti-HER3 monoclonal antibody designed to block HER3 activation, down-regulate HER3, and enhance ADCC. These different modes of action are demonstrated by biochemical studies, X-ray crystallography, detailed analyses of the effects on HER3 signalling and ADCC assays. They translate into convincing preclinical efficacy data in different xenografts as well as first signs of efficacy in the clinic.

5.40-6.00 **Mahendra Deonarain, CSO, PhotoBiotics Ltd & Honorary Reader in Antibody Technology, Imperial College London, UK**

Antibody-Drug Conjugates applied to Photodynamic Therapy

Antibody drug conjugates (ADCs) are enjoying a new lease of life thanks to exquisitely potent drugs, clever linkers and well-characterised antibodies. Two newly approved drugs and over 30 clinical stage candidates demonstrate the huge promise to overcome the limitations of standard monoclonal antibodies. We will review this area, highlighting where the innovation is coming from and what the outlook for the field is likely to be. Photobiotics' photo-active ADCs promise to combine the cosmetic benefits of laser therapy with the potency and selectivity of ADCs with fewer on-target toxicities. We have been developing single-chain Fv antibodies specifically optimized for accommodating covalently attached photodynamic therapy (PDT) drugs in a technology platform called "OptiLink". Antibody fragments, by virtue of their faster clearance dramatically improve the side-effect profile of PDT drugs. We show that scFv-based ADCs, activated by laser light are can destroy tumours in a range of challenging animal models for ovarian and prostate cancer. High drug loading can be achieved even with small fragments and we will compare photo-active with traditional cytotoxic-based ADCs.

8.30 pm until late ***Conference Dinner***

Wednesday 26th June 2013

9.20-9.40 **Shazad Ashraf & Walter Bodmer, Weatherall Institute of Molecular Medicine and Department of Oncology, Oxford University**

The use of a colorectal cancer cell line panel as a predictive tool in determining anti-IGF1R antibody responses

Initial trials using anti-IGF1R therapy (IGF1Ri) in colorectal cancer (CRC) have been disappointing (1). This is in contrast to preclinical studies that show susceptibility of CRCs to anti-IGF1R monoclonal antibodies (Mabs) or tyrosine kinase inhibitors (TKIs). However CRC is a heterogeneous disease with a broad spectrum of genetic and epigenetic changes. It is therefore not surprising that failure to tailor adjuvant therapy to a defined patient sub-group results in a dilution of clinical benefit and under-powering of clinical trials. As a clear example, cetuximab was initially administered to patients based on ERBB1 expression rather than genetic mutation status. *KRAS* testing is now mandatory prior to anti-ERBB1 therapy (2). This understanding has led to a search for signatures that predict response to IGF1Ri. We have recently demonstrated the effectiveness of a large CRC cell line panel in predicting response to established therapeutics, such as cetuximab and 5FU (3, 4). This panel is extremely well characterized at the biological and molecular level and therefore provides a powerful *in vitro* model for CRC. We have undertaken a high-throughput screen (SRB assay) to establish the direct growth response to IGF1Ri in a panel of over 60 CRC cell lines. As for anti-ERBB1 targeting, our data suggests that a similar proportion of colorectal cancers display sensitivity to IGFRi. We discuss predictive characteristics that may potentially be used in future anti-IGF1R clinical trials.

References

1. Reidy DL, Vakiani E, Fakih MG, Saif MW, Hecht JR, Goodman-Davis N, et al. Randomized, phase II study of the insulin-like growth factor-1 receptor inhibitor IMC-A12, with or without cetuximab, in patients with cetuximab- or panitumumab-refractory metastatic colorectal cancer. J Clin Oncol. 2010 Sep 20;28(27):4240-6.
2. De Roock W, De Vriendt V, Normanno N, Ciardiello F, Tejpar S. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. Lancet Oncol. 2011 Jun;12(6):594-603.

3. Bracht K, Nicholls AM, Liu Y, Bodmer WF. 5-Fluorouracil response in a large panel of colorectal cancer cell lines is associated with mismatch repair deficiency. Br J Cancer. 2010 Jul 27;103(3):340-6.
4. Ashraf SQ, Nicholls AM, Wilding JL, Ntouroupi TG, Mortensen NJ, Bodmer WF. Direct and immune mediated antibody targeting of ERBB receptors in a colorectal cancer cell-line panel. Proc Natl Acad Sci U S A. 2012 Dec 18;109(51):21046-51.

9.40-10.00 Surinder K Sharma and Kenneth D Bagshawe , University College London ,UK

Antibody Directed Enzyme Prodrug Therapy (ADEPT)

The fundamental principle of ADEPT is to retain an adequate concentration of enzyme in tumour with zero enzyme concentration in blood. To achieve this it was necessary to have a component that cleared enzyme from blood but not from tumours. This required a three component system which in a small feasibility trial in patients with advanced metastatic terminal disease resulted in 7 patients surviving for more than 6 months including 3 who survived 18, 25 and 36 months. Subsequent clinical trials using two component systems have not demonstrated similar effects which may result in loss of a promising three component ADEPT. However, there now exist ways that enhance the efficacy of the MFECP fusion protein two component ADEPT, as well as emerging developments of new multi component systems.

10.00-10.20 **Khaled Al-Qaoud, Yarmouk University-Irbid, Jordan Company for Monoclonal Antibody Production and Philadelphia University-Amman-Jordan**

Blocking of Histamine Release and IgE Binding to FcεRI on Human Basophils Using Antibodies Produced in Camels

The absence of the light chains and CH1 domain in *Camelidae* heavy chain antibodies (HCAbs) give them many special characteristics that are different and superior to conventional antibodies. HCAbs have the capability to resist heat and extreme pH without losing their activity in addition to their longer half life. These advantages of HCAbs render them eligible to be used for diagnostic and therapeutic purposes. For the purpose of production of camel anti-IgE blocking antibodies, camels were immunized with native human IgE (huIgE) and a synthetic loop peptide (SLP) that resembles FcεRI binding site to basophils and other effector cells mediating immediate hypersensitivity response. SLP was conjugated with multiple antigen peptide system (MAPS) forming SLP-MAPS immunogen. Polyclonal camel antibodies, both conventional and heavy chain (HCAbs) isotypes were produced, purified and characterized using Protein A, Protein G, ELISA and SDS-PAGE. Conventional IgG1as well as IgG2 and IgG3 HCAbs were detected and successfully purified from camel sera immunized with both native huIgE and SLP-MAPS. The potency of camel isotypesto block passive sensitization on human basophils was measured by flow cytometry and by inhibition of histamine release using an *in vitro* assay. The results of both methods were closely correlated and indicated that camel conventional (IgG1) and HCAbs (IgG2 and IgG3) had a high blocking potency with the HCAbs being much superior. Furthermore, the blocking potency of the HCAbs purified from SLP immunized camel was greater than that of HCAbs purified from the huIgE immunized camel.

SESSION 8 Chairman: Agamemnon Epenetos

CANCER STEM CELLS SYMPOSIUM

10.20-10.40 **N.J. Agnantis, MD, PhD, FRCPath, Emeritus Professor of Pathology; A.C. Goussia, MD, PhD, Ass. Professor of Pathology, Department of Pathology, Medical School, University of Ioannina, Greece**

Triple negative breast cancer: the role of cancer stem cells

Breast cancer (BC) is a complex and heterogeneous disease with respect to histology, cellular origin, progression, metastatic potential, therapeutic response and clinical outcome. Targeted therapeutic agents, such as tamoxifen and trastuzumab, were developed for use in subsets of patients expressing relevant biomarkers, estrogen (ER)/progesterone (PR) receptors and HER2 respectively. Utilization of targeted therapies in biologically preselected patient populations has significantly improved BC survival. The above treatment options are absent in patients diagnosed with tumors lacking ER, PR and HER2. These breast cancers are referred by definition as Triple Negative Breast Cancers (TNBC) and account approximately.15-20% of all BCs.

To better understand the heterogeneity of BC, genomic studies based on gene expression analyses, have identified intrinsic subtypes of breast tumors, with distinct molecular characteristics and important implications to outcome: luminal A and luminal B, HER2 positive, basal-like, normal-like, claudin-low, molecular apocrine and interferon subtypes. Because the majority of TNBC (approximately 70-80%) present with basal-like molecular characteristics and the majority of basal-like cancers are TNBC, it has been claimed that the triple-negative and basal like phenotypes are effectively synonymous. However, clinical, microarray and immunohistochemical data showed that TNBC encompass other molecular subtypes as well, such as the claudin-low tumors, which are tumors with cells that have properties similar to those of stem cells and features of epithelial-mesenchymal transition and the interferon-rich tumors, which encompass tumors with better prognosis than the prognosis of TNBC.

Several population-based studies have shown that TNBC often present at young age (<40 years old) and more frequently in African-American women and black ethnicities. Moreover, it is a well established link between BRCA-1 & BRCA-2 mutation status and the risk of developing TNBC. Histologically, TNBC is a heterogeneous group of cancers, being mostly invasive non-otherwise specified

(NOS) carcinomas and 90% of the cases are poorly differentiated with large tumor size. At the molecular level, they express epidermal growth factor receptor (EGFR) and genes associated with basal epithelium/ myoepithelium of the normal mammary gland (keratins 5/6, 14 and 17, caveolin 1 and 2, P-cadherin, nestin). In addition, TNBC are frequently associated with high expression of proliferation markers (e.g. Ki67), high levels of cyclin E, low levels of cyclin D1, mutations of p53 gene and activation of the beta-catenin pathway. TNBC are generally aggressive tumors with poor prognosis. The peak risk of recurrence occurs within the first 3 yrs after treatment with the majority of deaths presenting within the first 5 yrs. After diagnosis of metastatic disease, a significant shorter survival is observed. TNBC are more likely to metastasize early to lungs and brain than to lymph nodes and bones.

The reasons for this aggressive phenotype is currently the focus of intensive investigation. Research progress is limited due to the lack of suitable TNBC cell model systems. However, recent data suggest that one of the explanations for the heterogeneity of breast cancer is the presence of cancer stem cell (CSCs) and studies conducted by several laboratories suggest a strong association of CSCs phenotype with basal-like cancers or TNBC.

The basic structure of the normal human breast consists of a branching ductal-alveolar system lined by an inner layer of luminal epithelial cells and an outer layer of myoepithelial cells. Luminal epithelial cells express keratins 7, 8, 18 and 19, whereas myoepithelial cells express keratins 5/6, 14 and 17. Presumptive stem, multipotent progenitor and committed progenitor cells in mouse and human mammary gland have been identified. These cells, under growth conditions, differentiate to luminal/myoepithelial cells. Unlike pluripotent embryonic stem cells, that are able to give rise to all cells of the body, these stem cells are multipotent and they are restricted to produce cells within the breast gland. So, during normal mammary development, stem cells are ER-negative but differentiate into highly proliferative ER-positive progenitor cells. Under the influence of estrogen and progesterone hormones, the progenitor cells may interact with stem cells with paracrine signals (Wnt and growth factors). Finally, the proliferative ER-positive progenitor cells.give rise to quiescent ER- positive and ER-negative terminally differentiated adult cells.

A number of plethora of *in vitro* and mouse models have been developed in order to study the properties of mammary stem/progenitor cells. In assays carried out in the NOD/SCID mice, stem/progenitor cells can be form floating spherical colonies (“mammospheres”). Cells from “mammospheres”, can undergo multilineage differentiation after collagen coated dishes culture and subsequently, they can undergo morphogenetic differentiation forming ductal - alveolar structures in the cleared fat pads of the mice. These normal mammary stem cells have been identified based on the elevated expression of aldehyde dehydrogenase 1 (ALDH1). The $ALDH1^{+}$ cells express mainly the CK5/6 basal

keratin and can be differentiated to luminal and myoepithelial cells. Moreover, cell surface marker profiling with CD44 and CD24 has identified a $CD44^{+}/CD24^{-/low}$ subpopulation of normal mammary cells expressing elevated levels of stem cells enriched genes.

According to the old model of breast cancer progression, all breast cells have equal potential to become tumorigenic, under the influence of certain, repetitive insults. As more data were collected concerning the breast cancer heterogeneity and the nature of some aggressive breast tumors, a new model of cancer progression has been proposed. According to this model, the cancer progression is due to a subset of cells, with stem cell-like characteristics and with diffeent potentials for tumorigenesis. Therefore, breast cancer stem cells (BCSCs), are thought to be originated either from a normal stem cell that has acquired mutations and became tumorigenic or from a more differentiated progenitor or mature cell that has been changed into a dedifferentiated cell and acquired tumorigenic mutations as well as the ability of self renewal. BCSCs were initially characterized as $CD44^{+}/CD24^{+/low}$ and as few as 100 cells with this phenotype were able to produce tumors in NOD/SCID mice. Moreover, it has been demonstrated that as few as 20 $CD44^{+}/CD24^{+/low}/ALDH1^{+}$ cells were enough to be tumorigenic. As shown in some studies, the phenomenon of epithelial-mesenchymal transition (EMT) is capable of creating BCSCs, since a) overexpression of Snail or Twist (two important factors involved in EMT) may generate cells with $CD44^{+}/CD24^{+/low}$ phenotype and b) cells with $CD44^{+}/CD24^{+/low}$ phenotype are reported to be found on the leading or invasive front of BCs, a site where EMT is more apparent. However, BCSCs and cells resulting from EMT are not necessarily the same thing.

In several breast cancer cell lines, the correlation between BCSCs phenotype and intrinsic molecular subtypes of BCs was investigated. As with primary breast cancers, breast cancer cell lines can also be classified into distinct molecular subtypes. It was found, that basal-like breast cancers and TNBC had a higher percentage of $CD44^{+}/CD24^{+/low}$ expressing cells than other breast cancer subtypes. Consistent with this observation, patients with basal-like breast cancers or TNBC or $CD44^{+}/CD24^{+/low}$ phenotype displayed poor prognosis. Moreover, the most aggressive metastatic growth was observed in the above tumors that were $CD44^{+}/CD24^{+/low}$. Additional link between $CD44^{+}/CD24^{+/low}$ and both, invasive capacity and poor prognosis, was established by the demonstration of a unique 186-invasiveness gene signature in these cells. Importantly, the genes of the signature predicted distant metastases, to lungs and brain, where TNBC are more likely to metastasize. Moreover, clinical observations have indicated an increase of $CD44^{+}/ CD24^{+/low}$ cancer cells after chemotherapy treatment, suggesting that these cells may be resistant to therapy. This is an important issue of TNBC, for which systemic treatment options are limited and with a variable impact on long-term prognosis.

In conclusion, TNBC is a heterogeneous subset of BCs with aggressive clinical behavior, high metastatic ability and poor prognosis. Currently, TNBC are routinely identified in most Pathology Laboratories. Some patients respond to chemotherapy, but this is not the rule. Even after BCSCs have been identified, it is difficult to target these cells. Precise determination of the development and origin of BCSCs would be helpful in establishing specific treatments focused on them. Research has already discovered how important BCSCs are in the "war" against breast cancer.

Selected References:

1. Wicha MS. Targeting breast cancer stem cells. The Breast 18: S56-S58, 2009.
2. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. NEJM 363: 1938-1948, 2010.
3. Reis-Filho JS, Pusztai L. Gene expression profiling in breast cancer: classification, prognostication, and prediction. Lancet 378: 1812-1823, 2011.
4. Samaddar JS, Ritter E. Breast cancer stem cells-A review. In: Breast Cancer-Carcinogenesis, Cell Growth and Signalling Pathways. M. Gunduz (ed), In Tech, pp 505-522, 2011.
5. Chu JE, Allan AL. Cancer stem cells in breast cancer. In: Cancer Stem Cells in Solid Tumors. AL. Allan (ed), Springer Science & Business Media, pp 15-36, 2011.
6. Ricardo S, Vieira A-F, Gerhard R et al. Breast cancer stem cells markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype. J Clin Pathol 64: 937-946, 2011.
7. Kaur P, Nagaraja GM, Zheng H et al. A mouse model for triple-negative breast cancer tumor-initiating cells (TNBC-TICs) exhibits similar aggressive phenotype to the human disease. BMC Cancer 12: 120-112, 2012.
8. Ossovskaya V, Wang Y, Budoff A et al. Exploring molecular pathways of triple-negative breast cancer. Gene & Cancer 2: 870-879, 2011.
9. Gelmon K, Dent R, Mackey JR et al. Targeting triple-negative breast cancer: optimising therapeutic outcomes. Ann Oncol 1-12, 2012.
10. Brouckaert O, Wildiers H, Floris G, Neven P. Update on triple-negative breast cancer: prognosis and management strategies. Int J Womens'Health 4: 511-520, 2012.

10.40-11.10 ***Coffee break***

11.10-11.30 Walter Bodmer, Weatherall Institute of Molecular Medicine and Department of Oncology, Oxford University

Colorectal cancer stem cells and their differentiation in cell lines and primary cultures

Single cancer stem cells (CSCs) from colorectal cancer (CRC) derived cell lines can be functionally identified by their ability to form large lumen containing colonies in 3D Matrigel. These colonies contain the three types of differentiated colorectal epithelial cells, and single cells from them can reproduce themselves and form tumours efficiently in immuno-deprived mice. Lumens can be robustly identified by F-actin staining and express many characteristics associated with normal differentiated intestinal epithelium, including brush border enzymes, polarisation, and tight-junctions. The inhibiting effects of hypoxia on lumen formation and stem cell differentiation can be mimicked by prolyl-hydroxylase inhibition, which activates Hif1, suggesting that Hif1 is a major mediator of this inhibition. The cell line derived lumens are phenotypically more or less indistinguishable from colorectal tumour glandular structures used by pathologists to grade tumour differentiation. Parallel results to those obtained with long established cell lines are seen with primary cultures from fresh tumours. This in vitro approach to the functional characterization of CSCs and their differentiation refines the understanding of tumour differentiation, and facilitates the study of CRC stem cell differentiation and drug responses of the CSCs. It also enables high throughput screening for novel agents that induce differentiation and so tumour inhibition.

11.30-11.50 James West, Ph.D., CytomX Therapeutics, Inc., USA

The Probody™ Platform Enables Tumor Targeted Therapies

Antibodies have demonstrated therapeutic benefit in treating human disease through binding to specific target antigens in disease tissues. However, many potential disease targets are found in normal as well as diseased tissues, and the action of the antibody in normal tissues can result in toxicities that limit or preclude therapeutic utility of the antibody. We have engineered a novel proteolytically activated antibody scaffold, the Probody™ platform, designed to restrict antibody activity to the tumor. Probodies are comprised of a masking peptide that inhibits the activity of the antibody in healthy tissues, linked to the antibody through a protease sensitive linker engineered for activation by specific proteases upregulated in the tumor environment. When the Probody enters the tumor, the linker is cleaved resulting in the dissociation of the masking peptide, thereby releasing an active form of the antibody.

Although the anti-EGF antibody Cetuximab is approved for clinical use, anti-EGFR activity in the skin can limit its utility. We created a series of Probodies, based on Cetuximab, and have shown that the EGFR Probody™ therapeutic is stable and remains predominantly in an inactive form in healthy animals. We also show that the Probody is activated in xenograft tumors in mice, leading to accumulation and anti-tumor efficacy. Additionally, we have shown that the EGFR Probody is stable, has prolonged pharmacologic half-life, and has reduced dermatologic toxicity, as compared to cetuximab, in non-human primates. Finally we show that the anti-EGFR Probody is activated in preparations of primary tumors from both xenografts and human patients. These results show that an EGFR Probody can safely target EGFR expressing tumors and suggest that Probodies will be useful for targeting a wide variety of antigens for which conventional antibodies are not viable.

11.50-12.10 Jason Sagert, CytomX Therapeutics, South San Francisco, USA

An Anti-Jagged Probody Enables Tumor-specific Inhibition of Jagged-Dependent Notch Signaling

The Notch pathway has been the target of extensive therapeutic development, and monoclonal antibodies to Notch receptors and the DLL4 ligand have shown great promise for the treatment of neoplastic malignancies in preclinical models. However, the clinical benefits have been limited by toxicities associated with systemic Notch pathway inhibition. To restrict antibody activity to the tumor, we have developed the Probody™ platform. Probodies incorporate a peptide mask recombinantly linked to the antibody through a protease-sensitive linker. The linker is cleaved by specific proteases upregulated in the tumor environment where Probodies are activated and bind to their target. This approach results in tumor specific inhibition of the antibody target with reduced systemic toxicities.

CytomX is developing a pipeline of Probodies to targets within the Notch pathway and preclinical proof of concept for CytomX's anti-Jagged 1/2 Probody will be presented. Further, we show that human tumor samples are capable of activating anti-Jagged Probodies. These data illustrate the potential of the Probody platform to enable the drugging of the Notch pathway and a broader range of targets that have been avoided to date due to systemic toxicities.

12.10-12.30 Lioudmila Tchistiakova, PhD Senior Director, Global Biotherapeutic Technologies, Pfizer BioTx, 87 Cambridge Park Drive,Cambridge, USA

Development of Antibody-Drug-Conjugate that targets 5T4, an oncofetal antigen expressed on tumor-initiating cells

Antibody-drug conjugates (ADC) represent a promising therapeutic modality for the clinical management of cancer. We sought to develop a novel ADC that targets 5T4, an oncofetal antigen expressed on tumor-initiating cells (TIC), which comprise the most aggressive cell population in the tumor. Our strategy was to find synergy between the choice of targeting antibody, linker, payload and conjugation method in order to obtain a potent anti-5T4 ADC with a large therapeutic window. The anti-5T4 ADC, A1mcMMAF exhibited potent in vivo antitumor activity in a variety of tumor models and induced long-term regressions for up to 100 days after the last dose. Strikingly, animals showed pathologic complete response in each model with doses as low as 3 mg antibody/kg dosed every 4 days. In a non-small cell lung cancer patient-derived xenograft model, in which 5T4 is preferentially expressed on the less differentiated tumor cells, A1mcMMAF treatment resulted in sustained tumor regressions and reduced TIC frequency. These results highlight the potential of ADCs that target the most aggressive cell populations within tumors, such as TICs. The preclinical efficacy and safety data established a promising therapeutic index that supports clinical testing of A1mcMMAF.

12.30-12.50 Aleksandra Filipovic, Mahendra Deonarain, Ylenia Lombardo, Monica Faronato and RC Coombes. Imperial College London, UK

Antinicastrin antibody for the treatment of breast cancer

Nicastrin is a member of the gamma secretase enzyme complex and a therapeutic target on its own merit in breast cancer patients. We have previously shown that Nicastrin is overexpressed in breast cancer tissue where it confers worse overall survival in estrogen receptor negative the breast cancer cohort. Furthermore, nicastrin gene is amplified in a large proportion of basal like breast cancers. We have now developed, purified and characterised anti-nicastrin monoclonal antibodies and implemented them for treatment of invasive breast cancer *in vitro* and *in vivo*. Our study demonstrates that anti-nicastrin monoclonal antibodies inhibit proliferation, alter the morphology of 3D acini of invasive breast cancer cells to compact and small structures, inhibit invasion (> 60%) in transwell assays, inhibit mammosphere forming efficacy of breast cancer stem cells and impinge on Notch, as well as RhoGTPases and Akt pathways. BIACore and peptide based epitope mapping were used to elucidate binding epitopes of our lead antibody candidates. Importantly, using MDA-MB-231 Luciferase labeled cells we conducted an orthotopic breast cancer model, as well as a metastatic model by tail vein injection of cancer cells in BalbC nude mice. The 2H6 antibody candidate was potent to induce a 50% tumour growth reduction in the orthotopic model, while it conferred > 80% inhibition of secondary deposits in the metastatic model. In the same model, the gamma secretase inhibitor RO4929097 had negligible effects. We did not observe any treatment related toxicities, while we reproduced the *in vitro* inhibitory effects of the anti-nicastrin McAbs on pro-invasive molecules in tumours. We conclude that anti-nicastrin antibodies are valid novel therapeutics for the treatment of invasive breast cancer.

12.50 Conference Closing

POSTER PRESENTATIONS

These will be displayed in the Hall area during the whole period of the conference

- **Construction and Validation of Mouse Monoclonal Antibody and Single Chain Variable Fragment Antibody (scFv) against Canine CD20 for Antibody therapeutics**

Saurabh Jain, Jianguo Shi, Erin Worrall, Ted Hupp
Edinburgh Cancer Research Centre
University of Edinburgh
Western General Hospital
Crewe Road South
Edinburgh EH4 2XR

Naturally occurring tumours in dogs have become an important model for studying human cancer and therapeutics as dogs share about 73% genetic similarity with humans. The most important factor from a diagnostic and therapeutic point of view is to identify an appropriate molecular target. CD20 is a tetraspanning transmembrane phosphoprotein which is expressed in pre-B cells but the precise function of CD20 is still unknown. The characteristics that make CD20 a good target antigen is that it is over expressed in more than 90% of lymphomas and its natural ligand has not been defined. The approved monoclonal antibodies against this target such as Rituximab bind to human CD20 but not to canine CD20 due to a single amino acid change in the discontinuous epitope of the extracellular loop. Thus the need for monoclonal antibody targeting canine CD20 still remains.
Here we developed a mouse monoclonal antibody using standard hybridoma technology as well as constructed single chain antibody using phage display against canine CD20. Initially, the mouse was subjected to various rounds of immunization with canine CD20 and the antibody response was determined at various time points by ELISA using serum obtained from tail bleed.

Single chain variable fragment (scfv) construction: cDNA was used to amplify variable heavy and light chains via Polymerase chain reaction. These heavy and light chains were combined to make single chain (scFv) fragment of an approximate length of 800bp. The purified scFv was cloned into pCOMB3xSS and transformed into electro competent cells via electroporation. This library was subjected to process of bio-panning. The phage pools from round 3 and 4 were tittered plates. The active clones were grown again and the activity was compared between secreted antibody and antibody linked to phage. These clones were then cloned into pCDNA3.1 His and expressed in mammalian cells CHO cells. The activity of these active clones was tested using ELISA as well as western blot. Subsequently, PCR was carried out using the hybridoma cell's RNA to show that these two DNA from the same hybridoma cell code for two different sequence.

Comparison between mouse anti-canine monoclonal antibody and Rituximab: The mouse anti-canine antibody was compared to the FDA approved chimeric antibody Rituximab. Here we show that the rituximab binds to human CD20 but not to canine CD20 most likely due to an amino acid change in the extracellular loop, whereas the anti-canine monoclonal antibody binds to both human and canine CD20 protein. Another interesting aspect is that Rituximab only binds to the whole protein whereas anti-canine monoclonal binds to different peptides as well which were derived from the extracellular loop. When both antibodies were tested for binding activity onto the CD20 expressing cell lines (raji cells), Rituximab binds well as compared to anti-canine mouse monoclonal antibody.

Conclusion: The anti-canine monoclonal antibody binds better than Rituximab to human and canine CD20 epitope, whereas Rituximab binds better to the CD20 expressing human cell line. PCR using two different scFv set of primers shows single hybridoma cell can code for two different messages.

- **Future work:** Measuring the activity of mouse anti-canine antibody in different cancer cell lines and also determining the activity of two active scfv in comparison to mouse monoclonal antibody