31st International Conference

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

Santa Marina Hotel, Mykonos, Greece

23-25, June 2014
In Memory of Ferdinand Bach, Ph.D, 1951 – 2014

It is with deep regret that we heard the sad news of the passing of Ferdinand Bach

We dedicate this Conference to Ferdinand Bach

Ten years’ experience of Nimotuzumab in the treatment of high, malignant glioma in children, adolescents and adults -basis and outcome of four studies.

Final results of a double blind, placebo controlled phase IIb/IIIA study in advanced and/or metastatic pancreatic cancer – Gemcitabine + Placebo vs. Gemcitabine + Nimotuzumab

WE ARE GRATEFUL TO OUR SPONSORS FOR THEIR SUPPORT
SESSION 1 Chairman: Peter Hudson

9.45-10.15 Klaus Bosslet, Head Discovery Oncology Penzberg Pharmaceutical Research & Early Development (pRED), Roche Innovation Center Penzberg, Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg / Germany

Mode of action and clinical efficacy signals of the pRED early clinical development compounds

The clinical study results published by Stephen Hody et al. in 2010 (N Engl J Med 2010;363: 711-23.), with regard to the experimental drug Ipilimumab, opened the new area of successful cancer immuno-therapy. For the first time, overall survival of metastatic melanoma patients was significantly prolonged. Most interestingly, the Kaplan-Meier curve showed a tailing which indicates a certain percentage (10-30 percent) of potential long term survivors. The unique mode of action of Ipilimumab differentiates it from other targeted cancer therapies: CTLA4, the target structure inhibited by Ipilimumab, is expressed on T-cells as well as other immune cells, and its inhibition results in a T cell response directed towards tumor cells. (Lifting the immuno-suppressive brake).

Unfortunately, the immune-activation observed is frequently also directed towards certain normal tissues, resulting in severe immune related adverse events. Furthermore, combination studies with other immune check point inhibitors such as anti PD1 (Nivolumab, BMS) show additive efficacy signals in the clinic. For the time being, it seems that a few of the other check point inhibitors currently under development (anti PD1, Nivolumab, BMS or MK 3475, Merck, or anti PDL1, MPDL3280A Roche or MEDI 4736, MedImmune both inhibiting the PD1 Ligands on the tumor cell) can be more efficacious and better tolerated than Ipilimumab, the frontrunner molecule of this promising new class of therapeutics.
The exciting clinical results observed from our own pRED candidates RG7155 (anti CSF1R MAb), RG7116 (anti Her3 MAb) and RG 7221 (anti Ang2-VEGF) will also be presented and discussed in the context of their novel mode of actions and their potential contributions to the fast moving cancer therapy landscape.

Special emphasis will be devoted to RG7155 and its unique capability to eliminate immune suppressive M2 macrophages from PVNS patients’ joints and the tumor micro-environment of various solid tumors, suggesting great potential for combinations with other cancer therapeutics of synergistic modes of action.

Acknowledgements:

anti Ang2-VEGF bsAB:
Markus Thomas/Joachim Mueller, Oliver Krieter, Katharina Lechner, Kate Munro and team

anti Her3 MAb:
Birgit Bossenmaier, Martin Weisser, Maurizio Ceppi, Max Hasmann and team

anti CSF1R MAb:
Carola Ries, Dominik Ruettinger, Michael Cannarile, Monika Baehner and team

10.15-10.45 David J. King, AnaptysBio, 10421 Pacific Center Court, San Diego, California 92121, USA

Antibody Therapeutics with Multiple Mechanisms of Anti-Tumor Activity Generated by Somatic Hypermutation in vitro

Antibodies of interest for tumor therapy can encompass many potential mechanisms of action, including direct cell signaling, targeting of toxic moieties, for example using ADCs, and activation of host tumor immunity through inhibition of immune checkpoints. A novel approach to generate human antibodies of interest in all of these potential applications has been developed in which key features of the adaptive immune system, including somatic hypermutation (SHM) are replicated in vitro. This allows the generation of fully human antibodies under controlled conditions in which antibodies may be selected and evolved to meet
specific design goals. This can include specific functional activities, as well as meeting stringent criteria for development. Among the most promising approaches in the treatment of a number of different tumor types is the activation of anti-tumor immunity by blockade of immune checkpoints. These inhibitory immune checkpoints are crucial for maintaining self-tolerance in the normal immune system but can be co-opted in cancer to allow disease to escape from immune surveillance. Therapeutic validation has been provided using molecules generated to inhibit the CTLA-4 and PD-1 signaling pathways, which have shown significant clinical activity, both alone and in combination. A number of other immune checkpoints are of interest, and blockade of the T-cell inhibitory signaling molecules TIM-3 and LAG-3 has been shown to be effective in mouse models, especially in combination with blocking PD-1 signaling. Potential therapeutic molecules that target PD-1, TIM-3 or LAG-3 have been generated and inhibition of each pathway demonstrates activity. Assays have been tested and developed to identify assays in which multiple checkpoint inhibition can be assessed simultaneously. Combination of an anti LAG-3 or an anti TIM-3 antibody with an anti-PD-1 antibody can increase T cell activation over that seen with blockade of a single checkpoint alone and has the potential to lead to increased clinical efficacy.

Discoidin domain receptor 1 (DDR1) is a nonintegrin tyrosine kinase receptor for collagen implicated in diverse cellular roles, including cell adhesion, proliferation, and extracellular matrix remodeling. Multiple tumor types have been shown to overexpress DDR1, and a potential role for DDR1 in tumor pathology has been suggested. Inhibition of DDR1 signaling may therefore represent a novel therapeutic target for these cancers. High affinity anti- DDR1 antibodies have been generated with potent activity in inhibition of DDR1 signaling in multiple assays. These mAbs are being further explored for their potential for therapeutic efficacy in tumor types where DDR1 signaling plays a role in disease progression.

10.45-11.30 Coffee Break
SESSION 2 Chairman: David King

11.30-12.00  Ivan D Horak, MD, FACP, Chief Scientific and Medical Officer, Symphogen, Elektrovej Building 375, Lyngby, Denmark.

Antibody mixtures to target tumour 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.

12.00-12.30  Cecile Geuijen, Merus BV, Utrecht, the Netherlands

The use of bispecific antibodies to overcome resistance

The amplification and dimerization of HER2 promotes growth and survival of malignant cells. Tumor responses to available therapeutic agents targeting HER2 are variable. Re-activation of the potent HER2:HER3 signaling dimer by up-regulation of the HER3 ligand heregulin (HRG) has recently been identified as an important resistance mechanism. Treatment of patients with tumors expressing HER2 could be improved using agents that specifically target and potently inhibit the HER2:HER3 heterodimer.

Merus has developed and validated a powerful discovery engine for the discovery of potent and fully human bispecific antibodies based on common light chains (cLC) and CH3 engineering: Biclonics™. The cLC, selected for stability and high frequency in natural repertoires, facilitates rapid and comprehensive mining of antibody repertoires resulting in large and diverse antibody panels. Upon
transfection of cells with constructs encoding 2 different cLC mAbs and CH3-engineered Fc regions, pure bispecific antibody batches are obtained allowing direct screening of functional activity in the therapeutic format.

Using Merus’ proprietary single light chain discovery platform, a highly potent Biclonics™ targeting HER2 and HER3 was selected from a panel of > 500 bispecific antibody candidates. The lead Biclonics™ MCLA-128 blocks HRG mediated growth and shows higher potency compared to benchmark HER2/HER3 targeting agents. Finally, MCLA-128 shows potent activity in a trastuzumab resistant breast cancer xenograft model.

12.30-1.00 Claudio Sustmann, PhD, Senior Scientist Discovery Oncology, Pharmaceutical Research and Early Development (pRED), Roche Innovation Center Penzberg, Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg / Germany

Multivalent Antibody Formats for Cancer Therapy and Drug Development

Inhibition of oncogenic receptor tyrosine kinases with therapeutic antibodies is a proven and successful means of controlling tumor growth. At Roche we have a long history in targeting the epidermal growth factor family of receptors. Understanding receptor biology, interplay with other growth factor receptors and means to address these cross-talks with multivalent and/or multi-specific antibodies is one focus of our research activities.

Inhibition of receptor crosstalk with bispecific, tri- or even tetraspecific antibodies is an interesting avenue to address growth-factor receptor mediated tumor escape mechanisms. The potential and limitations of such approaches will be exemplarily addressed for antibodies simultaneously targeting ErbB-receptors, c-MET or IGFR. The potency of such antibodies on cells and strategies to select and develop them will be discussed. Limiting the focus on the hepatocyte growth factor receptor c-Met, the influence of binding geometries on inhibitory properties of a drug candidate will be highlighted. In a third part, the potential of bispecific-hapten binding antibodies for drug discovery approaches and therapeutic application will be introduced.

1.00-2.30 Lunch break
2.30-3.30 Open Air Workshop:  
Cancer Immunotherapy - Klaus Bosslet / David King

Session 3  Chairperson:  Cecile Geuijen

3.30–4.00    Surinder K Sharma,   ADEPT & Translational Therapeutics Group, Res Dept of Oncology, UCL Cancer Institute, University College London, UK

Antibody directed enzyme prodrug and combination therapy

Preclinical and clinical studies indicate that repeated ADEPT cycles are necessary for sustained tumour regression. We have studied the potential of PARP inhibitors to enhance the effect of each cycle of ADEPT. Our results indicate that PARP inhibition may be a useful combination therapy with ADEPT in the treatment of colon and pancreatic cancer.

4.00-4.30    ¹KD Bagshawe and ²S K Sharma  
¹Department of Medical Oncology, Imperial College London, Charing Cross Campus, London, UK  
²Research Department of Oncology, UCL Cancer Institute, University College London, UK

Suppressing the humoral immune response to a potent bacterial antigen

Bacterial enzymes have the potential value in the treatment of various cancers. However, they have been restricted by their immunogenicity in humans. We show that a widely used cytotoxic agent suppresses the humoral response to carboxypeptidase G2 in mice. This may allow repeated cycles of therapy to be given provided the human immune response is similarly disrupted by the drug.
A platform technology for glyco-optimization and high yield production of antibodies of various isotypes for tumor therapy

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 platform technology comprising a set of glycol-engineered human cell lines for the high yield production of fully human glycoproteins. Expression of antibodies and non-antibody glycoproteins result in improved biotherapeutics with respect to clinical efficacy and side effects. The system is biotechnologically superior in quality, reproducibility and yield compared to other including conventional production systems.

In order to produce antibodies of different isotypes for the potential improvement of tumor therapy we implemented vector systems for the overexpression of IgG1 as well as IgM, IgA1/2, IgE and others with different specificities. A robust, reproducible and high titer production of these antibody isoforms in GlycoExpress cells was achieved with the products optimized with respect to fully human glycosylation.

As an example GatoMab is developed as a humanized and fully human-like glycosylated antibody of the IgM isotype recognizing the tumor-specific Thomsen-Friedenreich (TF) carbohydrate tumor antigen. TF is a highly specific pancarcinoma antigen also present on various leukemia and cancer initiating tumor stem cells and is directly involved in metastasis formation. The multivalent IgM molecule is an attractive format for carbohydrate-specific antibodies. For GatoMab different modes of action have been observed against leukemic tumor cells including complement-dependent cytotoxicity (CDC) and direct cytotoxicity by induction of membrane lesions. Based on these findings GatoMab is a good candidate for treatment of leukemias as well as neoadjuvant therapy for the killing of disseminated tumor cells and prevention of metastasis. Results from preclinical testings will be presented.

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Molecular Pathology of Colorectal cancer
Colorectal cancer (CRC) is one of the most common cancer in economically developed countries and it is the second cause of death after lung cancer. Sporadic disease accounts for approximately 70% of CRC cases and fewer than 10% of patients have an inherited predisposition to CRC.
For years, a lot of studies have tried to identify the precursor lesions of CRC which has led to the concept that CRC develop via a variety of histologically distinct steps. Until recently, conventional adenomas of the colon were considered as precursor lesions of almost all sporadic CRC. Over the last few years, it has been demonstrated that a distinct group of colorectal lesions, called serrated lesions, are clonal epithelial proliferations with underlying genetic alterations and has been regarded as precursor lesions for a subset of CRC.
The adenoma-carcinoma sequence pathway
The adenoma-carcinoma sequence, is a term that describes the stepwise progression from normal to dysplastic epithelium to carcinoma associated with the accumulation of multiple genetic and epigenetic events. Some observations supporting the concept that CRC develop from adenomas include: similar anatomical distribution of both lesions, coexistence of CRC with adenomas in the same lesion, higher incidence of adenomas in colons containing carcinomas, reduction of CRC frequency after removal of adenomas, absence of carcinoma in situ outside the area of adenoma and similar molecular and genetic alterations in both lesions.
Although every adenoma has the capacity of malignant evolution only a small minority of them develop invasive cancer. Most adenomas stabilize their progression or regress. Features that are considered as risk factors for the malignant evolution of adenomas are their size, the growth pattern and the grade of dysplasia. Adenomas less than 1 cm in size have a very low (<5%) risk for cancer
development while larger adenomas have a greater tendency to progress to cancer. In adenomas with a villous architectural configuration the cancer risk rises to 17% and adenomas with high grade dysplasia have approximately 40% risk of cancer development. The average time for malignant transformation of adenomas is not exactly known, however it has been reported a range of median time from 7 to 11 in adenomas with high grade and low grade dysplasia, respectively.

Many years before, Fearon and Volgestein proposed the molecular basis for colorectal carcinogenesis in the adenoma-carcinoma sequence model and described an accumulation of genetic events each conferring a selective growth advantage to an affected colon cell. According to their descriptions mutational activation of oncogenes and mutational inactivation of tumor suppressor genes leads to the development of colorectal tumors. Mutations in the adenomatous polyposis coli (APC) tumor suppressor gene represents a crucial event in colorectal carcinogenesis. They occur early in adenoma-carcinoma sequence and several data support the role of the APC as a “gatekeeper” in cancer development. APC mutations has been found in 20-82% of adenomas and in 52-60% of CRC, suggesting that these mutations occur early in colorectal cancer development. Moreover, mutated APC has been demonstrated frequently in small adenomas compared to large adenomas, supporting the notion that such mutations are involved early in the adenoma-carcinoma sequence. KRAS mutations occur in 35-42% of CRC, 50% of large adenomas (>1 cm) and only in 9% of small adenomas (<1 cm). These data suggest that KRAS mutations occur early in the adenoma-carcinoma sequence, but are unlikely to be an initiating factor in colorectal carcinogenesis. Our experience in tissue sections from colorectal tumors showed that KRAS immunohistochemical expression was higher in adenomas than in carcinomas and in adenomas was increased according to the degree of dysplasia. Mutations at the “deleted in colorectal carcinoma” (DCC) gene represent the third step in the genetic pathway of Fearon and Vogelstein. The DCC is located in 18q region and loss of 18q has been observed in 10-30% of early adenomas and in 50-60% of late adenomas. Ther tumor suppressor genes in 18q region have been identified, such as SMAD2 and SMAD4 genes. Mutations of SMAD2 and SMAD4 genes have been reported in 25-30% of CRC. Alterations of p53 gene have been reported in 4-25% of adenomas, 50% of invasive foci within adenomas and in 50-75% of CRC. Functional inactivation of p53 protein is associated with the transition from adenoma to carcinoma. P53 protein overexpression has been found to be rare in adenomas with low dysplasia, frequent in adenomas with high dysplasia and more frequent in CRC.
The serrated neoplasia pathway
The serrated neoplasia pathway has been emerged recently as a distinct and alternative pathway to the traditional adenoma-carcinoma pathway. There is some evidence that the neoplastic progression within this pathway is faster than within the adenoma-carcinoma pathway. Some new colorectal precursor lesions have been recognized that may lead to a group of CRC characterized by morphological and molecular features distinct from conventional CRC. The serrated pathway consists of serrated polyps and its end point is a malignant lesion, the serrated adenocarcinoma. Serrated polyps form a heterogeneous group of mucosal lesions that includes non-dysplastic polyps, such as hyperplastic polyps and sessile serrated adenomas, and polyps that show overt cytologic dysplasia, namely serrated adenomas and mixed polyps. The morphological criteria for the diagnosis of serrated polyps are enough inconsistent and many of these lesions are misdiagnosed. Reproducible histopathological criteria have been recently proposed.

The earliest genetic alterations in serrated lesions are BRAF and KRAS gene mutations. BRAF mutations were found to be more common in sessile serrated adenomas (75-80%) and mixed polyps (40-90%) compared to hyperplastic polyps (19-36%) and serrated adenomas (20-33%). Moreover, studies have revealed mutations of BRAF gene in 43-76% of sporadic CRC. KRAS mutations were found in approximately 80% of serrated adenomas and less frequently in sessile serrated adenomas (7%) and in hyperplastic polyps (4-37%). Increased methylation is an early phenomenon in the serrated pathway. Promoter methylation is an important mechanism of gene silencing in carcinogenesis. GpG-island-methylation-phenotype (CIMP) describes an epigenetic methylation of GpG islands in promoter regions of the genome, that silences the transcription of genes. Depending on the number of methylated genes, CIMP has been distinguished into high (CIMP-H) and low (CIMP-L). CIMP-H has been detected in hyperplastic polyps and even in the normal mucosa of the proximal colon from patients with hyperplastic polyposis and in the mucosa from patients with CRC.

Microsatellite instability (MSI), due to DNA methylation, was first described in association with hereditary non-polyposis CRC (HNPCC), where germ-line mutations of mismatch repair genes (MMR) result in high frequency MSI (MSI-H) in over 90% of the cases. Sporadic CRC with MSI-H are developed as result of MMR gene MLH1 transcriptional inactivation through acquired promoter methylation. MLH1 methylation has been detected in 30% of hyperplastic polyps, 70% of sessile serrated adenomas and in 86% of sporadic MSI-H CRC. Low levels of MSI (MSI-L) is
associated with MMR gene O-6-Methylguanine DNA Metrhyltransferase (MGMT). MGMT methylation has been found in 22% of hyperplastic polyps, 25% of sessile serrated adenomas, 16-22% of serrated adenomas and in 50% of serrated adenocarcinomas.

Conclusion
Triggered by morphologic observations, molecular studies now provide evidence that, except the long held paradigm of the traditional adenoma-carcinoma sequence, an alternative pathway is responsible for colorectal carcinogenesis, the so called serrated neoplasia pathway. Serrated precursor lesions of CRC are not so homogeneous lesions and pathologists do not have relatively uniform criteria for their recognition. Further morphological and molecular determination of features is needed in order to obtain the best management strategies.

Selected References

9.30– 0.00 Michael Powell Ph D, Chief Scientific Officer DiaCarta, Inc. 3535 Breakwater Avenue Hayward, CA 94545


ABSTRACT NOT RECEIVED

10.00-10.30 Gordon Chiu, Grafoid Inc., 912-130 Albert St. Ottawa, Ontario, K1P 5G4

MesografTM: standardizing a scalable graphene from raw graphite ore

The greatest challenge in commercializing graphene for medical and other applications is how to produce the highest quality material, on a large scale at low cost, in a reproducible manner. Grafoid’s investment in a now patented production process for producing MesoGraf™ graphene directly from graphite ore overcome limitations by achieving those standards of quality, scalability, affordability on an environmentally sustainable basis.

10.30–11.30 Coffee Break
RG7787 a re-engineered cytolytic Fusion Protein (cFP) based on a de-immunized variant of Pseudomonas exotoxin with better tolerability and potent in-vivo efficacy

Development of immunotoxins into actual drugs has been greatly hampered by their immunogenicity and off-target toxicity. Particularly vascular leak syndrome is an adverse effect that is clinically difficult to manage. In collaboration with Ira Pastan’s lab from the NIH we have developed a new cFP format that is de-immunized, has much reduced off-target toxicity, and yet retains good in-vitro potency that translates into high therapeutic efficacy in multiple xenograft models.

The targeting moiety derived from the SS1 anti-mesothelin antibody has been fully humanized to avoid immunogenicity. In order to de-immunize PE, point mutations have been introduced into domain III and the entire domain II has been deleted reducing the size of the effector moiety to approximately 24 kD. Similar de-immunized and truncated variants of PE24 fused in the classical format with a disulfide-stabilized Fv fragment have previously been shown by the Pastan lab to be much better tolerated in rodents. However it could not be ruled out that this, at least in part, is attributable to their much reduced serum half-life and exposure. In order to restore PK parameters similar to those of SS1P, a classical SS1 dsFv PE38 fusion format, we substituted the mouse dsFv moiety by a humanized Fab fragment. We show that RG7787, unlike a dsFv PE24 fusion protein, has indeed comparable PK properties to SS1P.

Cell viability assays demonstrate that RG7787 has similar cytotoxic potency as SS1P on different cell lines. In mice RG7787 is more than 10 fold better tolerated than SS1P and dose-response studies show that maximum therapeutic efficacy is reached between 2-3 mg/kg. RG7787 achieves potent tumor growth inhibition and even tumor regressions in several xenograft models. We also observed clearly
synergistic efficacy with Taxol treatment in different tumor models making this a promising combination for clinical trials.

12.00-12.30 Peter J. Hudson (Director, Victorian Cancer Biologics: CSO, Avipep Pty Ltd and Co-Chair of HUPO-HAI Human Proteomics Antibody Initiative), VCB and Avipep Pty Ltd, 343 Royal Parade, Parkville, Victoria 3052, Australia

Engineered Antibody Fragments with efficient payloads for cancer diagnosis and ADC/RIT therapy

Antibody-drug-conjugates (ADCs) are rapidly expanding the biopharmaceutical market in oncology therapy. Avipep has designed and produced Antibody fragments (diabodies and Avibodies™) with unique surface disulphides for precise loading of either cytotoxic drug payloads (ADC-therapy) or radionuclides for PET-imaging (or RIT-therapy). With PEGylation, Tag72-targeting diabodies demonstrated remarkable xenograft-tumour uptake >70% ID/gm over 24-48hrs with fast blood clearance and low kidney uptake (<10% ID/gm). GMP-manufacture has exceeded 1gm/litre in bacterial fermentation and the product is stable for over 24 months. A first-in-man Phase-1 Biodistribution (PET imaging) trial in prostate/ovarian cancer was completed in May 2014, and demonstrated that the PEG-diabody format will deliver high tumour loads in man, with no specific uptake in normal tissues, including kidney and liver. Proprietary modifications enabled precise, site-specific loading of PEG, drug and isotope payloads onto surface disulphides and preclinical xenograft evaluation with the latest ADC/RIT therapeutic formulations will also be reported.
High turnover of Tissue Factor enables efficient intracellular delivery of antibody-drug conjugates

Therapeutic antibodies are currently used in the clinic to treat a variety of malignancies including cancer. The tumor-killing capacity of therapeutic antibodies can be greatly enhanced by conjugation with cytostatic toxins, this way combining antibody-mediated tumor targeting with the potent cytotoxic activity of toxins. This was also demonstrated through the FDA approval of brentuximab vedotin and transtuzumab emtansine. The number of antibody drug conjugates (ADCs) in clinical development has markedly increased in the last couple of years. This includes the development of a novel ADC designed to deliver the cytotoxic payload monomethyl auristatin E (MMAE) to tumor cells expressing the coagulation factor; tissue factor (TF). By carefully selecting a TF-specific antibody that interferes with TF:FVIIa-dependent intracellular signaling, but not with the pro-coagulant activity of TF, an ADC was developed (TF-011-MMAE) that efficiently kills tumor cells, with an acceptable toxicology profile.

To gain more insight in the efficacy of TF-directed ADC treatment, we compared the target characteristics of TF with the human epidermal growth factor receptors (HER) 1 and 2. Both in absence and presence of antibody, TF demonstrated more efficient internalization, lysosomal targeting and degradation than HER1 and HER2. By conjugating the toxin duostatin-3 to TF, HER1 and HER2 mAbs, we were able to compare cytotoxicity of ADCs with different tumor specificities. TF-ADC demonstrated effective killing against a variety of tumor cell lines with assorted target expression. This was confirmed in xenograft models where HER1- and HER2-ADCs induced significant inhibition of tumor growth. However TF-ADC induced even stronger inhibition of tumor growth, despite the lower expression of TF in these models. We hypothesize that the high turnover of TF on tumor cells, inherent to its biological role, makes this protein specifically suitable for an ADC approach.
1.00–2.30 Lunch

2.30-3.30 Open Air Workshop:
   Antibody Drug Conjugates - Peter Hudson / Yijie Gao

Session 6 Chairman : Gregory Sivolapenko

3.30-4.00  Steffen Goletz, CEO / CSO, Glycotope GmbH, Berlin

Using GlycoExpress™ to produce glycooptimized superior antibodies for cancer treatment

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.
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.

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4.00–4.30 Katherine A. Vallis, Neel Patel, Sarah Able, Fergus
Gleeson, Adrian L. Harris, CRUK/MRC Gray Institute for Radiation Oncology and
Biology, Oxford University, Oxford, UK

111In-Bevacizumab Imaging to Detect the In Vivo Response to Antiangiogenic
Therapy

Background: Vascular endothelial growth factor (VEGF) and associated receptors
play a pivotal role in tumor angiogenesis. The ability to image VEGF would enable
prospective, non-invasive determination of response to antiangiogenesis therapy.
Bevacizumab is a humanized monoclonal antibody that binds to VEGFA. The aim of
this study was to investigate the use of SPECT imaging with indium-labeled
bevacizumab as a predictive biomarker.

Methods: Bevacizumab was conjugated with benzyl-DTPA and radiolabeled with
111Indium (111In-BnDTPA-bevacizumab). Probe specificity was tested in vitro in
competitive inhibition assays and in vivo by comparing uptake in Balb/c nude mice
bearing tumours with variable VEGF expression with isotype-matched control
antibody (111In-bnDTPA-IgG) and an excess of cold bevacizumab. Intratumoral VEGF
was evaluated using ELISA and Western blot analysis. The effect of treatment on
tracer uptake was tested by administering rapamycin (20 mg/kg) to mice bearing
FaDu (squamous cell carcinoma) xenografts and compared to uptake of 111In-
bnDTPA-IgG. Uptake was measured using gamma counting of ex vivo tumours.
Mice received anti-CD31 antibody intravenously for vessel analysis using confocal microscopy.

**Results:** In inhibition assays the IC\textsubscript{50} values for \textsuperscript{111}In-BnDTPA-bevacizumab and unmodified antibody were similar (0.85 nM and 1.0 nM, respectively). Specific uptake of \textsuperscript{111}In-BnDTPA-bevacizumab in VEGF-expressing tumors was observed (%ID/g in FaDu xenografts: \textsuperscript{111}In-BnDTPA-bevacizumab =20.25; \textsuperscript{111}In-bnDTPA-IgG=3.13; \textsuperscript{111}In-BnDTPA-bevacizumab plus excess cold antibody=5.97). Rapamycin resulted in changes in tumor vascular morphology: relative vessel size increased (8.5 to 10.3, P=0.045) and mean relative vessel density decreased (0.27 to 0.22, P=0.002), and was associated with increased tumor uptake of \textsuperscript{111}In-BnDTPA-bevacizumab (70% increase) but not \textsuperscript{111}In-bnDTPA-IgG. Intratumoral VEGF increased post-treatment (from 283+/−56 to 419+/−47 pg/g of protein). Western blot analyses showed the increase in VEGF was due mainly to VEGF165.

**Conclusion:** \textsuperscript{111}In-BnDTPA-bevacizumab accumulates specifically in VEGF-expressing tumors and holds promise as an imaging tool for assessing response to therapy.

8.30 pm until late

*Conference Dinner*

**Wednesday 25\textsuperscript{th} June 2014**

**Cancer Stem Cells Symposium**

**Chairman: Agamemnon Epenetos**

9.30-10.00 Jon Aster, Boston USA

**Cancer Stem Cells**

It is now widely accepted that all cancers contain populations of cells with a seemingly unlimited capacity for self-renewal, and that elimination of these stem-like cells is essential for cancer to be cured. What remains uncertain is how prevalent or rare these cells are, whether they require specialized niches for preservation, and whether they have a strictly hierarchical relationship with other cells in individual cancers or instead can arise through “dedifferentiation” of other cancer cells in the population. This talk will focus on emerging data from animal models and human primary tumors that have begun to define the epigenetic state
that confers the property of “stemness” on tumor cells, and how such insights may help to develop rational anti-cancer stem cell therapies.

10.00 -10.30 Marianna Prokopi¹, Costas Pitsillides², Konstantinos K. Kapnisis², Andreas S. Anayiotos², Agamemnon A. Epenetos¹,³ and Christina A. Kousparou¹
1. Trojantec Ltd, The Bank of Cyprus Oncology Centre, 32 Acropoleos Avenue, 2006, Nicosia, Cyprus
2. Department of Mechanical Engineering and Materials Science & Engineering, Cyprus University of Technology, Limassol, 3036, Cyprus
3. Imperial College London, London, UK

Cancer Stem Cells, MicroRNAs & Therapeutic strategies including Stem Cell Microparticles

Introduction: In recent years, the cancer stem cell (CSC) model has been suggested to explain the functional heterogeneity and the carcinogenesis process of cancer. CSCs have the ability to initiate and sustain tumor growth, metastasis and resistance to therapy.

MicroRNAs (miRNAs) are small non-coding RNA molecules, which function in transcriptional and post-transcriptional regulation of gene expression. Aberrant expression of miRNAs has been implicated in numerous diseases including some if not all cancer types. Recent findings suggest that miRNAs could be involved in maintaining and regulating the stemness of CSCs. Therefore, miRNAs have been proposed as attractive targets for therapy and miRNA-related therapeutic strategies are under investigation. However, systemic delivery of miRNAs faces its own set of limitations because miRNAs may be degraded in the blood by enzymes such as RNases and excreted by the kidneys.

In this work we propose a novel system for therapeutic miRNA delivery applicable for both local and systemic administration with the use of umbilical cord mesenchymal stem cell (MSC) microparticles (MPs).

Methodology: MSCs were isolated from the Wharton’s jelly of human umbilical cords. The MSC cultures were subjected to stress conditions, leading to the formation of MPs (secreted membrane vehicles <1μm) which were harvested and characterized by SEM, PCR, FACS and Fluorescence Microscopy. Breast (MDA-MB-231 & MCF-7), colon (RKO & HT-29) and ovarian adenocarcinoma (SKOV3) cell lines
were then exposed to MPs. The response to treatment was evaluated by cell morphology, proliferation, migration, gene expression and apoptosis. Furthermore, the therapeutic potential of MPs was tested in vivo in xenograft tumor mouse models. Innovative imaging modalities such as in vivo flow cytometry and whole body fluorescence-bioluminescence were employed to dynamically investigate the biodistribution and homing kinetics of MPs in mice.

**Results:** *In vitro* experiments confirmed that MSC-derived MPs can be internalised by the various cancer cell lines and induce a biological effect as evidenced by membrane damage, cell shrinkage and blebbing in the recipient cell. Significantly, there was evidence that MPs induce apoptosis, inhibit cell proliferation and mediate tumor growth attenuation in a dose/time-dependent manner. The pro-apoptotic and anti-migrating effects of MPs in cancer cells were almost completely abrogated by RNase treatment before administration to cultures. Preliminary *in vivo* studies demonstrated that we were able to monitor and quantify fluorescently labelled MPs in circulation and to detect and image the biodistribution and incorporation in cells and organs in healthy and tumor-bearing mice.

**Conclusion:** MSC-derived MPs containing miRNAs possess tumor inhibitory properties both *in vitro* and *in vivo*. Administration of MPs after RNase treatment induces the loss of anti-cancer properties suggesting a horizontal transfer of small RNAs from MPs to cancer cells. MPs formulated to contain specific miRNAs, could affect the action of genes associated with carcinogenesis, neovascularization, metastasis and other cancer characteristics leading to therapeutic benefit.

10.30–11.30 Coffee Break
Discovery of a Notch1 inhibitory antibody with preferential activity against mutant receptor in T-ALL

The Notch1 receptor regulates cellular proliferation, differentiation, and survival through its intracellular domain, a transcriptional activator. Notch1 signaling plays essential roles in normal tissues processes such as cell fate specification, stem cell maintenance and angiogenesis. Compelling evidence has implicated Notch1 mutation and/or signaling dysregulation in both hematologic and solid tumors. We have developed a panel of potent and selective anti-Notch1 antibodies that inhibit ligand-dependent signaling by stabilizing the NRR domain in an autoinhibited state thus preventing proteolytic cleavage and release of the Notch1 intracellular domain. T-cell leukemias often harbor mutations within the NRR of Notch1 and potentially escape targeting with an anti-Notch1-NRR inhibitory antibody strategy. However, biochemical, structural and cell based analyses have indicated that our anti-Notch1-NRR antibodies are able to inhibit ligand-induced and constitutive signaling from wild-type as well as certain mutant Notch1 receptors. Moreover, one such antibody exhibited more potent inhibition activity against mutant Notch1 NRR in T-ALL with reduced activity against wild type Notch1. This differentiated inhibition profile was confirmed in in vivo efficacy studies. Co-crystallography and affinity analysis revealed potential insights into the mechanisms of this differentiated activity against wild type and mutant Notch1 receptors. This unique antibody activity profile may potentially provide a strategy to target mutant Notch1 in T cell leukemia with a better safety profile.
NOTCH-antibody drug conjugates have a different mechanism of action than NOTCH signaling inhibitors and induce tumor regression

There are four different NOTCH receptors in mammalian cells that have overlapping patterns of expression in embryonic and adult tissues, but fulfill non-redundant roles during hematopoietic stem cell specification, T cell development, intestinal crypt cell specification and vascular development. NOTCH receptors are over-expressed or amplified in certain human tumors and regulate cell proliferation, differentiation, and survival through an intracellular domain that functions as a transcriptional activator. Several strategies are in development to block NOTCH signaling for therapeutic purposes in cancer, including gamma-secretase inhibitors that block all NOTCH signaling and antibody-based targeting of individual receptors. However, blocking pathway activation with inhibitory antibodies has proven to be less efficacious than originally anticipated. We have generated antibody-drug conjugates (ADCs) that combine the specificity of high affinity anti-NOTCH antibodies with the cytotoxicity of microtubule inhibitors. These ADCs significantly enhance efficacy and also allow targeting in tumors that overexpress NOTCH but are not driven by its signaling. NOTCH-ADCs inhibited the in vitro growth of lung, breast and ovarian cancer cell lines in the low ng/ml range and in vivo regressed the growth of established human tumor xenografts. Our data demonstrate that NOTCH-ADCs are potent therapeutics capable of inducing sustained tumor regressions in pre-clinical models.
POSTER PRESENTATIONS

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

CLINICAL VALUE OF IMMUNOSCINTIGRAPHY IN THE RECTAL CARCINOMA SURGERY
M. Petrovic, V.Artiko, N.Petrovic, V.Obradovic
Clinical Center of Serbia, Faculty of Medicine University of Belgrade

Background: The aim of this study was to evaluate the clinical validity of immunoscintigraphy with radiolabeled monoclonal antibodies for the detection of metastases and recurrences of rectal carcinomas.

Methodology: Immunoscintigraphy was performed using 30-min infusion of IMACIS 1, a cocktail of 111 MBq 131I MAb 19-9 F(ab’)2 and MAb anti CEA F(ab’)2, in 75 patients. The main indication for examination was suspicious rectal cancer recurrence and/or metastases.

Results: Sensitivity of the method was 93.7%, specificity 82.3%, positive predictive value 91.5%, negative predictive value 86.0% and accuracy 89.6%. There was statistically significant relationship between immunoscintigraphy findings and rectoscopy findings ($r_s$=0.417, $p=0.013$), as well as significant relationship between immunoscintigraphy findings and US findings ($r_s$=0.336, $p=0.001$). Tumor marker levels were in positive correlation with findings of immunoscintigraphy ($r_s$=0.844, $p=0.001$), especially raised CEA level ($r_s$=0.817, $p=0.004$). Patients with higher CA19-9 level had higher Duke stage ($p=0.025$).

Conclusion: We can conclude that immunoscintigraphy is an useful method in the detection of metastases and recurrences of colon carcinomas, and has a potential use in radioimmunoguided surgery and radioimmunotherapy.