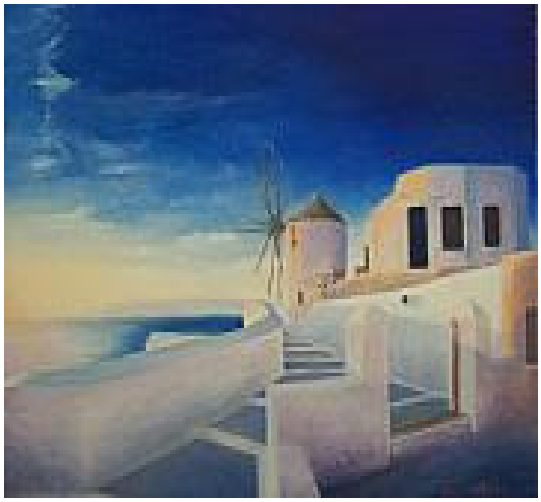
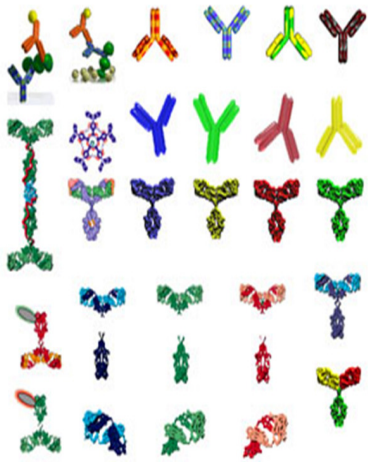
28th International Conference

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

Santa Marina Hotel, Mykonos, Greece

20- 22, June 2011





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PROGRAMME

Monday 20th June 2011

9.00- 9.45 ***Registration***

9.45- 9.55 Welcome Agamemnon Epenetos

SESSION 1 Chairman: Steven Rust

10.00- 10.30

The Application of Phage Display for the Identification of Novel Target Antigens in Cancer

Steven Rust, MedImmune, UK

Following the success of rituximab, trastuzumab and bevacizumab, antibody therapy is now established as a key treatment strategy in clinical oncology. These monoclonal antibodies (mAbs) play a significant role in modern cancer therapy and for mAbs to continue playing a significant role a number of challenges have to be addressed. One of these challenges is the continued identification of novel target antigens. To date, the majority of mAbs approved for clinical use or in clinical development target a relatively small number of targets; e.g. EpCAM, MUC1, EGFR, CD20, CEA and HER2.

The revolution in genomics and proteomics has led to the identification of some potentially novel antigens; however, more can be done. This presentation will introduce the use of phage display, of both antibody fragments and antibody mimetics, to identify new targets. Unlike other target identification techniques, the process also provides a tool reagent, which can be used for target characterisation and validation. The method comprises screening by phage display against primary cancer cells from NSCLC patients and subsequent functional assays on the same primary NSCLC cells, some of which were performed using cells grown in 3D culture. Antibodies and mimetics were assessed for anti-proliferative and pro-apoptotic activity; by assigning antibody function at the earliest opportunity the process is biased towards the identification of novel target antigens with potential therapeutic application. This approach has successfully resulted in the isolation of leads able to demonstrate in vivo activity directly from our libraries without prior optimisation of antibody sequence.

10.30- 11.00 a.m.

Discovering and validating new biomarkers in the cancer arena; the CRC-BT approach

Andrew M. Coley CSO, Cooperative Centre for Biomarker Translation, Melbourne, Australia

If we are to advance the field of antibody therapy for cancer it is likely that we will need to discover new and more effective biomarkers. The era of 'grind and find' and serendipitous approaches to biomarker discovery is past and discovery of 2nd generation biomarkers requires a degree of innovation. This applies to either the identification of the markers or their validation as bona fide markers of disease or targets for intervention. The CRC-for Biomarker Translation has a multi-pronged approach to biomarker identification and verification that encompasses 'omics', reagent generation, biomarker distribution and evaluation of potential use in diagnostics or therapeutics. This approach will be discussed and validated with some examples of CRC-BT biomarkers recently discovered that have real potential in the clinic.

11.00- 11.30 ***Coffee break***

SESSION 2 Chairman: Christina Kousparou

11.30- 12.00

Ang-2-VEGF CrossMab, a novel bispecific human IgG1 antibody blocking VEGF-A and Ang-2 function mediates potent anti-tumoral, anti-metastatic and anti-angiogenic efficacy

Klaus Bosslet, Markus Thomas, Werner Scheuer, Jörg Regula, Monika Bähner, Jürgen Schanzer, Rebecca Croasdale, Christian Gassner, Guy Georges, Hubert Kettenberger, Harald Dürr, Sabine Imhof-Jung, Manfred Schwaiger, Kay Stubenrauch, Michael K. Weidner, Wolfgang Schäfer, Christian Klein (Roche , Germany)

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 blood vessel remodeling and tumor angiogenesis. In tumors Ang-2 is up-regulated and a bad prognostic factor. Recent data demonstrated that Ang-2 inhibitors, both as a single agent or in combination with chemo- or anti-VEGF therapy mediate anti-tumoral effects.

We have recently described a novel generic method for the production of bivalent bispecific human IgG1 antibodies (CrossMabs) based on the crossover of the CH1 and CL domains within the Fab region of one half of a bispecific antibody combined with the

knobs-into-holes technology to enforce heterodimerization of the Fc portion. Subsequently, we have applied the CrossMab technology for the generation of a bispecific antibody recognizing VEGF-A with one arm based on bevacizumab and recognizing Ang-2 with the other arm based on LC06, a highly Ang-2 selective human IgG1 antibody. The Ang-2-VEGF CrossMAb could be produced with good yields and purity by eukaryotic. Surface Plasmon resonance studies showed that the two different arms of the Ang-2-VEGF CrossMab retained their antigen binding affinity for VEGF-A and Ang-2 and interfered with VEGF-induced HUVEC proliferation and Ang-2 induced Tie2-phosphorylation in a similar manner than the parental antibodies. Crossmab showed very potent tumor growth inhibition in orthotopic (KPL-4) and in subcutaneous xenograft tumors (Colo205). In the orthotopic KPL-4 and the s.c. Colo205 xenograft we observed a strong inhibition of angiogenesis by ex vivo analysis. In the VEGF-induced cornea pocket assay Crossmab resulted in a complete shutdown of angiogenesis. We have generated a bispecific human IgG1 antibody blocking VEGF-A and Ang-2 function simultaneously. Our data indicate that the Ang-2-VEGF CrossMab mediates potent anti-tumoral, anti-metastatic and anti-angiogenic efficacy and represents a promising therapeutic agent for the therapy of cancer.

12.00-12.30

Preclinical Development of Anti-VEGF Antibody Blocking VEGF Binding to VEGFR2 (KDR) but not VEGFR1 (FLT-1)

Sergej Kiprijanov, Affitech Research AS, Oslo, Norway

Affitech is developing a fully human antibody AT001/r84 which is currently in late pre-clinical testing with initial clinical trials planned for 2011. The antibody is specific for human and mouse vascular endothelial growth factor A (VEGF-A) and selectively blocks interaction of VEGF only with VEGF receptor 2 (VEGFR2; KDR), the receptor primarily responsible for tumour angiogenesis. This feature distinguishes AT001/r84 from Avastin® (bevacizumab) and other drugs in development that block VEGF binding to both VEGFR2 and VEGF receptor 1 (VEGFR1; Flt-1). The antibody AT001 is as effective as bevacizumab in inhibiting growth of established tumours in preclinical models of human breast, lung and colorectal cancer. In addition, it shows no significant side effects after chronic high dose therapy in mice. Selective blocking of VEGF binding to VEGFR2 may ultimately offer safety or efficacy benefits over the non-selective approaches.

12.30- 14.00 ***Lunch break***

14.00- 15.00 ***Open Air Workshop*****: Tumor Angiogenesis-what is missing?**
Klaus Bosslet & Christina Kousparou

SESSION 3 MEET THE PROFESSOR

Chairman: Surinder Sharma

17.00- 17.45

RADIOIMMUNOTHERAPY - QUO VADIS? (που πάτε)

Robert Waibel and Roger Schibli- CRS, Paul Scherrer Institute, CH-5232 Villigen PSI, Switzerland

Looking back at past meetings of "The International Conference", e.g. at halftime – the 14th International Conference –, about 30% of the presentations dealt with radioimmunotargeting. At last year's 27th Conference in Mykonos there was only one presentation dealing with radioimmunotargeting (presented by the Paul Scherrer Institute PSI). We try to address some of the explanations for the decline in the excitement on the use of radionuclides. Our hypothesis is that the tightened regulatory requirement for bringing antibodies to the clinic is one of the main reasons for slowing down patient studies. Even for a "first in human" trial the antibody quality requires a GMP production, which is virtually impossible for academic institutions for financial reasons. A second reason could be a technical/logistical problem. Despite increasing evidence of the high efficacy of radioimmunotherapy in NHL, the low toxicities and the approval by the FDA and the EMA, these therapies are seldom used most likely because each hospital has to establish its own labeling service. The concept of centralized production of radiotracers for Nuclear Medical Centers – routinely accepted for the most widely used PET tracer [18F]-2-Fluoro deoxyglucose - would counteract the gross underuse of the highly effective therapy using e.g. 90Y-Zevalin or 131I-Bexxar against relapsed or refractory, low-grade or follicular B-cell NHL. A third reason might be the cost and availability of new, more suitable radionuclides for diagnosis and therapy since many of the necessary large infrastructures and facilities (high neutron-flux nuclear reactors and high-current cyclotrons) have reached the end of their lifespan and will in the near future cease production. In this lecture I will introduce the concept of the Center for Radiopharmaceutical Science at the Paul Scherrer Institute in Switzerland and highlight some of the goals with respect to radionuclide therapy. New data on successful radioimmuno-therapeutic and -diagnostic approaches in experimental tumors, ranging from small molecules (peptides) to medium size molecules (DARPins) to larger molecules like monoclonal antibodies as well as the production of new radionuclides will be presented.

17.45-18.00 ***Discussion***

20.00- 22.00 ***Welcome Reception Cocktail***

Tuesday 21st June 2011

SESSION 4 Chairman: Spyros Stylianou

9.00-9.30

The role of molecular markers in the definition of histologically aggressive breast cancer

Niki Agnantis and Anna C. Goussia, University of Ioannina, Greece

Despite its unique origin in the terminal duct-lobular unit (TDLU), invasive breast cancer is a very heterogeneous disease concerning clinical presentation, histological features, expression of tumour markers, biological behavior and response to treatment. The recent edition of WHO Histological Classification of breast carcinomas recognizes the existence of 19 distinct entities with some subcategories and distinct ICD-O codes. Some histologic types are more common and some others are rare. Invasive ductal carcinomas of non otherwise specified (NOS) are the most common types of breast carcinomas and represent approximately 50-80% of all breast cancers. Moreover, they constitute a diagnosis of exclusion; i.e. a tumour that does not fill the morphological criteria for any of the other special types. The remaining 20-25% accounts to breast carcinomas of special type. The prognostic value of these special types, that reflects to tumour behavior, can be divided into groups with good or poor prognosis, according to some clinicopathological parameters. From the Pathologist's point of view, there are some markers that predict the tumour behavior and these markers come mainly from the tumour characteristics. Therefore, markers predicting the tumour behavior are: tumour size, histologic type, tumour grade, vascular invasion and lymph node involvement. Other factors that are under investigation are stromal elastosis, tumour necrosis and immunological reaction of the regional lymph nodes. Although histopathology is our standard for giving information about the probable clinical course of breast cancer disease there are some carcinoma types that have an expected aggressive behavior. These types are: small cell/oat cell neuroendocrine carcinoma, micropapillary, pleomorphic lobular, metaplastic and lipid-rich carcinoma. Recent molecular studies based on gene expression profiling, using DNA microarrays, have defined molecular subtypes of breast cancer; i.e. molecular classification was created. Thus, breast cancer has been divided into molecular subgroups with distinct biological features, such as metastatic propensity, prognosis and response to therapy. In this molecular classification there are distinct ER+ and ER- groups of breast carcinomas.

The HER2 and basal-like molecular subtypes are associated with an aggressive clinical outcome and a shorter survival. The prognostic significance of apocrine, interferon and claudin-low molecular phenotypes is yet undetermined. While invasive ductal carcinomas of non otherwise specified (NOS) and lobular carcinomas contain all molecular breast cancer types (luminal, basal and HER2 phenotypes), histological special types are more homogeneous and belong mainly to one or two molecular subtypes. Micropapillary and pleomorphic lobular carcinomas are preferentially of HER2 or luminal phenotypes.

Metaplastic and medullary carcinomas are preferentially of basal-like phenotype and some of them may display a claudin-low phenotype. In conclusion, understanding these specific histologic types of breast carcinomas with an expected aggressive behavior, is not an academic exercise but may lead to a better understanding of the biology and the clinical behavior of breast cancer and therefore to the planning better therapeutic approaches. Pathologists should not be only "graders" or "typers", but must be the key players in the field of breast cancer research.

9.30-10.00

Intracellularly-targeted recombinant antibodies as cancer therapies

Silvia Colucci, Imperial College London, UK, and Trojantec Ltd, Cyprus

Using the cell-penetrating shuttle protein antennapedia (Antp), a dominant-negative version of Mastermind-like 1 (MAML-1) can be transported intracellularly, leading to inhibition of Notch signalling both in vitro and in vivo. Notch expression is deregulated in several cancers and implication in cancer stem cell maintenance. Based on this innovative approach, we aim to show that intracellular signalling can be a general approach by delivering a membrane-translocating scFv directed to the same Notch signalling pathway. The strategy for signalling inhibition consists of blocking tripartite complex formation by targeting MAML-1 interactions with N^{IC} and CSL. So far, research was focused on generating the essential tools including production of Antp and selection of a specific Notch pathway inhibitory scFvs through phage display. We believe this novel approach of signalling inhibition, targeting intracellular pathways, will enable us to overcome some of the issues encountered in the battle against cancer such as targeting of drug resistant cancer stem cells and tissue penetration.

10.00-10.30

Elimination of cancer by restoration of apoptotic pathways

Christina A. Kousparou, Mahendra Deonarain, Agamemnon Epenetos Trojantec Ltd, Nicosia, Cyprus and Imperial College London, UK

Cancer can be the result of a malfunction in cell-cycle control, which in turn is largely regulated by cyclins and cyclin-dependent kinases. The cdk inhibitor p21 plays a key role in G1 cell cycle arrest in response to DNA damage by blocking cycD/cdk4 complex formation. As a result, the retinoblastoma protein remains in a low phosphorylation state, tightly bound to E2F, inhibiting its activity. The balance of p21 activity appears to shift depending on cell state, not only in differentiated cancer cells, but also in cancer stem cells, and can therefore be considered a putative marker. It also affects response to treatment and drug sensitivity. We reasoned that anti-mitotic therapy will be less effective if administered to a tumour which has compromised p53/p21 activity. If wild-type tumour suppressor protein

functionality is restored by complementation, cells should regain drug sensitivity. We have utilised an effective, non-toxic, non-immunogenic, biologically-active pharmaceutical formulation to restore p21 activity in cancers that have abolished this functionality. The protein transduction domain antennapedia which was part of the recombinant drug overcame the obstacles of membrane penetration and successfully delivered functional p21 protein to all tissues in mice, including the brain. This resulted in cell division arrest and apoptosis. Intravenous administration of Antp-p21 to tumour-engrafted animals inhibited cancer growth and prolonged survival, an effect enhanced by anti-mitotic chemotherapy. Efficacy was superior with improved treatment regimens, and resulted to complete cancer eradication.

10.30-11.00

SIMPLE Antibody™ antagonists of c-Met give unprecedented insight into receptor function in cancer

Torsten Dreier, Belgium

c-Met is an exciting and compelling cancer target, although its function in tumor development, angiogenesis and metastasis is complex and incompletely understood. One major reason is the technical difficulty in generating antibodies that can block receptor signal transduction without mediating agonistic effects. We have applied our SIMPLE Antibody™ technology to c-Met and identified mAbs representing 24 distinct V gene families – unprecedented diversity against this target. All selected mAbs are highly potent antagonists of the receptor, with 8 lacking any detectable agonistic activity. Epitope mapping of mAbs from all 24 families reveals extensive coverage of all extracellular domains of recombinant c-Met. Five of the 8 pure antagonists bind and act through the high affinity HGF binding site, with the remaining 3 specific for the low affinity site. Highly selective binding to a panel of human tumor cell lines over-expressing c-Met (BxPC-3 - pancreatic carcinoma; MKN-45 - gastric adenocarcinoma; U-87MG - glioblastoma-astrocytoma) has been successfully demonstrated. To correlate c-Met binding of these mAbs with their functional effects, all 8 antagonists have been analyzed in tumor cell-based bioassays. None of the panel induce c-Met phosphorylation upon binding of A549 (alveolar adenocarcinoma) cells, which supports their non-agonistic profile. Several mAbs have been shown to block HGF-independent phosphorylation of the constitutively activated receptor in MKN-45 cells. All selected antagonists show potent blocking of both c-Met-mediated scatter and tumor cell proliferation (using HPAF and BxPC3 pancreatic tumor lines, respectively), with no evidence of agonism. In summary, these SIMPLE Antibodies™ have highly attractive properties when compared to c-Met mAb antagonists in clinical development. Given their impressive potency and functionality, as well as their fully human sequence composition, these mAbs have exciting potential as development candidates for solid tumor immunotherapy.

11.00-11.30 ***Coffee break***

SESSION 5 Chairman: Andrew Coley

11.30-12.00 **Bispecific TandAbs for the activation of immune effector functions**

Uwe Reusch, Affimed Therapeutics AG, Germany

Affimed Therapeutics AG develops unique antibody therapeutics for improved treatments of cancer and inflammatory diseases. Affimed generated a comprehensive pipeline of antibody product candidates based on their proprietary TandAb technology platform. TandAbs are tetravalent bispecific human antibody formats that have two binding sites for each antigen. RECRUIT-TandAbs bind to target molecules on the surface of tumor cells and can activate immune effector cells like cytotoxic T cells or natural killer (NK) cells for tumor cell lysis. Latest in vitro data from the development and characterization of the RECRUIT-TandAbs AFM13 for the indication Hodgkin Lymphoma, and AFM11 and AFM12 for the treatment of Non-Hodgkin Lymphoma will be presented. TandAbs possess the same avidity and affinity for each target as an IgG. Combined with their bispecificity, this format offers unique advantages over first generation antibody formats/scaffolds. In addition, the lack of an Fc portion may contribute significantly to the impressive safety profile observed in preclinical and clinical studies.

12.00-12.30

Angiolix (HuMc3 Monoclonal Antibody) for Breast Cancer Therapy

Laura Boney, **Agamemnon Epenetos and** Mahendra Deonarain, Imperial College London, UK

Lactadherin has recently grown in interest as a vasculature-related glycoprotein implicated in a range of cancers, particularly breast. It is a 46kDa milk-fat globule-related protein which binds to integrins and modulates vascular endothelial growth factor (VEGF) receptor-integrin signalling. Angiolix is a humanised form of the Mc3 monoclonal antibody. We have shown this to be a high affinity (sub-nanomolar Kd) antibody able to disrupt tumour-matrix interactions leading to direct tumour cell and indirect anti-angiogenic responses resulting in tumour death in human tumour xenograft animal models. We will present data to support the further evaluation of Angiolix as a combination therapy with VEGF-inhibiting therapies and propose mechanisms by which this would work .

12.30- 14.00 ***Lunch***

14.00- 15.00 ***Open Air workshop*****: Cancer stem cells. Do they exist? Steven Rust & Ronan O'Hagan**

SESSION 6

MEET THE PROFESSOR Chairman: Sergej Kiprijanov

17.00- 17.45

ADEPT- what have we learnt, and the way forward

Surinder Sharma, University College London, UK

Antibody directed enzyme prodrug therapy (ADEPT) was designed to generate cytotoxic drug within tumours for optimum therapeutic benefit without systemic toxicity. To achieve this required a multidisciplinary approach and consideration of parameters such as target antigen, enzyme, prodrug, pharmacokinetics, pharmacodynamics as well as immunogenicity. Preclinical studies provided valuable information for first in man studies but it is only through clinical trials that the real challenges can be defined and despite widespread interest in the ADEPT approach, after more than 20 years, the only clinical reports have, to the best of our knowledge, come from our group. This presentation will review ADEPT from concept to first in man studies and the way forward.

17.45- 18.00 ***Discussion***

20.00 until late ***Gala Dinner***

Wednesday 22nd June 2011

SESSION 7 Chairman: Ronan O'Hagan

9.00-9.30

The role of Notch in non small cell lung cancer (NSCLC) and its implication in epidermal growth factor receptor (EGFR)

D. Kotsirilou, **E. Giannopoulou**, E. Papadimitriou, T. Makatsoris and H.P. Kalofonos University Hospital of Patras, Greece

The Notch signaling pathway is evolutionary conserved and has crucial roles in the development and maintenance of embryonic and adult tissues. Recently, Notch has attracted the interest of many researchers regarding its role in cancer. Indeed, there are

data for the role of Notch signaling in leukemia, breast, lung and cervical cancers. It has been shown that the functions of Notch pathway are highly cell-type dependent in different embryonic and adult tissues, as well as in cancers. Depending on the cell type, Notch may behave as an oncogene or a tumor suppressor gene. Targeting Notch is a challenge for the investigators and the last few years Notch inhibitors have been developed. The majority of them are γ-secretase enzyme complex inhibitors and recently promising monoclonal antibodies have been developed increasing the arsenal of cancer therapeutics. Although, the importance of Notch pathway in lung cancer is under investigation, the contribution of EGFR signaling in lung cancer development is well established. So far, it has been found that the cross-talk between the Notch and EGFR signaling can function in either an antagonistic or synergistic fashion, depending on tissue and developmental context. The aim of this study is to investigate the interaction between the pathways of Notch and EGFR in human NSCLC cell lines. In vitro experiments were performed on H23, H661, HCC827 and A549 human NSCLC cell lines. HCC827 cells express mutated EGFR and all the other cell lines express wild type EGFR. Notch pathway was blocked using DAPT, an inhibitor of gamma-secretase enzyme which participates in enzymatic cleavage/activation of Notch. Cell proliferation was determined using the MTT (methyl tetrazolium) assay. The Notch protein levels were determined using western blot analysis. Notch was differentially expressed in human NSCLC cell lines: H23 and A549 cells express the maximum and the minimum levels of Notch protein, respectively, with H661 and HCC827 cells expressing median, equal amounts of Notch protein. DAPT decreased H23, H661 and A549 cell number in a concentration-dependent manner, 48 hours after its addition in cells but had no effect on the number of HCC827 cells. Furthermore, DAPT reversed the stimulatory effect of EGF in H661 proliferation in a concentration-dependent manner, an effect that was not observed in H23 and A549 cells. In H661 cells, DAPT decreased Notch protein levels 24 h after treatment. In the same cells, EGF activated Notch receptor 60 min after its addition in cells and this effect was reversed at basal levels 90 min after EGF treatment.

Previous data have shown that Notch interacts with molecules like ERK, mTOR and Wnt; however, this study is the first that presents indications of a possible direct interaction between EGFR and Notch signalling, at least in some types of NSCLC cells. In the current study a nonselective strategy was used but further research is required using a selective strategy including monoclonal antibodies for the evaluation of Notch as a therapeutic target for the NSCLC.

9.30-10.00

The Akt Pathway as Drug Target in Human Cancer

Spyros Stylianou, Trojantec, Nicosia, Cyprus

The protein kinase Akt is associated with tumor cell survival, proliferation, and invasiveness. The activation of Akt is one of the most frequent alterations observed in human cancer and tumor cells. This activation results in enhanced resistance to apoptosis through multiple mechanisms. Tumor cells that have constantly active Akt may depend on Akt for survival. Therefore, understanding Akt and its pathways is important for the

creation of better therapies to treat cancer. AKT signalling regulates apoptosis through many different routes. One is through the inactivation of p53, which occurs in two ways. Firstly AKT phosphorylates and targets Mdm2 to the nucleus, and blocks p53 function by nuclear export and proteasome-mediated degradation. In addition, AKT phosphorylates and inhibits ASK1 which activates the JNK signalling pathway JNK activates p53 by phosphorylation at Thr-81. In non stressed cells Mdm2 and JNK bind and target p53 for degradation by the proteasome. Upon DNA damage, JNK dissociates from p53 and is activated. Activated JNK then further participates in p53 activation by phosphorylating Thr-81 and displaces Mdm2 away from p53. Because of the Akt functions above, Akt inhibitors may be able to treat cancers. Some Akt inhibitors are undergoing clinical trials. We are a developing a direct inhibitor of Akt activation. We are engineering a fusion protein, consisting of Antennapedia (ANTP) and a dominant negative (DN) Akt protein which may have the potential to inhibit the Akt transactivation complex.

10.00- 10.30

Immunomodulatory Properties of Hypothalamic Proline-rich Polypeptide Galarmine and Anticancer Applications

A.A. Durgaryan, M.B. Matevosyan, T.E. Seferyan and A.A. Galoyan

H.Buniatian Institute of Biochemistry, Yerevan, Armenia

Cytokines could play an important role as potential modulators of immunity and can function to inhibit tumour development and progression. The operative injury affects the immune system what results in cytokine production--mediators of immune response. It has been shown that the implementation of the postoperative cytokines (IL-6, IL-10, TNF-alpha) correlates with the improvement of postoperative course in gastric cancer patients (Szczepanik A.M. *et al*, 2010).

Proline-rich polypeptides are new brain cytokines isolated from neurosecretory granules of hypothalamus with broad-spectrum of biological activities including antibacterial, antitumor, and immunomodulatory properties (Galoyan A.A., 2008). PRP-1 (Galarmin) action on human chondrosarcoma JJ012 cells demonstrated the abolishment of Myc oncogene activity usually upregulated in chondrosarcoma cells and other malignancies (Galoyan K. *et al*, 2009). Galarmin, even at low concentrations (0.5-1 μg/ml), reduces viable sarcoma JJ012 cell numbers in comparison with control (89% growth inhibition) (Galoyan K. et al, 2010).

It is of great interest to address the role of Galarmin as an immunostimulatory agent and its impact on the synthesis of the main pro- and anti-inflammatory cytokines. The immunostimulatory properties of Galarmin were studied using an *in vivo* mouse model and the levels of IL-6, IL-10, and TNFa were measured with commercially available enzyme-linked immunosorbent assay (ELISA) after 96h of Galarmin and its analogues administration both in plasma and peritoneal cavity.

It has been shown that administration of Galarmin and its analogues significantly increased the levels the main pro- (IL-6, IL-1b, TNFa) and anti-inflammatory cytokines (IL-

10). The most significant increase (6.8 fold) was observed for IL-10, an immunoregulatory cytokine. According literature data, dependent on the experimental model, IL-10 seems to favor or inhibit the existence and progression of tumors (Sabat and Asadullah, 2002).
Therefore, received results indicates that Galarmin represent a perspective immunomodulatory agent and can be eventually tested as a usefull modulator in immunosuppressed patients after chemotherapy or chirurgical operation in order to improve host defense systems and antitumor immunity.

SESSION 8 Chairman: Agamemnon Epenetos

11.00-11.30

Antibodies against Notch receptors

Ronan O'Hagan, AVEO Pharmaceuticals, USA

AVEO has developed a series of inducible mouse models of cancer which, through the preservation of critical tumor/stromal interactions, facilitate identification of cell-surface and secreted proteins that represent viable targets for therapeutic antibodies and other biologics. Functional genetic screens performed in vivo in these models identified the Notch pathway as a critical regulator of tumor maintenance. This finding is consistent with emerging evidence that activation of Notch signaling via receptor point mutation, receptor amplification, and elevated receptor and ligand expression, plays a key role in various human cancers. Moreover, the Notch pathway controls diverse aspects of tumorigenesis and tumor maintenance, regulating tumor autonomous processes and interactions with the microenvironment, including angiogenesis. To further understand the role of the Notch pathway in tumor maintenance, and to assess the therapeutic potential of targeting the Notch pathway in cancer, we have generated monoclonal antibodies that inhibit various Notch receptors. Characterization of monoclonal antibodies targeting Notch1 or Notch3 through cell-based and biochemical studies demonstrated that these antibodies bound with high affinity and high specificity to the ligand binding domains of the Notch receptors, prevented ligand mediated activation of the target receptor, and specifically repressed Notch-dependent signaling with high potency.

Effective inhibition of functional angiogenesis was observed upon anti-Notch1 antibody treatment in both in vitro and in vivo models. Significantly, specific inhibition of Notch1 by this antibody did not result in the dose-limiting gut toxicity observed with pan-Notch inhibitors such as gamma-secretase inhibitors. Humanized versions of the Notch1 monoclonal antibodies have been generated and characterized. Inhibition of tumor growth by the Notch3 monoclonal antibody was effected through tumor cell autonomous mechanisms. To identify tumors that are dependent upon tumor autonomous Notch signaling, gene expression profiles were correlated with Notch pathway dependence in human cancer cell lines. Expression of specific downstream targets was highly correlated with sensitivity of human cancer cell lines to inhibition of ligand-dependent Notch signal. This biomarker of Notch pathway dependence successfully predicted that a subset of Kras

mutant pancreatic and colon cancer cell lines would be highly sensitive to Notch pathway inhibition. Moreover, identification of Notch1 and Notch3-specific target genes further enables selection of tumors that will respond to monoclonal antibodies specifically targeting one or other, or both of these receptors.

11.30- 12.00

Expression of ABCB5 in the human breast: Cell surface expression and stem cell population

Hazem Ghebeh, Abdullah Adhfyan, Safiah Olabi, Khalid Al-Faqeeh and Chaker Adra (King Faisal Specialist Hospital and Research Center, Riyadh Saudi Arabia and Transplantation Center, Children's Hospital Boston and Brigham & Women's Hospital Harvard Medical School, Boston, USA

ABCB5 is a multi-drug resistant protein that has been described as a marker of cancer stem cells in melanoma. Very little is known about the expression of this molecule in other organs. We used several immunological and molecular methods to characterize this molecule particularly in human breast. Immunohistochemistry demonstrates abundant expression of ABCB5 in both the cytoplasm and the nucleus of several normal tissues including the breast, while many other tissues showed only nuclear expression. In the breast, ABCB5 was expressed in the cytoplasm of the luminal cells and the nucleus of both the luminal and myoepithelial cells. In contrast to the ubiquitous nuclear/cytoplasmic expression, the cell surface expression of ABCB5 was restricted to $CD44^{high}$, $CD24^{neg}$, $Ep\text{-}CAM^{low}$, $CD10^{high}$, CD49f+ consistent with the phenotype of breast normal breast cell. In vitro ABCB5 sorted cells formed significantly more mammospheres than ABCB5 negative cells. On the other hand, in breast cancer cells, ABCB5+ cells were $CD44^{high}/CD24^{low}$ and were ALDH+ consistent with breast cancer stem cell phenotype. In addition, sorted ABCB5+ cancer cells formed significantly more mammospheres than ABCB5 negative cells Our findings suggest that cell surface ABCB5 is associated with cells that have stem cell/progenitor phenotype in a fashion similar to the skin and encourage for further studies to examine its importance in breast cancer and other types of tumors.

12.00-12.30

Light-activated ADCs: The best of both worlds?

Ioanna Stamati, Gokhan Yahioglu, Hayley Pye, Marina Kuimova and Mahendra Deonarain, Imperial College London and PhotoBiotics Ltd, London, UK

Targeted photodynamic therapy (PDT) promises to combine the cosmetic benefits of laser therapy with the potency and specificity of antibody-drug targeting. We have been developing optimized single-chain Fv frameworks specifically for accommodating

covalently attached PDT drugs in a technology platform called "OptiLink". We show that scFv-based ADCs, activated by laser light can destroy tumours in a range of challenging animal models for ovarian and prostate cancer. We will present data on a range of ADCs with differing and improving properties. We also suggest that the fluorescent properties of such ADCs can be used for imaging purposes.

12.30-12.35 Farewell: Agamemnon Epenetos

NOTES