The symposium on Antibodies as Drugs, organized by Keystone Symposia and chaired by J. Marks, (University of California Los Angeles, USA), E.S. Ward (University of Texas Southwestern Medical Center, USA) and L. Weiner (Georgetown University Medical Center, USA), was held in Whistler, British Columbia. This Canadian Rockies village, which will host the 2010 Olympic Games, served as an enchanting backdrop to the meeting. The more than 350 speakers and attendees included scientists from major pharmaceutical firms, e.g., Abbott, MedImmune/Astra Zeneca, Bristol-Myers Squibb, Merck & Co., Pfizer, Sanofi-Aventis, Schering, GlaxoSmithKline, Eli Lilly, Hoffmann LaRoche, Novartis, Wyeth; and biotechnology companies, e.g., Ablynx, Medarex, Morphosys, GenMab, Amgen, Genentech, ImmunoGen, Agensys, Domantis, Biogen Idec, Centocor, LFB, Micromet, PDL Biopharma, Borean Pharma, Dyax Corp., Symphogen and Syntonix. Academic research groups at Imperial College London, University of Oxford, ETH Zürich, Scripps, Institute Cochin, Karolinska Institute, Utrecht University, Harvard Medical School, Massachusetts Institute of Technology, Baylor College, Paul Ehrlich Institute, University of California San Francisco, University of California San Diego, University of Nantes, University of Tours and Ludwig Institute for Cancer Research, were also represented, as were regulatory authorities, including the US Food and Drug Administration, National Institutes of Health and the Public Health Agency of Canada. The meeting was very interactive and included thoughtful exchanges during the different sessions and networking events.

Keynote Address

The meeting’s keynote address on the use of chemical biology and protein aptamer approaches to target validation in cancer therapy was given by Professor Sir David Lane (Cancer Research UK, Dundee UK; Institute for Molecular and Cell Biology, Singapore). Sir David is Director of the Cancer Research UK Transformation group at the University of Dundee, and Executive Director of the Institute for Molecular and Cell Biology in Singapore. He is internationally recognized for his original discovery of the p53 protein/SV40 T antigen complex and for many subsequent contributions to the field.

The lecture was focused on novel therapeutic approaches involving p53 protein, and how to target the multiple mutant forms of p53, which are found in about 50% of cancer patients and can escape conventional therapies. Mutations are likely to modulate the thermodynamic stability of p53, thereby destabilizing the protein. Novel drug designs include stabilization to reactivate the functions of mutant p53 or activation of the wild-type protein. The target corresponds to the interaction domain between p53 and MDM2 (HMD2), an ubiquitin ligase. One approach based on protein aptamers derived from thioredoxin insert proteins (or TIP) was described. Some lead candidate TIPs selected via a peptide phage display approach can act as p53 activators. Similarly, the MDM2 and eIF4E pathway was targeted by the TIP aptamer approach. In addition, the discovery of tenovins, which are novel p53 activators, was highlighted. These molecules were discovered during a cell-based screen for p53 activation and decrease of tumor growth. Via a yeast genetic screen, their cellular targets were identified as proteins of the sirtuin family with protein deacetylation activities. Finally, the utility of zebra fish as a good model for in vivo studies on p53 protein was discussed. One zebra fish protein, Delta113p53, is a target for transcriptional activation by p53, and can, in turn, inhibit the activity of the full-length p53 protein, thereby establishing a novel negative feedback loop centered on the p53 locus itself.

Novel Cancer Drug Scaffolds

This session focused on novel technologies to create next-generation, post-antibody therapeutics, ranging from small modified peptides, macrocycles and protein scaffolds to large antibody structures with improved binding domains.

A. Plückthun (University of Zürich; Molecular Partners, Switzerland) presented an overview of the designed ankyrin repeat proteins (DARPin) technology, which is based on the natural ankyrin repeat scaffold. An example of a tri-specific DARPin
targeting human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR) and a human IgG-Fc region was presented. This molecule showed extended half-life due to the binding to IgG in human serum. A very strong avidity effect was observed upon dimerization of DARPinS. Best binders showed better tumor accumulation compared to weak binding molecules. An immunotoxin was constructed by fusion of anti-epithelial cell adhesion molecule (EpCAM) DARPin with Pseudomonas exotoxin A. In silico immunogenicity, determined using technology from Antitope, was predicted to be low.

The drug discovery potential of Ensemblins, orally bioavailable small molecule macrocyclic compounds that have the potential to behave like biologics, and can disrupt protein-protein interactions, was presented by N. Terrett (Ensemble Discovery Corp., USA). DNA-Programmed Chemistry™ (DPC) technology is applied to control chemical reactions. DPC reactions occur between pairs of chemical building blocks attached to complementary DNA strands. Under mild aqueous conditions, specific hybridization of the DNA strands brings the building blocks into close proximity, thereby increasing their relative concentration by a factor of > 10,000. This hybridization also controls reaction stoichiometry and lowers energies of activation, thus enabling highly specific reactions. The specificity achieved by DPC is analogous to single turnover enzyme catalysis. Applications of DPC include synthesis of diverse chemical libraries, discovery of new chemical reactions, and in situ generation of signal-emitting compounds for use in biodetection applications. The most advanced program involves Ensemblin macrocycles that competitively antagonize the activity of TNFα on TNF receptors in both biochemical and cell-based assays. Three leads that are active in primary cell assays and in vivo in a rat edema model for anti-inflammatory activity have been isolated; the best of these is E-32224.

G. Verdine (Harvard University, USA) introduced the concept of stapled peptides to target ‘undruggable targets.’ According to Dr. Verdine, about 80% of proteins exhibit limited targetability by either small molecules or biologics due to the poor physico-chemical properties of the binding surface area or to intracellular localization. The concept is to design synthetic biologics by identifying the functional area of a target, i.e., the ligand binding interface, and then designing a stable peptide to inhibit the ligand-receptor interaction. The amide bond of natural peptides is converted into a less polar, more metabolically stable structure such as ethylene bond. Three examples were discussed. The first approach targets the death pathway via BH3-only protein domain. A candidate peptide (SAHBA) showed mitochondrial apoptosis activation upon binding to BAX protein. The second example involves the p53/HDM2 interaction. The last approach involves targeting the Notch pathway and the Notch/CSL/MAML interface. Peptide SAHMI showed potent activity in in vitro cytotoxicity assays, and in an in vivo mouse leukemia model. Stapled peptides are being commercially developed by Aileron Therapeutics.

CovX-Bodies are created by covalently linking a pharmacophore, i.e., a peptide, via proprietary linkers to the binding site of a specially designed monoclonal antibody (mAb), thereby reprogramming the activity or specificity of the mAb. All CovX-bodies share the same mAb structure, so only a novel pharmacophore has to be designed. R. Lappe (CovX, USA) presented data on an anti-angiogenesis candidate, CVX-060, which is a selective angiopoietin-2 antagonist, and highly selective for Ang2. The molecule comprises the fusion of two Ang2 sequestering peptides linked to CovX’s proprietary mAb. The half-life of such molecules is about 80 hours. CVX-060 demonstrated similar anti-tumor activity compared to bevacizumab in a Colo205 mouse xenograft model, and decreased microvessel density and increased tumor necrosis. The combination of CVX-060 with either bevacizumab or docetaxel showed strong synergistic anti-tumor activity. The candidate is currently in Phase 1 clinical studies. Bi-functional CovX-bodies, e.g., molecules linking an Ang-2-peptide and a vascular endothelial growth factor (VEGF) binding peptide to the same mAb, are currently being developed.

Two short talks by Y. Guillen (Massachusetts General Hospital, USA) and J. Cochran (Stanford University, USA) highlighted alternative methods to develop modified peptides as potential therapeutics or diagnostics. Dr. Guillen discussed the PURE technology, which is based on artificial translation machinery to incorporate unnatural amino acids. The engineering of knottin peptides that target integrins was presented by Dr. Cochran. These molecules have potential as useful tools for tumor imaging.

Workshop 1: Novel Approaches to Cancer Drug Development

L. Jermutus (MedImmune Ltd., UK) discussed various technologies implemented at MedImmune to discover novel biologics. For example, Dr. Jermutus noted that human antibody libraries are applied to select for cross-reactive antibodies or for very high affinity binders (pM), and transgenic mice technologies that produce human mAbs are applied to “find the unexpected.” Fc domain engineering has been used to produce mAbs with modulated functions. This approach was used to produce afucosylated mAbs, e.g., anti-IL-5 mAb MEDI563, and motavizumab, which is an anti-RSV mAb with mutations in the Fc region. The bispecific T-cell engager (BiTE) bispecific technology of Micromet was also utilized in some projects, e.g., development of CEA-CD3 BiTE. Screening has been performed using human antibody libraries to profile several disease-specific cell lines and primary cells to identify antibody binders with a specific cell recognition signature. They were further screened on a specific function such as internalization, or agonistic properties for a given target.

Work done in the laboratory of Irv Weissman on CD47 antigen as a target for acute myelogenous leukemia (AML) was presented by R. Majeti (Stanford University, USA). Their results suggest that there is increased expression of CD47 on human AML leukemia stem cells (LSC) compared to normal hematopoietic SC. Differential CD47 expression on Lin-CD34 + CD38-cells can be utilized to prospectively separate normal (CD47lo) from leukemic (CD47hi) progenitors in the same patient sample. They hypothesize that increased CD47 expression on human AML contributes to pathogenesis by inhibiting phagocytosis of leukemia cells. Anti-CD47 mAbs able to disrupt the CD47-SIRP-α interaction
and enable phagocytosis of AML LSC by both mouse and human macrophages in vitro were generated. Moreover, coating of AML LSC with anti-CD47 mAb completely eliminated in vivo engraftment upon xenotransplantation into NOD/Shi-scid IL2Rγ(null) (NOG) mice. Finally, treatment of NOG mice engrafted with human AML LSC with daily intraperitoneal injections of anti-CD47 mAb resulted in complete elimination of circulating leukemia, and a significant reduction in bone marrow leukemia.

T. Junttila (Genentech, USA) discussed the design and evaluation of an afucosylated version of trastuzumab (Herceptin). As a humanized IgG1, trastuzumab binds to FcγRIIIa, and is a potent mediator of antibody-dependent cell-mediated cytotoxicity (ADCC). Afucosylated trastuzumab was produced in CHO-K1/FUT8- cells. The mAb demonstrated increased affinity to human FcγRIIIa, but not to the other human or mouse FcγRs. The lack of fucose increased the ability of trastuzumab to mediate in vitro ADCC while retaining the ability to inhibit tumor cell proliferation. To test the effect on in vivo efficacy, mice that lack murine FcγRI and FcγRIII, but express the human FcγRIIIa (F158) transgene, were generated. Afucosylated trastuzumab had superior in vivo efficacy when compared to trastuzumab in treating two xenograft models of HER2 amplified breast cancer (KPL-4 and MDA-MB-361.1) in this transgenic mouse model.

The concept of cancer cell metabolomics was introduced by M. Yuneva (University of California Los Angeles, USA). Preferred metabolism of glucose through glycolysis as the main energy source is considered one of the important characteristics of tumor metabolism. Glutamine is metabolized through the Krebs cycle in mitochondria, and can serve as a major adenosine triphosphate source in some cancer cells. Metabolomics and microarray approaches were combined to evaluate changes in glucose and glutamine metabolism pathways in liver tumors induced by tissue specific over-expression of Myc oncogene and V12, an activated mutant of Ras. In tumors, the level of glycolysis was up-regulated in comparison with normal liver, e.g., lower glucose and higher lactate levels, associated increased mRNA and production of key glycolytic enzymes were observed. In contrast, the activity of the Krebs cycle was higher in Myc, but not in RasV12-induced tumors, e.g., increased levels Krebs cycle intermediate metabolites and increased mRNA level of the Krebs enzymes were observed.

Generating and Engineering Novel Antibody Binding Sites

G. Georgiou (University of Texas at Austin, USA) presented exciting work on the production in E.coli of full-length mAbs that retain full effector functions. Anchored periplasmic expression (APEX) and E-clonal technologies\(^5\) that allowed expression of properly assembled full-length IgGs in the bacterial periplasm upon co-expression with an inner-membrane-tethered Fc-binding protein were described. Upon outer-membrane permeabilization, bound mAbs were selected by fluorescence-activated cell sorting (FACS). The introduction of mutations in the CH3 domain (M428I/E382V) restored FcγRI binding, and allowed retention of FcRn binding. In vitro cytotoxicity was observed in assays with dendritic cells in combination with SKBR3 cells. This approach can be used to modulate binding to various FcγRs.

J. Marks (University of California San Francisco, USA) presented work on drug delivery systems that was conducted at Hermes Biosciences. Systemic delivery of therapeutic molecules requires the ability to target specific cell types, and deliver a therapeutic across the cell membrane into the cytosol. Phage antibody technology was applied to generate single chain variable fragments (scFv) capable of delivering therapeutics into the cytosol of tumor cells. Non-immune phage antibody libraries were selected directly on triple negative breast tumor cell line (MDA-MB 231). The targeted antigen was identified using a combination of immunoprecipitation and tandem mass spectrometry, correlation of transcriptional profiling with cell staining, and antigen cDNA display. Using this approach, a number of tumor-targeting scFv were isolated (anti-CD44 and anti-EPHA2). scFv-targeted immune liposomes capable of delivering small molecules, e.g., doxorubicin, in vivo were generated. The strong avidity effect observed was likely due to the scFv density on liposomes.

An overview of the Modular Antibody technology developed by f-star was provided by M. WOIsETSCHLÄGER (f-star, Austria). This technology involves engineering of novel binding sites into protein loops of an antibody that are not in the complementarity determining region (CDR). Two major structures have been designed: (1) Fcab, which corresponds to an Fc portion with antigen binding functions inside CH3 domain, and (2) mAb\(^2\), which is a bi-functional molecule with a conventional variable domain and a second binding interface constructed in a non-CDR CH3 loop. Two loops ([AB] and [EF]) of the CH3 domain could be engineered. Fcab libraries were expressed in yeast and as fusion to a yeast transmembrane domain allowing membrane expression. The example of H10-03-6, which displays strong binding to Her2 and has been shown to strongly induce ADCC in experiments with SKBR3 cells, was discussed by Dr. Woisetschläger. The molecule also has a good half-life through binding to FcRn. An example of mAb\(^2\) that corresponds to the variable domain of rituximab with an additional TNF-α binding site in the CH3 domain was also presented. The resulting mAb\(^2\) yielded simultaneous binding to CD20 and TNF-α.

R. Beerli (Cytos Biotechnology AG, Switzerland) gave a short presentation on the validation of human mAbs isolated by mammalian cell display. Pools of B cells specific for antigens of interest are directly isolated from peripheral blood mononuclear cells of human donors, e.g., immunized volunteers, donors with neutralizing mAbs against pathogens or auto-reactive antibodies, using highly repetitive arrays of antigen. Recombinant, antigen-specific scFv libraries are generated from these B cells and screened by mammalian cell surface display using a Sindbis virus expression system, which allows identification of antigen-specific antibodies in a single round of FACS. After cloning of the variable regions from positive clones, recombinant mAbs are produced as whole IgG or any other desired format. In this manner, fully human mAbs can be isolated within short timelines. As an example, isolation of anti-nicotine mAbs that showed in vivo activity by decreasing the amount of nicotine going to the brain was presented.
Engineering Fc Regions for Optimized ADCC and Pharmacokinetics

L. Weiner (Georgetown University Medical Center, USA) presented recent data from his lab on the parameters, either cellular or antibody-linked, that are important for optimization of ADCC. Dr. Weiner first reviewed knowledge of the importance of ADCC in the therapeutic activity of mAbs. He then discussed various parameters important for improving ADCC that were discovered through study of C6.5, an anti-HER2 prototypical mAb, and several engineered variants with modulated binding affinities (10^-7 to 10^-11 M). For high molecular weight binders, affinity has less impact than for small biologic scaffolds. Another parameter discussed was the affinity of mAbs for FcγRs obtained by various mutations in the Fc portion. As a result, the affinity of mAbs for FcγRs had more impact on ADCC response as monitored on SKOV3 cells than on the affinity for Her-2; thus, it is more important to increase effector functions via FcγRs than to enhance binding to the target. Attempts to decipher the influence of various immune cell types by using different mouse strains combined with TLR4 agonists were made, but no clear picture was obtained.

Data on a large set of proprietary mutants in the Fc portion that were selected by a team at Xencor was presented by J. Desjarlais (Xencor, USA). These mutants selectively modulate binding to the various FcγRs and FcRn, therefore allowing precise tuning of half-life, ADCC, complement-dependent cytotoxicity (CDC) functions. Serum half life could be extended up to three-fold. Another mutant yielded 40-fold and 10-fold increased affinity for FcγRIIa and FcγRIIb, respectively. Novel series of mutations yielded up to 400-fold enhanced binding affinity for inhibitory FcγRIIb, the only FcγR expressed on B cells. This improved Fc mutant has been associated with an anti-CD19 variable domain, a target antigen also part of the B cell antigen co-receptor complex. Enhanced FcγRIIb binding that prevents B cell activation may be beneficial in the treatment of autoimmune and inflammatory diseases.

P. Umana (GlycArt Biotechnology AG, Switzerland, a subsidiary of Hoffmann LaRoche) discussed a next-generation anti-CD20 mAb (GA101) that was generated by applying the glyco-engineering (Glycomab) technology of Glycart. During the humanization process, several ‘elbow’ hinge structures between the VH and CH1 domains of the heavy chain were tested; these demonstrated strong enhancement of GA101 caspase-independent apoptotic properties. GA101 recognizes a type II epitope on CD20 and has the following properties: low CDC, strong homotypic aggregation, not localized on lipid rafts, half number of CD20 binding sites per B cell, strong induction of caspase-independent cell death. The afucosylated GA101 is produced in CHO-cell at 3-6 g/l at the 10,000 liter scale. GA101 showed extremely potent and efficacious activity in various in vitro and in vivo models, and was more efficacious than rituximab, e.g., complete tumor remission and long term survival in aggressive diffuse large B cell and mantle cell lymphomas. The candidate inhibited progression of tumors previously treated with rituximab. GA101 is currently in Phase II clinical studies in B cell follicular non-Hodgkin lymphoma.

E.S. Ward (University of Texas Southwestern Medical Center, USA), who is a pioneer in the study of FcRn, gave a comprehensive overview of the functions and mode of action of FcRn and mAb recognition. She made the important point that higher binding affinity of human IgGs for mouse FcRn results in longer serum half life in mice. FcRn binding is not dependent on N-glycosylation. When engineering novel Fc versions with modified FcRn binding properties, it is important to evaluate the pH sensitivity—high affinity binding must be achieved at pH 6.0, but low affinity at pH 7.4 is required to allow release of the IgGs into the circulation. In contrast, IgG with Fc regions engineered to bind with higher affinity and reduced pH dependence to FcRn potently inhibit FcRn-IgG interactions and induce a rapid decrease of IgG levels in mice. Such FcRn blockers, called ‘Abdeg’ (antibodies that enhance IgG degradation), may have uses in reducing IgG levels in antibody-mediated diseases, and in inducing the rapid clearance of IgG-toxin or IgG-drug complexes. Dr. Ward also discussed development of an elegant transgenic mouse model showing site-specific deletion of floxed FcRn in endothelial and hematopoietic cells, but functional FcRn in other cell types.

Workshop 2: The How and Why of Bispecific Antibodies and Antibody Combinations

The topic of bispecific or bifunctional antibodies was quite strongly emphasized during the meeting, both during the dedicated workshop, as well as plenary and poster sessions. This clearly indicates the current importance of these potential therapeutic agents, although bispecific antibody fragments have been constructed and evaluated for more than 20 years.

R. Mabry (ZymoGenetics, Inc., USA) discussed the design and development of a novel bi-specific antibody fragment format based on fusion of two different scFv fragments N-terminal and C-terminal of human IgG Fc CH2-CH3 domains. A natural hinge was maintained at the N-terminus of CH2, and, in the C-terminus of the CH3 domain, a 10 amino acid linker was introduced in front of the scFv fragment. The selection process for scFv fragments was stress-guided; stresses included pH and temperature. Selected targets were VEGF-A and platelet-derived growth factor receptor (PDGFR) beta. The bispecific construct was produced in CHO-K1, remained mostly as a monomer, and yielded excellent thermal stability. Assessment of pharmacokinetics in mice indicated that the candidate had a plasma half-life of about 460 hours.

Multimerization of anti-CD3 scFv (up to eight blocks) was reported by C.R. Wagner (University of Minnesota). These protein macrocycles could be obtained by fusion of scFvs to dihydrofolate reductase (DHFR) moieties, and by co-incubating with the chemical dimerizer MTX-Cg. Using two different types of DHFR moieties, bivalent or octavalent ‘nanoring’ scFvs could be constructed. Of interest, these nanorings can be disassembled by adding a non-toxic DHFR antagonist compound.

www.landesbioscience.com mAbs 321
W. Yan (Amgen Inc., USA) discussed production of bispecific antibodies through use of mutations made in the CH3 domain of antibodies that force heterodimerization, rather than homodimerization. Indeed, CH3 is an important domain for Fc homodimerization. In contrast to the ‘knob-into-hole’ type of mutations designed by Paul Carter’s group, Amgen’s strategy is based on the introduction of charged residues to promote Fc heterodimers via electrostatic interactions. As an example, Dr. Yan noted that K392D/K409D mutation on one CH3 domain combined with D356K/D399K on the second CH3 domain forced Fc heterodimerization, and allowed production of bispecific antibodies with low homodimer contamination.

Increasing therapeutic activity of anti-CD23 (IgE low affinity receptor) lumiliximab by multimerization was reported by A.P. MacLaren (Biogen Idec, USA). Because lumiliximab-induced apoptosis is dependant on CD23 aggregation, a tetravalent anti-CD23 mAb was expected to have better activity. To produce this molecule, an anti-CD23 scFv was fused either to N- or C-terminal heavy chain of lumiliximab, thus forming tetravalent anti-CD23 mAbs. Of these recombinant molecules, fusion of anti-CD23 scFv to the C-terminal end showed greater biological activity compared to scFv fused at the N-terminal part. Binding to FcγRs was maintained.

K. Koefoed (Symphogen A/S, Denmark) reported generation of 83 unique anti-EGFR chimeric antibodies using their proprietary Murine Simplex™ technology. Using ELISA assays and surface plasmon resonance, mapping of the targeted epitope was realized in order to select future optimal antibody combinations. After in vitro and in vivo evaluation, a combination of two antibodies, Sym004, was selected. These two antibodies target non-overlapping domain III of EGFR, and are able to generate potent anti-tumor activity in vivo. Sym004 is currently in preclinical development.

Using a similar synergistic strategy, R. Roovers (Utrecht University, The Netherlands) described how his group developed CONAN-1. They first selected two llama anti-EGFR nanobodies (9G8 and 7D12) that compete either with cetuximab or matuzumab. These two nanobodies were fused together to form a dual-specificity anti-EGFR nanobody, and then this molecule was linked to an albumin-binding nanobody, which increased serum half-life, to provide CONAN-1. In vitro and in vivo experiments have confirmed the strong activity of CONAN-1.

Antibodies for the Treatment of Cancer

M. Slawowski (Genentech, Inc, USA) presented an overview of the mechanisms of action of trastuzumab (Herceptin), and described new data on the inhibition of HER3 and AKT signaling by this mAb in breast cancer treatment. The crucial role of HER3 was demonstrated in vitro by siRNA technology, and by immunohistochemistry in breast cancer patients whose HER-2-amplified tumors showed activation of HER3. Based on the results, a new mechanism of action involving disruption of ligand-independent HER2/HER3 interactions was proposed for trastuzumab. The action of this mAb on HER2 phosphorylation and downregulation seems less important, but a correlation was found between the anti-proliferative effect of trastuzumab and the dephosphorylation of HER3 and downregulation of proximal and distal AKT signaling in HER2-amplified cells. While trastuzumab has shown clinical efficacy in HER2—overexpressing breast cancers, primary or acquired therapeutic resistance is also observed in patients. Several hypotheses for this have been raised, including PTEN loss, activation of alternative pathway or p85/PI3K mutations. In this context a selective PI3K inhibitor, GDC-0941, has shown a strong synergistic action in combination with trastuzumab both in tumors and in trastuzumab-resistant cells (BT-474 and SKBR3 cells).

Study results for mAb 806, which targets a novel EGFR epitope on cancer cells, were provided by A. Scott (Ludwig Institute for Cancer Research, Australia). mAb 806 recognizes a conformational epitope on domain II that is exposed only on overexpressed, mutant (EGFR variant III) and ligand-activated forms of EGFR. Unlike other anti-EGFR antibodies, mAb 806 is selective for activated receptors on tumor cells, and does not bind to EGFR on normal tissues. It is thus an interesting therapeutic candidate because of the potential for decreased toxicity. The mAb bound and internalized EGFR, and showed specific tumor uptake upon biodistribution analysis. Other effects on vasculature normalization have also been observed, e.g., increased VEGF and IL-8 levels. Dr. Scott reported on a series of preclinical studies of human tumor xenograft models in which mice received mAb 806 alone, or combined with chemotherapy, another EGFR inhibitor (TKI or m-Ab) or radiotherapy. Data showed the efficacy of mAb 806 in tumor regression and anti-tumor activity. In a Phase 1 clinical study, chimeric IgG1 806 mAb was administered to eight patients; no adverse effects were observed at the evaluated doses.

K. Chester (University College London, UK) presented her work in taking Antibody-Directed Enzyme Prodrug Therapy (ADEPT) from the laboratory to Phase 1 clinical studies. A recombinant fusion protein composed of an anti-CEA scFv fragment (MEF-23) and the bacterial enzyme carboxypeptidase G2 coupled to a histidine tag, was manufactured in yeast (Pichia). In mouse pharmacokinetic (PK) studies, the recombinant protein was cleared rapidly (5 hours) via the liver, but showed good and selective accumulation at the tumor site. Treatment with the candidate was associated with an effective anti-tumoral effect in a colorectal xenograft model. Furthermore, promising results were observed in a Phase 1 clinical study in refractory chemotherapy patients, with 50% showing positive response. However, the effects of repeated cycles of ADEPT were strongly decreased by human anti-mAb responses, especially against the bacterial enzyme. Evaluation of an ADEPT candidate, comprising modified human enzyme (pancreatic RNase) and a humanized version of the anti-CEA scFv, that might be less immunogenic is ongoing.

The potential use of phosphatidylserine (PS) on cancer blood vessels as a therapeutic target was discussed by P. Thorpe (University of Texas Southwestern Medical Center, USA). PS is an anionic phospholipid located normally on the inner leaflet of the plasma membrane in mammalian cells. In the tumor microenvironment, PS becomes externalized on vascular endothelium. Different mAbs targeting PS have been described including
bavituximab, a chimeric IgG1, and 1N11, a fully human IgG1. These mAbs bind with high affinity to complexes of the phosphatidylserine-binding plasma protein 2-glycoprotein I (2GP1) and anionic phospholipids, and promote an inflammatory response against tumor blood vessels, which results in reduction of tumor growth. A synergistic effect of bavituximab combined with chemotherapy or radiotherapy was obtained in lung and brain xenograft models. An increase of PS expression has been observed after chemotherapy or radiotherapy treatment. Different mechanisms of action of bavituximab are proposed, including activation of natural killer cells and macrophages at cancer blood vessels, ADCC and blockade of PS-mediated immunosuppression via dendritic cells. Bavituximab has entered several clinical studies. A Phase 2 study comparing bavituximab to carboplatin/paclitaxel in locally-advanced or metastatic breast cancer yielded 43% of patients with partial responses (PR) and 7% patients with complete responses (CR). Another Phase 2 study in previously untreated non-small cell lung cancer patients compared carboplatin/paclitaxel to bavituximab: 35% and 6% of patients had PR and CR, respectively.

K. Davis (Massachusetts Institute of Technology, USA) gave an overview of pretargeted radio-immunotherapy (PRIT). This strategy requires a bifunctional mAb to target a specific cancer antigen, and a chelated radionuclide that is captured by the pretargeted mAb while the unbound hapten rapidly clears from the body. In order to improve tumor retention in PRIT, Dr. Davis presented a novel approach using only 1,4,7,10-tetraazacyclododecane-N,N',N''N'''-tetraacetic acid (DOTA) as the hapten in conjunction with a high-affinity DOTA-binding bifunctional mAb. This hapten yielded good biodistribution, and, interestingly, resulted in lower bone marrow dose and improved tumor retention, especially in regions with low antigen density.

The advantages of non-fucosylated antibodies were described by M. Satoh (Kyowa Hakko Kirin, Japan). He presented work on the physiological mechanisms responsible for the higher therapeutic efficacy of non-fucosylated therapeutic IgG1, and why these antibodies can exhibit much higher ADCC. The data showed that non-fucosylated rituximab can activate human neutrophil functions involving phagocytes and increase MHC class II expression, which may favorably potentiate the adoptive immune response in cancer patients.

Workshop 3: Therapeutic Antibodies in the Neurosciences

After an introduction to the physiology of the blood brain barrier (BBB), E. Shusta (University of Wisconsin, Madison, USA) described use of yeast display on brain endothelial capillary cells to select scFv specific to BBB.12 One selected scFv recognized neural cell adhesion molecule (NCAM), which is involved in cell-cell adhesion. The ability of these BBB targets to promote transcytosis remains unclear.

The use of intrabodies for neurodegenerative diseases like Huntington disease was reported by A. Messer (Wodsworth Center, USA). An engineered scFv targeting the N-terminal portion of huntingtin can counteract the pathogenic aggregation phenotype in transfected brain slices, and in a Drosophila model. Similar strategies were used to select scFv targeting α-synuclein for Parkinson disease.

On the topic of prion-based diseases, A. Williamson (Calmune Corporation, USA) described antibodies selected to prevent interactions between PrPc and PrPsc. Some of the selected anti-PrPc mAbs induced an interesting decrease of PrPsc formation, but others yielded the opposite response. In vivo experiments using intracerebral injections of adenoviruses expressing the scFv of interest showed a decrease in formation of PrPsc.

Identifying and Predicting Antibody Efficacy and Toxicity

P. Senter (Seattle Genetics Inc, USA) shared data for antibody drug conjugates (ADC) employing auristatin as the drug payload linked to the antibody via two different linkers, first a protease-cleavable moiety containing a valine-citrulline molecule or second an uncleavable linker. In vivo studies demonstrated that even with an uncleavable linker, free drug could be found in tumors. The SGN-75 ADC targeting CD70 was exemplified, it yielded strong anti-tumor activity in a 786-o renal carcinoma xenograft model with a mouse maximum tolerated dose of about 100 mg/kg more than 10-fold above its anti-tumor efficacy. Decreasing the size of the targeting agent is highly beneficial for APCs to improve tumor penetration, thus ADCs based on diabodies against CD30 were designed, but they showed a poor tumor/tissue free drug ratio as compared to ADCs on whole mAbs. Their lead candidate, SGN-35 targeting CD30 received fast track designation from the US Food and Drug Administration (FDA) for Hodgkin lymphoma, and orphan drug designation from the FDA and the European Medicines Agency (EMEA) for both Hodgkin lymphoma and anaplastic large cell lymphoma.

B. Schoeberl (Merrimack Pharmaceuticals, USA) presented a systems biology analysis of key pathways of ErbB signalling, and explained why HER3 is an interesting target. MM-121 is a fully human anti-HER3 antagonist mAb that prevented heterodimerization of HER3 with other ErbB members, induced HER3 internalization and degradation, and inhibited PI3K and Akt activation. MM-121 showed in vivo anti-tumor activity alone or in combination with chemotherapy in DU145 and OVCAR3 xenograft models, and has entered Phase 1 clinical study. Original data were presented on MM-111, a bispecific structure based on two scFv binding to HER2 and HER3 linked via modified human serum albumin which increased its half-life.

The new Guidelines for Information About Antibody Therapy Experiments (GIAATE) that were designed to help accelerate drug development were discussed by R. Begent (University of College London, UK). GIAATE, which can be found at www.antibodysoociety.org, is a database of biological information needed to characterize any given antibody therapeutic. Examples from the TeGenero TGN1412 Phase 1 study and a radioimmunotherapy study of CHT25, a 131I-labeled chimeric anti-CD25 antibody, in Hodgkin disease were reported.13

M. Warncke (Novartis, Switzerland) presented data on the similarities and differences between human and cynomolgus macaque (Macaca fascicularis or crab-eating macaque) FcγRs. Data were based on recombinant expression of all types of FcγRs from...
Antibodies in the Treatment of Autoimmunity, Allergy and Infectious Diseases

I. Wilson (The Scripps Research Institute, USA) presented his group’s work on neutralizing mAbs and vaccines against Influenza viruses. Understanding how the immune system reacts against the virus allows the design of improved vaccines capable of providing efficient protection. The main target of neutralizing mAbs is the major envelope glycoprotein hemagglutinin (HA), which mediates viral attachment and membrane fusion. Dr. Wilson and colleagues investigated the hetero-subtype and the diversity of epitopes on HA for a variety of anti-influenza mAbs and their cognate HA isolated from elderly pandemic survivors. Co-crystal structures were determined for a broadly neutralizing human mAb CR6261 Fab in complex with HA derived from viruses responsible for the 1918 H1N1 influenza pandemic, as well as a recent case of H1N1 avian influenza. Notably, the CR6261 mAb binds exclusively via its heavy chain CDRs, and contains a very hydrophobic CDR-H2 derived from one particular human V-gene germline (IGHV1-69). The epitope identification of these mAbs may assist development of improved vaccines for prevention of influenza epidemics and therapeutics for treatment of influenza.

The advantages of employing the human immune system to produce mAbs against infectious agents were discussed by A. Lanzavecchia (Institute for Research in Biomedicine, Switzerland). One strategy is to immortalize human memory B cell by infection with EBV plus CpG, and then clone and sequence the genes from the antibodies of interest. Using this method, Dr. Lanzavecchia and colleagues isolated and developed human neutralizing mAbs against Dengue virus, human immunodeficiency virus (HIV) and Influenza from immunized donors. A cocktail of neutralizing mAbs directed against different epitopes of the Dengue virus E protein may be more efficient than monotherapy. Similarly, different neutralizing mAbs that recognize different subtype of Influenza were isolated from B cells of immunized patients. In the case of HIV, a series of 63 mAbs from memory B cells were isolated; three of these mAbs were broadly neutralizing.

D. Burton (The Scripps Research Institute, USA) presented the concept of ‘retro-vaccinology’ or ‘analytical vaccinology’, which is based on rational design of the best immunogen to be used in the development of an effective HIV vaccine for prophylactic and therapeutic use. The method involves generation of a large number of neutralizing antibodies selected from phage libraries, i.e., from hybridomas derived from patients’ B cells. Major difficulties with HIV are the antigenic variability of circulating strains. An international ‘Neutralizing Antibody Consortium’ collected sera from about 1,800 donors worldwide; the neutralizing capacities of the samples were determined via a screen on about 60 different HIV isolates. Elite neutralizers were then identified. Upon cloning of these mAbs, a reconstituted neutralizing serum could be obtained by mixing 5–6 different mAbs.

Symplex™ technology, which involves isolation of large repertoires of high affinity mAbs specific for human antigens by cloning from single antibody producing cells of immune individuals, was described by J. Haurum (Symphogen, Denmark). A key feature of this method is the ability to capture immune antibody repertoires while preserving the diversity and the original pairing of VH and VL regions. A set of unique, high-affinity mAbs against vaccinia virus, tetanus toxoid or respiratory syncytial virus (RSV) were isolated from human plasma cells of immunized volunteers or diseased patients. As an example, anti-RSV Sym003 comprises a mixture of six different mAbs directed against both RSV protein G and E. In a next step, the Sympress™ technology is applied to produce recombinant polyclonal antibodies.

Imaging in Cancer Drug Discovery

Polycationic sequences called cell penetrating peptides (CPPs) can drag various payloads onto and into cells, but in a non-cell-selective manner. R. Tsien (University of California San Diego, USA) presented a novel approach using activatable CPP (ACPP) for imaging active matrix metalloproteases (MMP). These ACPP consisted of a polyarginine sequence quenched by a polyanionic sequence making electrostatic interactions. These two sequences were linked together with a MMP2/MMP9-cleavable sequence. The CPP activity of the polyarginine is only active upon cleavage of the linker and dissociation of the polyanionic counterpart. As an example, the molecule [Glu]₈-xPLLAG-[Arg]₈-Cy5 showed a strong and selective tumor labeling six hours after intravenous injection due to specific MMP activity at the tumor site.
K. Wittrup (Massachusetts Institute of Technology, USA) discussed Pretargeted-Radioimmunotherapy (PRIT). One major advantage of PRIT corresponds to the targeting capacity and the amount of bispecific antibody localized in tumors. Using in vivo results from molecules of different size and advanced mathematic modeling, data could be obtained concerning the best format of antibody to be used for PRIT. For large molecules (>100 kDa), strong affinity is apparently not a key issue for good tumor localization. In contrast, for small molecules below 15 kDa, strong affinity (<100 pM) is required. Concerning transport parameters, the larger the molecule, the worse the outcome. Dr. Wittrup also described a novel bispecific format corresponding to a conventional mAb with an additional scFv fused to the C-terminus of the Cκ domain.

A. Wu (University of California Los Angeles, USA) gave an overview of the best antibody formats for imaging. The serum half-life of a prototypical anti-CEA scFv-Fc fusion protein was modulated by mutation in its Fc portion; t1/2 ranging from 200 hours (wild type Fc) to eight hours (Fc with two point mutations His310Ala/His435Gln) were obtained. The minibody structure corresponds to scFv-CH3 fusion, and represents a good balance between tumor penetration and half-life. An anti-PSCA minibody generated good image quality 20 hours after injection (good signal/noise ratio). In contrast, whole IgG required 168 hours post injection to yield enough background reduction.

S. Ram (University of Texas Southwestern Medical Center, USA) discussed ways in which three-dimensional (3D) intracellular trafficking pathways of mAbs in live cells can be used to understand their role in mediating immune responses. Multifocal plane microscopy and quantum dot technologies were applied to perform quantification and characterization of 3D intracellular events. 3D videos of trafficking itineraries for IgG and FcRn from the plasma membrane to intracellular compartments in living cells via endocytosis or exocytosis were presented.15

References
1. Dey A, Verma CS, Lane DP. Updates on p53: modulation of p53 degradation as a therapeutic approach. Br J Cancer 2008; 98:4-8.
2. Lain S, Hollick JJ, Campbell J, Staples OD, Higgins M, Aoubala M, et. al. Discovery, in vivo activity, and mechanism of action of a small-molecule p53 activator. Cancer Cell 2008; 13:454-63.
3. Walensky LD, Piter K, Morash J, Oh KJ, Barbuto S, Fisher J, et. al. A stapled BID BH3 helix directly binds and activates BAX. Mol Cell 2006; 24:199-210.
4. Bernal F, Tyler AF, Korsemeyer SJ, Walensky LD, Verdin GL. Reactivation of the p53 tumor suppressor pathway by a stapled p53 peptide. J Am Chem Soc 2007; 129:2456-7.
5. Mazor Y, Van Blarcom T, Iverson BL, Georgiou G. E-clonal antibodies: selection of full-length IgG antibodies using bacterial periplasmic display. Nat Protocol 2008; 3:1766-77.
6. Adams GE, Weiner LM. Monoclonal antibody therapy of cancer. Nat Biotechnol 2005; 23:1147-57.
7. Chu SY, Vostier I, Karki S, Moore GL, Lazar GA, Pong E, et. al. Inhibition of B cell receptor-mediated activation of primary human B cells by coengagement of CD19 and FcγRIIB with Fc-engineered antibodies. Mol Immunol 2008; 45:3926-33.
8. Vaccaro C, Zhou J, Ober RJ, Ward ES. Engineering the Fc region of immunoglobulin G to modulate in vivo antibody levels. Nat Biotechnol 2005; 23:1283-88.
9. Mi W, Wanjie S, Lo S-T, Gan Z, Pickl-Herk B, Ober RJ, et. al. Targeting the neonatal Fc receptor for antigen delivery using engineered Fc fragments. J Immunol 2008; 181:7550-61.
10. Montoyo HP, Vaccaro C, Haefner M, Ober RJ, Mueller W, Ward ES. Conditional deletion of the MHC class I-related receptor FcRn reveals the sites of IgG homeostasis in mice. Proc Natl Acad Sci USA 2009; 106:2788-93.