

# 6<sup>th</sup> Annual European Antibody Congress 2010

## November 29–December 1, 2010, Geneva, Switzerland

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The 6<sup>th</sup> European Antibody Congress (EAC), organized by Terrapinn Ltd., was held in Geneva, Switzerland, which was also the location of the 4<sup>th</sup> and 5<sup>th</sup> EAC.<sup>1,2</sup> As was the case in 2008 and 2009, the EAC was again the largest antibody congress held in Europe, drawing nearly 250 delegates in 2010. Numerous pharmaceutical and biopharmaceutical companies active in the field of therapeutic antibody development were represented, as were start-up and academic organizations and representatives from the US Food and Drug Administration (FDA). The global trends in antibody research and development were discussed, including success stories of recent marketing authorizations of golimumab (Simponi<sup>®</sup>) and canakinumab (Ilaris<sup>®</sup>) by Johnson & Johnson and Novartis, respectively, updates on antibodies in late clinical development (obinutuzumab/GA101, farletuzumab/MORAB-003 and itolizumab/T1 h, by Glycart/Roche, Morphotek and Biocon, respectively) and success rates for this fast-expanding class of therapeutics (Tufts Center for the Study of Drug Development). Case studies covering clinical progress of girentuximab (Wilex), evaluation of panobacumab (Kenta Biotech), characterization of therapeutic antibody candidates by protein microarrays (Protagen), antibody-drug conjugates (sanofi-aventis, ImmunoGen, Seattle Genetics, Wyeth/Pfizer), radio-immunoconjugates (Bayer Schering Pharma, Université de Nantes) and new scaffolds (Ablynx, AdAlta, Domantis/GlaxoSmithKline, Fresenius, Molecular Partners, Pieris, Scil Proteins, Pfizer, University of Zurich) were presented. Major antibody structural improvements were showcased, including the latest selection engineering of the best isotypes (Abbott, Pfizer, Pierre Fabre), hinge domain (Pierre Fabre), dual antibodies (Abbott), IgG-like bispecific antibodies (Biogen Idec), antibody epitope mapping case studies (Eli Lilly), insights in FcγRII receptor (University of Cambridge), as well as novel tools for antibody fragmentation (Genovis). Improvements of antibody druggability (Abbott, Bayer, Pierre Fabre, Merrimack, Pfizer), enhancing IgG pharmacokinetics (Abbott, Chugai), progress in manufacturing (Genmab, Icosagen Cell Factory, Lonza, Pierre Fabre) and the development of biosimilar antibodies (Biocon, Sandoz, Triskel) were also discussed. Last but not least, identification of monoclonal antibodies (mAbs) against new therapeutic targets (Genentech, Genmab, Imclone/Lilly, Vaccinex) including Notch, cMet, TGFβRII, SEMA4D, novel development in immunotherapy and prophylaxis against influenza (Crucell), anti-tumor activity of immunostimulatory antibodies (MedImmune/Astra Zeneca) and translations to clinical studies including immunogenicity issues (Amgen, Novartis, University of Debrecen) were presented.

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**Day 1: November 29, 2010**

**Alain Beck**

The EAC chairman, Alain Beck (Centre d'Immunologie Pierre Fabre), opened the meeting with a presentation on strategies and challenges for the next generation of therapeutic antibodies.<sup>3</sup> By analyzing the regulatory approvals of IgG-based biotherapeutic agents in the past ten years, we can gain insights into the successful strategies used by pharmaceutical companies so far to bring innovative drugs to the market. Strategies to optimize the structure of IgG antibodies and to design related or new structures with additional functions were presented. A detailed knowledge of antibody structure and activity now allows researchers to engineer primary antibodies on a more rational basis. Most approved antibodies are chimeric, humanized or human IgGs with similar constant domains. Numerous studies looking at the structure-function relationships of these antibodies have been published in the past five years with the aim of identifying antibody microvariants<sup>4</sup> and investigating the influence of these variants on antigen binding, stability, pharmacokinetics (PK) and pharmacodynamics (PD). This knowledge is now being used to increase homogeneity and mitigate the chemistry, manufacture and control (CMC) liabilities of preclinical antibody candidates by genetic engineering. The removal by mutation of instability or aggregation hot spots in the antibody complementarity-determining regions (CDRs), and the use of hinge-stabilized or aglycosylated IgG4, are just a few examples of antibodies with improved pharmacological properties, including decreased heterogeneity, that are currently in development.

Dr. Beck explained that the variable fragment (Fv) of an antibody is responsible for interactions with antigens and dictates essential properties such as binding affinity and target specificity. The origin of the Fv in therapeutic antibodies can be diverse, e.g., hybridomas, human antibody libraries, rodents with a human antibody repertoire or primatized or humanized antibodies from various species. Affinity maturation allows the binding affinity of the Fv to be improved or target selectivity to be modulated. The constant fragment (Fc) of an antibody is responsible for interactions with immune cells, and the associated properties of the Fc can also be modulated by engineering at several levels:<sup>5</sup> altering the glycosylation status to regulate anti- and pro-inflammatory properties, modulating antibody-dependent cellular cytotoxicity (ADCC) by site-directed mutagenesis to alter binding to Fc receptors, increasing the serum half-life by Fc engineering to increase binding to the neonatal Fc receptor (FcRn), thereby preventing IgG degradation, and increasing complement activation by isotype chimerism. Additional functions can be endowed on antibodies by conjugation to other drugs. To date, the clinical success of antibody-drug conjugates (ADCs) has been limited. Nevertheless, promising new ADCs that include linkers with optimized properties (e.g., hydrolysable in the cytoplasm, resistant or susceptible to proteases or resistant to multi-drug resistance efflux pumps) and highly cytotoxic drugs are being studied in advanced clinical trials

(e.g., trastuzumab emtansine, inotuzumab ozogamicin and brentuximab vedotin).<sup>6</sup> IgGs have also been engineered to contain unique drug conjugation positions to obtain uniform and more homogeneous drug conjugates, such as ThioMab-drug conjugates, which have a uniform stoichiometry of approximately two coupled drugs per antibody molecule. Collectively, these advances should open new therapeutic avenues to deliver highly cytotoxic drugs with increased tolerability.

### **Antibody and Related Products: Choosing the Right Format for the Right Indication**

**Andrew Goodearl** (Abbott) discussed the strategies and solutions to ensure that the right indication is met with the best antibody isotype.<sup>7</sup> All the currently approved therapeutic antibodies are G-type immunoglobulins (IgGs) and derivatives of mouse, human or mixed origin. Human IgGs are divided into four subclasses or isotypes defined by different heavy chains ( $\gamma$ 1,  $\gamma$ 2,  $\gamma$ 3 and  $\gamma$ 4 in a 66/23/7/4 ratio in plasma) and different disulfide pairings. The 3-dimensional structures of IgGs are maintained by non-covalent interactions and by disulfide bridges, with specific numbers and characteristic connections for each isotype. These precise linkages can be established by liquid chromatography coupled to on-line mass spectrometry, as illustrated by peptide maps of IgG1, IgG2 and IgG4. IgG3s are characterized by a longer and more flexible hinge domain and the presence of 11 inter-heavy chain disulfide bridges (vs. two for IgG1s and IgG4s, and four for IgG2s). Despite a high ADCC potential, IgG3s are generally not selected for therapeutic antibody development mainly because the plasmatic half-life is shorter than that for the three other isotypes (7 vs. 21 days, respectively).

The case studies shown by Dr. Goodearl demonstrate that IgG1, IgG2 and IgG4 preclinical properties should be evaluated to characterize influence on activity. If an effector function active isotype required is required for efficacy, IgG1 should be selected.<sup>8</sup> On the safety side, effector function inactive isotype selection helps to minimize inadvertent non-mechanistic toxicity. However, ineffective clearance of immune complexes may also cause undesirable consequences. Isotype-dependent oligomerization properties (IgG2-dimerization and IgG4-half molecules) should be taken into account. IgG mutants with eliminated/enhanced effector function (IgG1) or modulated quaternary structure (IgG2, IgG4) may be deployed. Isotype selection should also be conducted in parallel with variable domain selection, with careful evaluation of isotype vs. epitope and, last but not least, drug-like properties should be included in the isotype evaluation.

**Mike Schlittler** (Pfizer Global Biologics) provided an overview of the complexity of the human IgG2 isotype.<sup>9</sup> Recent studies of human IgG2 antibodies have revealed several disulfide-linked structural isoforms.<sup>10</sup> The accepted structure of human IgG2 that contains four symmetrical disulfide bonds in the hinge region was inferred from the crystallographic structures of murine antibodies and via enzymatic digestion of myeloma protein. This historical form is referred to as IgG2-A, but it has proven to be the least abundant form in most of the human IgG2

antibodies studied. Two other newly identified disulfide isoforms are referred to as IgG2-B and IgG2-A/B. IgG2-B is defined by a symmetrical arrangement of both Fab regions covalently linked to the hinge. IgG2-A/B represents an asymmetrical arrangement involving one Fab arm covalently linked to the hinge. The combination of RP-HPLC with other orthogonal techniques (e.g., capillary gel electrophoresis and cation exchange chromatography) permits characterization of disulfide-mediated IgG2 heterogeneity during the drug development process. During development and commercialization, monitoring the IgG2 disulfide isomer distribution is necessary to ensure drug product consistency. Dr. Schlitter showed that the *in vitro* potencies of isolated disulfide isomers were not different in ELISA and cell based assays. Cation exchange chromatography was a useful mode for separation and isolation of IgG2 disulfide isomers. Mobile phase is “gentle” compared to other solvents and more amenable to subsequent bioassay measurements. Isolated fractions can be tested in bioassay by simple dilution to minimize handling. Chromatography pH was identified as critical for separation of isomers, and it separates other species as well, e.g., acidic species, methionine oxidation.

In summary, Dr. Schlitter demonstrated that multiple analytical methods (nrCGE, RP-HPLC, Imaging Capillary Electrophoresis and peptide mapping) were valuable for further characterization and identification of cation exchange chromatography pools and fractions.<sup>11,12</sup> Sample handling is critical during isolation of fractions, so having accurate protein quantitation allows testing of more dilute (less manipulated) samples for proper data interpretation. Accurate protein quantitation is essential before doing *in vitro* potency assessments. The method selected should have a wide dynamic range (ug/mL to mg/mL). Plate-based methods may help if sample size is limited—bicinchoninic acid (BCA) assay was optimal for this study and other ongoing studies. Analysis of many fractions across the cation exchange HPLC separation confirmed the findings that the pooled isolates were not different in *in vitro* potency assays.

### Improving Efficacy and Druggability of mAbs

The use of structural analytical methods and molecular modeling to improve antibody “druggability” was discussed by **Alexey Lugovskoy** (Merrimack Pharmaceuticals).<sup>13</sup> Several pathways exist whereby marginally stable proteins may lose their function as they form either soluble or insoluble aggregate structures. Such non-native aggregates are encountered routinely in biopharmaceutical processing during fermentation, refolding and purification of materials, as well as shipping and product storage. For mAbs, these aggregation issues are often more pronounced because of the complex multidomain structure of these molecules. An increasing number of therapeutic molecules are based on re-engineered antibody fragments. Although these fragment-based molecules may possess more favorable therapeutic profiles compared with those of their parent IgGs, they may not be as stable, which emphasizes the need for further understanding and engineering to improve protein stability. A variety of experimental and computational methods are being used to increase

molecular stability of proteins, including directed evolution and computational protein design. Although these methods have been largely successful in generating more stable molecules, achieving the ultimate goal of understanding what, at the molecular level, contributes to stabilization and arriving at rapid and robust tools for protein engineering has remained challenging.

Dr. Lugovskoy explained that, based on a hierarchical structure-based mutant selection strategy using the stepwise evaluation of the components of the energy function, a molecular modelling program has been developed. This new approach, featuring multiple filter criteria based on the patterns of hydrophobic packing and hydrogen bonding within antibody domains, enhanced the ability to identify stabilizing mutations and led to the identification of a new stabilizing mutation that was not detected by other design methods. This strategy provides a general method for identifying and assessing the value of stability-altering mutations, which can be applied to the engineering of other antibody systems or multi-domain proteins to enhance thermal and chemical stability, leading to optimized manufacturing processes, minimal non-native aggregate formation and an extended product shelf-life. Mechanistic modelling can serve as an important tool in designing an optimal antibody-like biotherapeutic. New antibody-like scaffolds present an inherent risk of suboptimal pharmaceutical properties that can limit therapeutic efficacy and the speed of development, but this risk can be mitigated by designing diverse sets of antibody modules in parallel during therapeutic design project stage. Triage based on suboptimal activities or limited stabilities at microscale is informative and practical. The approach presented by Dr. Lugovskoy successfully combines rational design and yeast display to engineer molecules with improved potency and favourable pharmaceutical properties.

**Thierry Wurch** (Centre d’Immunologie Pierre Fabre) presented data highlighting the importance of the antibody hinge region, located between the heavy chain CH1 and CH2 domains, in the control of the functional activity, including not only effector functions, but also antigen responses. This particular region provides structural flexibility to both variable and constant domains via Fab arm rotation and waving and Fc rotation, and controls Fab-Fc planar folding. Numerous Cys residues are involved in interchain (H-H or H-L) bounds. These major structural features are directly associated with the primary amino acid sequence of the hinge region, and thus to the antibody isotype. Numerous mutants or chimeric hinge regions, constructed by swapping portions from different Ig isotypes and especially human IgG1 and IgG3, have been evaluated for their effector functions.<sup>14</sup> A clear implication of the lower hinge portion, which is part of the CH2 domain based on genetic criteria, in complement activation was demonstrated. Some mutants located in the middle hinge portion affected FcγR binding and ADCC.

On the other hand, much less information is available on the role of the hinge region in antigen binding. Dr. Wurch and colleagues constructed a large series of mutants in this hinge region that modulate the flexibility and rigidity of the Fab portion; mutations included insertion of additional cysteine residues and amino acid deletions (1 to 4). These modifications were

associated with a strong impact on the intrinsic activity of the resulting antibody mutants towards the target antigen: both agonistic and antagonistic activities were strongly modulated, ranging from weak to strong partial agonist and weak to efficacious antagonist. Introduction of additional cysteine residues was in some cases associated with mis-pairing of heavy and light chains, as already mentioned for wild-type human IgG2 mAbs.

**Sandeep Kumar** (Pfizer Global R&D) presented methods that interweave sequence optimization and affinity maturation, including comprehensive site-directed saturation mutagenesis and high-throughput antibody optimization routinely applied in his company.<sup>15,16</sup> The process was shown to be stable, universally applicable and automation-supported. Aggregation is the most common degradation pathway for antibodies. Besides their potential to impact drug potency, aggregates are also considered a risk factor for immunogenicity. In particular, cross  $\beta$ -aggregates in biotherapeutics have the potential to be immunogenic. Hence, fundamental understanding as to why some molecules are more prone to aggregation than others would go a long way towards reducing or even eliminating this risk factor. Contribution of short aggregation-prone regions (APRs), which may self-associate via cross- $\beta$  motifs and were earlier identified in therapeutic mAbs, towards antigen recognition may be evaluated via structural analyses of antibody-antigen complexes. Similar to small proteins and peptides, the potential APRs in the antibodies are also short sequence regions. Hence, one or a few changes in sequences that disrupt the CDR-localized APRs may significantly reduce aggregation propensity of the mAbs. From a product formulation and developability point of view, improvement in mAb stability and solubility is desirable. This may help improve expression levels in cell lines, facilitate high concentration dosage forms and increase shelf-life of the product. However, developability-related sequence mutations should not adversely impact the potency of the therapeutic mAbs. In this regard, the observed incidence of potential APRs in the CDR loops and adjacent framework  $\beta$ -strands is significant. Disruption of these APRs without affecting therapeutic mAb potency could be difficult and time-consuming without a rational approach. A structure-based input that simultaneously considers all these issues may lead to more “druggable” therapeutic candidates.

### Antibody PK and Metabolic Studies

**Ivan Correia** (Abbott Bioresearch) discussed stability of IgG isotypes in serum and the potential implications for drug development.<sup>17-19</sup> Analysis of antibody therapeutics recovered after circulation in blood show altered physicochemical characteristics. In many instances, therapeutic antibodies recovered from serum show lower potency. Technologies that can facilitate rapid screening of candidate antibody therapeutics directly from blood were highlighted. Antibody therapeutic development programs must incorporate understanding of the basic biology of the isotype and its stability in serum, which is the intended environment of the therapeutic. Engineering stabilizing mutations in the molecule and monitoring for new characteristics after circulation in the blood may help mitigate potentially adverse effects. PK studies and appropriate technologies to

recover and rapidly evaluate candidate molecules directly from blood can help in choosing an appropriate IgG isotype for further development.

The effects of Fc glycan structure on clearance for different therapeutic antibodies should be evaluated on a case-by-case basis, preferably during clinical PK studies. These studies are relevant not only for clinical justification for setting glycan specifications, but they also establish bioequivalence for the therapeutic, especially if a particular glycan is preferentially cleared from serum. A total of 16 disulfide bonds are found in IgG1 molecules. Correct pairing of the 16 disulfide bonds takes place in the endoplasmic reticulum (ER), and the process is aided by chaperones and enzymes such as protein disulfide isomerase. However, IgG1 molecules will occasionally have a population of molecules where the intra-chain disulfide bond in the VH domain between Cys22-Cys96 residues is not paired. Further, the unpaired cysteine residues can be found in molecules extracted from the ER/Golgi, suggesting that, despite the unpaired cysteine residues, they are not targeted for degradation via the ubiquitin pathway (Correia IR and Alessandri L, unpublished results). In a significant finding, Ouellette et al. recently reported that the unpaired cysteine residues reformed their disulfide bonds when the mAb is exposed to serum.<sup>19</sup>

**Tomoyuki Igawa** (Chugai Pharmaceutical) presented data on antibody PK improvement by guided molecular design. Fc engineering to increase the binding affinity of IgG antibodies to FcRn has been reported to reduce the elimination of IgG antibodies. Dr. Igawa presented two novel non-FcRn-dependent approaches to reduce the elimination of IgG antibodies.<sup>20,21</sup> PK studies conducted in normal mice of various humanized IgG4 antibodies that had identical constant regions, but different variable region sequences, revealed that an antibody with a lower isoelectric point (pI) has a longer half-life. These antibodies exhibited comparable binding affinity to FcRn, and with the antibodies with lower pIs, a longer half-life was also observed in *b2*-microglobulin knockout mice, suggesting that differences in PK were due to a non-FcRn-dependent mechanism. On the basis of these findings, the PK properties of a humanized anti-IL6 receptor IgG1 antibody were engineered. Selected substitutions in the variable region, without substitution in the Fc region, lowered the pI but did not reduce the biological activity, and showed a significant reduction in the clearance of the antibody in cynomolgus monkey. These results suggest that lowering the pI by engineering the variable region could reduce the elimination of IgG antibodies and provide an alternative to Fc engineering of IgG antibodies.

Engineering the variable region to lower the pI to reduce elimination can be applied to the optimization of a lead antibody such as a humanized antibody, in tandem with affinity maturation, stability improvement and deimmunization. Using a combination of optimization technologies, a humanized antibody can be engineered to have a longer half-life, a stronger affinity to antigen, a more stable formulation and minimum immunogenicity in human. Selection of a lead antibody or clinical candidate from various clones could be simplified by focusing on antibodies with a lower pI and reduce the number of laborious PK studies.

**Andreas Plückthun** (University of Zurich) addressed PK/PD comparisons between antibodies and small protein scaffolds.

Slow-clearing, tumor-targeting proteins such as mAbs typically exhibit high tumor accumulation, whereas intermediate-sized proteins such as scFvs show faster clearance but only moderate tumor accumulation. For both, tumor targeting does not seem to improve above an optimal affinity.<sup>22</sup> Dr. Plückthun showed that limits can be overcome with very small high-affinity proteins such as designed ankyrin repeat proteins (DARPs). Dr. Plückthun and colleagues systematically investigated the influence of molecular mass and affinity on tumor accumulation with DARPs with specificity for HER2 in SK-OV-3.ip nude mouse xenografts. DARPs with a mass of 14.5 kDa and affinities between 270 nmol/L and 90 pmol/L showed a strong correlation of tumor accumulation with affinity to HER2, with the highest affinity DARP reaching 8% injected dose (ID)/g after 24 h and 6.5% ID/g after 48 h (tumor-to-blood ratio >60). Tumor autoradiographs showed good penetration throughout the tumor mass. Genetic fusion of two DARPs (30 kDa) resulted in significantly lower tumor accumulation, similar to values observed for scFvs, whereas valency had no influence on accumulation. PEGylation of the DARPs increased the circulation half-life, leading to higher tumor accumulation (13.4% ID/g after 24 h), but lower tumor-to-blood ratios.

Dr. Plückthun explained that affinity was less important for tumor uptake of the PEGylated constructs. Two regimes exist for delivering high levels of drug to a tumor in which the importance of affinity is less pronounced: (1) small proteins with very high affinity, e.g., unmodified DARPs; and (2) large proteins with extended half-life, e.g., PEGylated DARPs. DARPs and other small, very soluble proteins with picomolar affinity also show high tumor accumulation with very fast clearance through the liver and kidney. This is probably due to high extravasation and tumor penetration, and requires a very slow off-rate from the target, which leads to efficient tumor retention. Targeting is proportional to affinity and no plateau with affinity was seen, suggesting that still higher affinities might further increase tumor targeting. Very large proteins such as mAbs or PEGylated DARPs achieve even higher tumor loads due to the long circulation and (slow) equilibration with the tumor, but the elimination from blood and non-tumor tissue is also slow, and tumor-to-tissue ratios remain lower. Intermediate size molecules (fused DARPs, scFv fragments) show lower tumor accumulation because elimination through the kidney is equally rapid, but extravasation, and perhaps tumor diffusion, is diminished.

### **Fab/Antigen or Fc/Fc $\gamma$ Receptors Co-Crystallization Studies to Gain Insights on Structure and mAb Mechanisms of Action**

Marion Espeli (University of Cambridge) highlighted the role of Fc $\gamma$ RIIB in autoimmunity and infection and discussed the evolutionary and therapeutic implications. Receptors for the Fc region of IgG (Fc $\gamma$ Rs) are expressed by many immune cells and are important in both promoting and regulating the immune and inflammatory response to immune complexes.<sup>23</sup> Most Fc $\gamma$ Rs are activating receptors and include the high-affinity receptor Fc $\gamma$ RI and a family of low affinity receptors, including

Fc $\gamma$ RIIA, Fc $\gamma$ RIIC, Fc $\gamma$ RIIAA and Fc $\gamma$ RIIIB in humans, and Fc $\gamma$ RIII and Fc $\gamma$ RIV in mice. Fc $\gamma$ RIIB is the only Fc $\gamma$ R that has an inhibitory function. Tumor necrosis factor (TNF) upregulates the expression of activating Fc $\gamma$ RIIA on neutrophils *in vitro*, and amplifies immune complex-induced activation of neutrophils *in vivo*. TNF blockade with infliximab in patients with rheumatoid arthritis led to a decrease in expression of Fc $\gamma$ RIIA and increased expression of Fc $\gamma$ RIIB on neutrophils in some patients. The increased expression of Fc $\gamma$ RIIB was associated with a decrease in disease severity, suggesting that the efficacy of TNF blockade may be in part due to modulation of Fc $\gamma$ RIIB expression levels. Fc $\gamma$ RIIB, which was identified as the mediator of Fc fragment induced B-cell suppression over 30 years ago, is now known to be widely expressed and to control key aspects of immunity. Variation in its complex and often subtle regulation is crucially involved in determining susceptibility to autoimmunity and defence against infection. Better understanding of this variation is beginning to provide insight into the evolution of disease susceptibility and to open new opportunities for therapy.

Victor Obungu (Eli Lilly & Co.) discussed Fas ligand therapeutic antibody epitope mapping. Fas ligand (FasL) is a 40 kDa type II transmembrane protein belonging to the TNF family of proteins and binds to its specific receptor Fas, a member of the TNF receptor family. Membrane-bound FasL can be processed into a soluble form by a metalloprotease similar to that which cleaves TNFR.<sup>24</sup> Elevated levels of FasL have been implicated in a wide variety of diseases ranging from cancer to inflammatory abnormalities that could be targeted by antibody therapy. A human high-affinity antibody against FasL that binds to and neutralizes the activity of both soluble and membrane-associated human FasL was generated. In order to elucidate the mechanism of the function of this antibody, the epitope region was mapped, as well as critical residues involved in the recognition of FasL, using a combination of homology modelling, immunoprecipitation, hydrogen-deuterium exchange mass spectrometry (H/DXMS) and alanine scanning site-directed mutagenesis. These studies revealed the antibody binding site on human FasL. Furthermore, through molecular homology modelling, a mechanism was proposed for the neutralizing activity of this antibody that involves interference with the docking of the ligand to its receptor by the antibody. LA296 antibody is an IgG4 mAb with a binding affinity of 100 pM for soluble FasL. LA296 binds to and neutralizes the activities of both soluble and membrane-associated human and cynomolgus monkey FasL, but not that of mouse or rabbit, as determined by ELISA and BIAcore measurements.

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## Day 2: November 30, 2010

Thierry Wurch

The morning of the second day, chaired by **Paul Parren** (Genmab), was dedicated to recent advances in treating autoimmune, immunology and cancer diseases with antibody therapeutics. The afternoon was split into two parallel sessions. Stream A focused on novel antibody targets, the safety and immunogenicity of antibodies, and the clinical development of novel antibodies; and Stream B focused on antibody production scale-up, process transfer and comparability, as well as the future of bio-similar antibody development in Europe.

### Antibody Advances and Developments: Autoimmune and Immunology

**Janice Reichert** (Tufts University) summarized the most recent advances in the clinical development of antibodies and related molecules such as Fc-fusion proteins and immunoconjugates.

Her data set includes more than 600 products that entered clinical development, focusing on company-sponsored studies only. In 2010, two novel mAbs were approved and four more are under regulatory review by the US Food and Drug Administration (FDA). During the 1990–2009 period, about half (51%) of the antibodies that entered clinical trials was for cancer indications and 28% for immunological-related diseases. The mean duration for clinical and approval phases was 8.2 years (mean for 23 US-approved mAbs). Thirteen mAbs are currently in clinical Phase III, several of them targeting novel antigen classes like folate alpha receptor (farletuzumab) or carbonic anhydrase IX (girentuximab). Of interest, three immunoconjugates reached late-stage clinical trials, one being a derivative of the naked mAb trastuzumab. In the longer term, small protein scaffolds that may or may not be derived from antibody structures have also progressed towards clinical trials. These novel structures should be watched carefully in the future.<sup>1</sup>

**Omid Vafa** (Johnson & Johnson) summarized the most relevant preclinical and clinical data for the recently approved antibody golimumab (Simponi™), approved in 2009 (US, Canada) for three major indications: rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis. One important feature of the marketed product is its unique subcutaneous (s.c.) administration using the SmartJect™ autoinjector. Golimumab is a human anti-TNF antibody (Medarex HuMab technology) produced as a human IgG1/kappa isotype. Its preclinical activity profile showed high-affinity binding to TNFα, potent neutralization of bioactive TNFα, and no lysis of human monocytes. Golimumab was more potent than infliximab in the Tg197 mouse polyarthritis model. Golimumab was formulated as a 100 mg/ml solution to be used in s.c route via injector (monthly administration as 0.5 mg in 0.5 ml volume). Five Phase 3 studies that included more than 2,300 patients were initiated simultaneously in 2006 and completed between 2007 and 2008. Additional indications are currently being pursued (ulcerative colitis Phase 2/3 trials are ongoing, sarcoidosis POC study is in progress). A general review of golimumab's development was recently published.<sup>2</sup>

The last presentation of the session, given by **Thomas Jung** (Novartis), summarized the preclinical and clinical development process that led to the 2009 approval of canakinumab (Ilaris™) as an orphan drug for the treatment of cryopyrin-associated periodic syndromes and Muckle-Wells syndrome in both the US and EU. Canakinumab is a human anti-IL-1β antibody (Medarex UltiMab technology). IL-1β is a mediator of many pivotal biological functions such as fever, chills, anorexia, somnolence, myalgia, arthralgia, hypotension; it controls IL-6, sTNF receptors, IL-1Ra, IL-1 itself and G-CSF release. The preclinical activity profile of canakinumab showed picomolar binding to human IL-1β but not to rodent, and strong neutralization of human IL-1β-induced fever in a rat model. One pivotal Phase 3 study (D2304; NCT00465985) was designed: it corresponded to a 3-part randomized, 48-week duration study in patients with CAPS. Patients were treated for eight weeks (part 1) with canakinumab (150 mg), responders were randomized in a 24 week withdrawal study (part 2) and re-entered treatment for 16 weeks (part 3). In part 1, 97% of patients responded

to canakinumab and remained in remission during part 2. In contrast, 81% of the patients relapsed in the placebo group. Ninety percent of patients completed part 3 in full remission. Canakinumab is currently being investigated in several Phase 2 and Phase 3 trials in other inflammasome-mediated indications such as gouty arthritis and systemic onset of juvenile idiopathic arthritis. A general review of canakinumab's development was recently published.<sup>3</sup>

### Antibody Advances and Developments: Oncology

**Pablo Umana** (Roche Glycart) presented an overview on the research activities and preclinical development of GA101, a next-generation anti-CD20 antibody carrying glyco-engineering improvements. GA101 represents the first Fc-engineered, type II humanized IgG1 antibody against CD20. Relative to rituximab, GA101 has increased direct and immune effector cell-mediated cytotoxicity and exhibits superior activity in cellular assays and whole blood B-cell depletion assays. In human lymphoma xenograft models, GA101 exhibited superior antitumor activity, resulting in the induction of complete tumor remission and increased overall survival. In non-human primates, GA101 demonstrated superior B-cell depleting activity in lymphoid tissue, including in lymph nodes and spleen.<sup>4</sup>

An update on the clinical development of farletuzumab (MORAb-003) was given by **Martin Phillips** (Morphotek/Eisai). Farletuzumab is a humanized monoclonal antibody directed against folate receptor  $\alpha$  (FRA). This receptor is overexpressed in >90% of ovarian cancer and the degree of FRA expression correlates with the grade of malignancy in vivo. Farletuzumab has been shown to suppress phosphorylation by Lyn kinase, and it was active in ADCC and CDC assays as well as in a SKOV3 mouse xenograft model.<sup>5</sup> Farletuzumab showed a favorable toxicity profile in non-human primate studies.<sup>5</sup> During a standard Phase 1 dose escalation study in heavily-pretreated, platinum-resistant ovarian cancer patients, no dose limiting toxicity was observed and trends toward clinical efficacy were suggested. A Phase 2 study was designed with both single-agent and carboplatin/taxane (paclitaxel or docetaxel) combination therapy arms. CA125 biomarker status was normalized in approximately 89% of subjects in platinum-sensitive relapse treated with carboplatin, taxane and farletuzumab. Patients with first platinum-free interval of less than 12 months responded as did those with first platinum-free interval above one year.<sup>6</sup> Approximately 21% of second intervals are as long or longer than the first interval based on CA125 status. Randomized clinical studies are ongoing to evaluate farletuzumab in platinum-sensitive and platinum-resistant relapsed ovarian cancer.

**Norman Neville** (Wilex AG) presented a progress report on the ongoing pivotal Phase 3 trial of their investigational drug girentuximab (Rencarex®, WX-G250). Girentuximab is a chimeric antibody that binds to carbonic anhydrase IX (CA IX or G250 antigen). CA IX is expressed on the cell surface in over 90% of all clear cell renal cell cancer (ccRCC), but not expressed in normal kidney tissue. The antigen is also present in bladder cancer and a variety of other solid tumors, including cervical,

colon, breast and non-small cell lung cancer. The antigen is expressed at low levels in the gastric epithelium, upper intestinal tract epithelium, and the bile ducts, but is undetected in normal kidney and other tissues. Girentuximab is also able to trigger strong antibody-dependent cellular cytotoxicity (ADCC). Through this mechanism, effector cells, e.g., natural killer (NK) cells, of the immune system are activated to kill the tumor cells.<sup>7</sup>

Dr. Neville explained that in Phase 1 and 2 studies, with more than 100 ccRCC patients treated, girentuximab has shown good safety and tolerability and a promising efficacy profile. Girentuximab is currently in a pivotal Phase 3 trial (ARISER; NCT00087022) as an adjuvant therapy of patients with non-metastatic ccRCC. ARISER is a double-blind, placebo-controlled Phase 3 study to assess the effect of adjuvant treatment with girentuximab on disease-free survival and overall survival in RCC patients with a high risk of recurrence following nephrectomy. ARISER has recruited 864 patients from over 140 centers in 14 countries. An interim analysis by the Independent Data Monitoring Committee showed positive results after 100 relapses. Recruitment of the study was completed in mid-2008, and the last patient finished the 24-week treatment in February 2009. Relapses for the 864 patients in the trial are taking longer than originally anticipated. As of the end of September 2010, a total of 335 relapses had been reported.

### Stream A: Generation and Identification of mAbs Against New Targets

**Kyla Driscoll Carroll** (ImClone Systems/Eli Lilly) discussed preclinical data on novel antibodies targeting TGF $\beta$  receptor II (TGF $\beta$ RII). These antibodies may ultimately suppress TGF $\beta$  mediated cancer cell growth and metastasis, block TGF $\beta$  signaling-induced angiogenesis and enhance anti-tumor immunity or block immunosuppression. Two mAbs were isolated, one (TR1) specific for the human isoform of TGF $\beta$ RII and another (MT1) that was selective for the mouse TGF $\beta$ RII ortholog. Both mAbs yielded potent anti-tumor activity on primary tumors and metastases in human (BxPC3, MDA-MB-231) and mouse (EMT6, 4T1, CT26) tumor models. A dose-dependent inhibition of the TGF $\beta$ -induced phosphorylation of SMAD2 was observed in vitro. The antitumor effect of TGF $\beta$ RII inhibition is partly attributed to improved antitumor immunity since CD8a<sup>+</sup> T cells are required for inhibition of primary tumor growth, NK cells are required for inhibition of metastasis, CTL and NK killing activity is enhanced in MT1 treated mice. MT1 (mAb specific for mouse TGF $\beta$ RII) treatment improved the efficacy of cyclophosphamide treatment in an EMT6 mouse tumor model.<sup>8</sup>

**Yan Wu** (Genentech/Roche) presented preclinical data on novel antibodies targeting specifically either Notch1 or Notch2 receptors. Importance of the Notch pathway in oncology was summarized in the first part of the presentation. Antibodies were generated using phage display on a naïve human antibody library. Receptor selective and high affinity binders were isolated; they were cross-reactive for their cognate receptor from human, mouse and cynomolgus species. Co-crystal structures were obtained with each receptor NRR domain (negative regulatory

region) and its specific Fab fragment, showing that the inhibitory mechanism relies on stabilizing the NRR quiescence. Selective blocking of Notch1 inhibited tumor growth in a MT3 mouse xenograft model through direct inhibition of cancer cell growth and deregulation of angiogenesis. Whereas inhibition of Notch1 plus Notch2 yielded severe gut toxicity, inhibition of either receptor alone reduced or eliminated this adverse effect.<sup>9</sup>

Discovery and preclinical data on novel antibodies targeting the c-Met receptor were discussed by **Paul Parren** (GenMab). HuMax-c-Met<sup>TM</sup> is a human IgG1 antibody that targets the c-Met oncogenic protein. c-Met is the receptor for hepatocyte growth factor, which is overexpressed or shows abnormal activity in a wide variety of solid tumors. HuMax-c-Met was selected from a panel of over 75 antibodies and targets the c-Met protein at a unique site, binding strongly and showing anti-cancer activity in experimental laboratory and animal cancer models.

**Ted Kwaks** (Crucell) provided an overview on the design and development of novel immunotherapeutic agents against influenza viruses. Antibody CR6261 was isolated from the immune repertoire of a healthy, vaccinated individual using phage display selection on recombinant H5 hemagglutinin (HA).<sup>10</sup> Despite no known exposure to H5 viruses, several clones capable of neutralizing H5 viruses were obtained. Human IgG1 CR6261 neutralized multiple group #1 influenza subtypes, including H1, H2, H5, H6, H8 and H9, and protected mice from lethal challenge with H1N1 and H5N1 viruses when administered up to five days post-infection. Crystal structures of CR6261 Fab were obtained in complex with HAs from the human 1918 H1N1 pandemic virus and from a highly pathogenic avian H5N1 virus; the epitope corresponds to a highly conserved helical region in the membrane-proximal stem of HA1/HA2. CR6261 neutralizes the virus by blocking conformational rearrangements associated with membrane fusion. In a model mimicking human flu infection in ferrets, prophylactic administration of 30 and 10 mg/kg CR6261 prior to viral challenge completely prevented mortality and largely prevented H5N1-induced lung pathology.<sup>11</sup> When administered therapeutically 1 day after challenge, 30 mg/kg CR6261 prevented death in all animals and blunted disease.<sup>11</sup> Another series of mAbs, especially CR8020 and CR8043, neutralized multiple group #2 influenza subtypes, including H3, H4, H7, H10, H14 and H15 and protected mice from lethal challenge with H3N2 and H7N7 viruses. These mAbs seem to block the pH-induced conformational change of HA, thereby preventing the proteolytic cleavage necessary for virus replication.

The last talk of this session was delivered by **Ross Stewart** (MedImmune/Astra Zeneca). He discussed the discovery and preclinical development of MEDI4736, a mAb intended to repress immunosuppression to enhance tumor rejection via the B7-H1/PD-1 axis and the control of T-cell activation. B7-H1 is a member of the B7 protein superfamily, which includes B7.1 and B7.2 interacting respectively with CD28 and CTLA4 to activate or inhibit T-cell activation. The B7-H1/PD-1 axis is also a negative regulator of T-cell activation; its antagonism may thus lead to the reactivation of immune responses. Such inhibition has been shown to activate anti-viral responses.<sup>12</sup> In addition, B7-H1 is frequently expressed in a range of cancers and often correlates

with poor clinical outcome and advanced disease. Thus, B7-H1 blockade may eliminate the negative inhibitory signals delivered through PD-1 and may promote anti-tumor immunity and tumor cell killing.

Dr. Stewart explained that anti-B7-H1 antibodies were generated by classical hybridoma technology and screened for binding to human and non-human (but not mouse) B7-H1, and for the inhibition of B7-H1 and PD-1 interaction. Several additional in vitro selection assays and humanization were applied, leading to selection of MEDI4736 as the final lead candidate. For in vivo evaluations, tumor cells were co-implanted with human T cells (CD4<sup>+</sup> and CD8<sup>+</sup>; 1:1) or a surrogate antibody against mouse B7-H1 was used in a syngeneic mouse model. MEDI4736 showed potent and efficacious anti-tumor activity that is dependent on the presence of T cells supporting a T-cell dependent tumor killing mechanism. Combination with oxaliplatin resulted in complete regression in >50% of treated animals.

### Stream A: Developments for Safety and Immunogenicity

**Frank Brennan** (Novartis) discussed how potential immunotoxicity and more generally the pharmacology of immune responses of mAbs should be evaluated. MABs have generally proven to be safe and efficacious in the clinic. Nevertheless, because wider arrays of mAbs targeting different immune molecules have been generated during the last years, these may result in a broader spectrum of immunopharmacological activity associated with potentially increased toxicity due to the targeted protein, and not only to effector functions. mAbs for inflammatory disease may suppress or modulate the function of immune cells and of cytokine mediators, inhibit antigen presentation, prevent homing of immune cells, induce depletion/anergy of immune cells, or cause a Th1 to Th2 switch. Immunotoxicity can be evaluated at various levels, e.g., in silico (literature review for mAbs with similar mode of action or studies with anti-rodent mAbs against the same target), in vitro testing (desired immunopharmacology, potential immunotoxicity, cytokine release, immunogenicity, off-target binding) and in vivo studies (GLP toxicology studies and efficacy, immunotoxicology). Some undesired immunotoxicity effects may be circumvented by rational antibody design and early screening. For each antibody in preclinical development, it is crucial to understand the immune mode of action, and also the limitations of the preclinical data for predicting human safety. As a result, the estimates for the clinical starting dose for first-in-man studies should take into account both immunotoxicology and immunopharmacology data and apply minimum anticipated biological effect level (MABEL) rules.<sup>13,14</sup>

**Michael Rudolf** (Kenta Biotech) provided an update of the discovery, preclinical and clinical development of panobacumab (KBPA101). The mAb was generated by applying Kenta's proprietary MabIgX<sup>®</sup> technology based on human hybridomas from human blood lymphocytes obtained using a unique specific heteromyeloma fusion cell line. Panobacumab was designed against *Pseudomonas aeruginosa*, a ubiquitous, Gram-negative environmental bacterium and an important pathogen in people



whose immune system is compromised, e.g., cancer patients undergoing immunosuppressive treatment, people with burns or patients being artificially ventilated in an intensive care unit. *P. aeruginosa* infection has a crude mortality rate of about 39%, which is the highest rate observed for bacteria-induced nosocomial blood stream infections.

Dr. Rudolf explained that panobacumab is a vaccine-induced human monoclonal antibody of the IgM isotype (pentameric structure), that is specific for the O-polysaccharide of IATS serotype O11 of *P. aeruginosa* and has no cross-reactivity with other serotypes. The antibody mediates complement-dependent phagocytosis at low concentration (0.2 ng/ml) and protects mice from systemic and local respiratory infection in vivo. Panobacumab completed a clinical Phase 2a program that included 13 treated patients: all patients experienced 100% survival and 100% resolution of pneumonia, although two relapsed. Two inadequately treated patients showed clinical resolution of pneumonia despite resistance of *P. aeruginosa* to the antibiotics initially used. The mAb reached the inflamed lung tissue; no local or systemic infusion reactions were observed. Panobacumab was safe and well-tolerated and no immunogenicity was observed. Early administration of panobacumab correlated with clinical resolution.

**Andrew Erdman** (Amgen) highlighted the potential clinical consequences of immunogenicity and the development of anti-drug antibodies upon administration of mAbs in humans. All currently approved mAbs are associated with some level of immunogenicity and anti-drug antibody (ADA) formation, even human mAbs. The pathophysiology of ADA formation is not fully understood, but seems similar to the establishment of an immunogenic response for other foreign proteins. Typically, it consists of an early response (mainly IgM-based, low concentration, low affinity) and in a late response (IgG-based, higher concentration, higher affinity, longer duration), and includes a T-cell response. Thus, late response has drug neutralizing effects. Occasionally, ADA can exist prior to drug exposure. Multiple factors can affect immunogenicity: (1) product-linked factors (e.g., antibody type, manufacturing, formulation, glycosylation, impurities, degradation products, aggregation), (2) patient factors (e.g., genetics, age, underlying disease, concomitant medications), (3) dosing factors (e.g., route, regimen). Out of 22 mAbs reviewed for their safety, six mentioned an association between ADA and safety outcome (muromonab, abciximab, infliximab, natalizumab, ranibizumab and tocilizumab). Most critical clinical concerns are about hypersensitivity (HST) or HST-like reactions. In many cases, local inflammatory reactions were observed with mAb treatment. But, surprisingly, data on the safety impact of ADA are limited. In general, more data and more studies are needed to establish associations between ADA and safety outcomes. In his conclusion, Dr. Erdman argued companies developing mAbs should study immunogenicity and safety association to a greater extent.

### **Stream A: Clinical Development of Novel Antibodies**

The last session included discussions of two antibodies targeting novel types of antigens: CD6 for the treatment of immunological disorders, and Sema4D in oncology indications.

**Arun Anand** (Biocon) discussed the discovery, preclinical and clinical data on itolizumab (T1h), a novel mAb targeting CD6 antigen for T-cell mediated immunological disorders. CD6 is a type I cell membrane glycoprotein belonging to the scavenger receptor cysteine-rich (SRCR) superfamily group B. It is predominantly expressed by T cells and binds activated leukocyte cell adhesion molecule (ALCAM). ALCAM is expressed on activated T cells, B cells, monocytes, skin fibroblasts and keratinocytes. CD6-ALCAM interaction has been implicated in cell adhesion, T-cell maturation and activation. Recent genetic analyses suggested CD6 to be a susceptibility gene for pathological autoimmunity leading to tissue inflammation. Itolizumab is a humanized IgG1 that: (1) inhibits human lymphocytes proliferation induced by CD6 costimulation; (2) inhibits Th1, Th2 and Th17 cytokines, which may be associated with internalization of CD6 bound to itolizumab in T cells; (3) downregulates the gene transcription of pro-inflammatory and adhesion molecules in human activated lymphocytes; (4) reduces pro-inflammatory cytokines production and T-cell infiltration in psoriatic lesions; and (5) does not induce T- or B-cell depletion mediated by CDC, ADCC or apoptosis.

Dr. Anand discussed the results of a multicenter Phase 2 clinical trial that evaluated the safety and efficacy of itolizumab in patients with active psoriasis.<sup>15</sup> Itolizumab was well-tolerated, with chills and pyrexia as the most common adverse events. Significant reductions in mean Psoriasis Area and Severity Index (PASI) score from baseline were observed: PASI50 and 75 responses were 72.5 and 45%, respectively.<sup>15</sup> Another multicenter Phase 2 trial of itolizumab in combination with methotrexate (MTX) was conducted in India in patients with rheumatoid arthritis who were MTX inadequate responders. The antibody was well-tolerated, with the most common side effects being chills, fever and headache. Itolizumab-treated patients experienced significant improvements in tender and swollen joints, and their quality of life and disability. At a weekly dosing of 0.4 mg/kg of itolizumab, 37% of the patients reached an ACR50 score. Evaluation of itolizumab in several other indications in the auto-immune area, such as psoriatic arthritis, multiple sclerosis and diabetes mellitus, will be explored in the future.

The last presentation of this session disclosed a novel target in oncology, the SEMA4D (or CD100). **Maurice Zauderer** (Vaccinex) presented data on the discovery and preclinical development of their lead antibody candidate, VX15/2503, which targets SEMA4D. SEMA4D is a potent pro-angiogenic molecule. Its signaling through plexin-B1 induces activation and migration of endothelial cells resulting in the formation of new blood vessels promoting tumor growth in vivo. SEMA4D is overexpressed in a wide array of tumor types, and is also produced by inflammatory cells present in the tumor microenvironment. In addition to its effects on endothelial cells, Sema4D enhances Plexin-B1-mediated transactivation of c-Met, which is co-expressed on tumor cells. It has been reported that overexpression of Plexin-B1 together with c-Met in prostate and breast cancers is a negative prognostic factor. A high frequency of mutations in Plexin-B1 that enhance oncogenic activity has

been reported in prostate cancer. Collectively, these results suggest that expression of SEMA4D, either by cancerous cells or by tumor-associated macrophages, is a widely used strategy by tumors to induce angiogenesis and tumor invasion, thereby enhancing their growth, survival and metastatic potential. Antibody neutralization of SEMA4D to block its interaction with Plexin-B1 receptor may represent a new and effective therapeutic strategy for cancer treatment.

Dr. Zauderer then discussed Vaccinex's high affinity human IgG4 antibody that is specific for both mouse and human SEMA4D. VX15/2503 efficiently blocked the interaction of SEMA4D and Plexin-B1. In CT26 and EM6 tumor models in mice, VX15/2503 efficiently inhibited tumor growth and vascularization. In January 2011, Vaccinex received clearance from the FDA to initiate a Phase 1 clinical study to evaluate the mAb as a treatment for advanced solid tumors. A Phase 1 study of VX15/2503 in multiple sclerosis is planned by the company.

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## Stream B: mAb Production, Scale-Up, Process Transfer and Comparability

Alain Beck

Olivier Cochet (Centre d'Immunologie Pierre Fabre) showcased the strategy, choices and challenges to creating a new facility

dedicated to production of mAb clinical material. The new building, located beside Pierre Fabre's existing research and development center in St. Julien en Genevois, France, was planned with five key factors in mind: capacity, regulatory requirements, flexibility, life-quality and sustainability. The conceptual design was made with Biopharm Services, a UK-based company with recognized expertise in single-use systems implementation. Based on the existing process and the perspective of producing clinical material for Phase 1 and ultimately Phase 2, the layout was defined to accommodate a 1,000 L bioreactor and appropriate downstream equipment. Additional floor space was also kept in the process rooms to further expand the capacity with a second 1,000 L bioreactor. Dr. Cochet then presented the technologies to be implemented in the facility and emphasized that a deliberate choice was made to implement 100% disposable systems. The benefits were highlighted, including the advantages in flexibility, general engineering simplification (e.g., no steam in the building, no cleaning systems) and the capital savings by using disposables. The concept also allowed design of smaller classified areas compared to traditional facilities. The footprint of disposable systems is not only smaller, but all non-critical components such as media and buffer tanks will be located in a central low-classified hall. Liquids will be transferred through the wall to process equipments with specifically designed hatches.

Dr. Cochet emphasized the importance of sustainability aspects that were taken into account during the conception and the construction. The project is the first French pharmaceutical facility committed to build according to the French Haute Qualité Environnementale (HQE) environmental label specifically for industrial activities. The project took into consideration many aspects, such as quality of construction, isolation, energy and water savings, nuisance reduction, health condition and life quality. Of note, several requirements of the HQE label were clearly facilitated by implementation of single-use systems. Dr. Cochet also noted that the selection of the appropriate material providers is challenging because the field of disposables is emerging and the supply chain for the plastic components is highly critical. This new facility will open its doors late 2011 and will streamline the antibody discovery up to GMP production of material for early clinical phases.

## The Future of Biosimilar Antibody Development in Europe

Mark McCamish (Sandoz) presented a perspective on the development of biosimilar mAbs in Europe; to wit, antibodies that are not highly similar should undergo greater preclinical and clinical evaluation and, conversely, abbreviated clinical programs should be done for true biosimilar mAbs. At generics companies, attempts to increase the degree of similarity are based on extensive characterization and on iterative process development. Pre-clinical and clinical studies can be streamlined if a proposed biosimilar is shown to be "highly similar" to a reference product at the analytical level. Preclinical and clinical study requirements are increased if the product quality attributes of a proposed biosimilar deviate from the reference.

Dr. McCamish explained that manufacturing process changes are tightly regulated, as described in the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) document ICH Q5E. Changes of quality attributes are only acceptable if they do not alter safety and efficacy. For demonstrating biosimilarity, it is therefore acceptable to use the upper and the lower limit of the pre- and post-shift material. However, the biosimilar release specification should be as tight as the current originator specification. Guidelines already exist in EU, US and in Japan for biotechnology product manufacturing changes. Developing “highly similar” comparability standards before and after manufacturing changes will help to deliver “highly similar” mAbs based on product quality attributes.

Another point emphasized by Dr. McCamish is that biosimilars must be systematically engineered to match the reference product for glycoforms and charge variants. Differences of such biosimilars are not inherently more relevant than those observed after manufacturing changes for an originator mAb. The comparability of the reference products of one original manufacturer marketed in different highly regulated countries (e.g., US, EU, Japan) can be clearly established by stringent analytical and functional studies. In summary, analytical characterization is a fundamental component of successful biosimilar product development. The biosimilar mAb approval pathway to be established in Europe based on the draft guideline released for consultation on November 18, 2010 will follow established regulatory science precedence, and is likely to include requirements for studies such as Fc-receptor binding and activation, FcRn binding and PK.

**Harish Iyer** (Biocon) expanded the discussion on biosimilars by detailing opportunities, and the potential impact on the European market, of biosimilar antibodies from Asia. From India’s public health perspective, the current annual spending on drugs is estimated to be approximately \$4 billion of total healthcare cost of \$24 billion. Biologics, with costs at approximately \$300 million, represent a small portion of the total, but these costs are expected to top \$3 billion because healthcare spending is likely to increase as a consequence of increases in both life expectancy and per capita income. Early acceptance of biosimilar antibodies will be essential to contain high healthcare costs, and is likely to be driven by a much-expanded insurance industry. In this scenario, India will play a critical role as a key, emerging hub for biosimilars in the world. India’s regulators have been proactive in evaluating biosimilars, and India was among the first regions to approve peptide hormones such as insulin and more complex molecules such as rituximab. Regulators in China have also been fast to approve biosimilar products.

Dr. Iyer explained that the key criteria to approving such biosimilars should continue to be quality, safety and efficacy. Decisions should be informed by good scientific judgment, and include an evaluation of the risk of an abbreviated approval pathway compared to the expected benefit to an increased patient population. The first biosimilar to be approved in India was recombinant human insulin. The price of biosimilar insulin in India is approximately 50–60% of a comparable innovator

product in India. However, India’s biosimilar prices are themselves significantly lower and often only 20% of that in either the US or Europe. A similar pricing differential exists for biosimilar antibodies as well, although the cost of a dose of rituximab in India is approximately twice that of other biosimilar products in India. These differences are not as large as those seen for small molecule drugs. However, approval of biosimilar therapeutics has increased access to such products, and further competition is expected to bring down the price of medicine in countries like India. Dr. Iyer also noted that Biocon has partnered with Mylan to sell biosimilar products globally.

### **Day 3: December 1, 2010**

Janice M. Reichert

On the morning of the final day of the European Antibody Congress, attendees were greeted with a snow-covered Geneva (and a closed airport), but the indomitable Chairman **Clive Wood** (Bayer Schering Pharma) kept the conference on track with some help from fast-working IT personnel who arranged teleconferencing for a few speakers who could not travel to Geneva. The topics for the final day included the review of antibody products, antibody-drug conjugates and radio-immunoconjugates, bispecific and recombinant oligoclonal antibodies, as well as domain antibodies and new scaffolds.

### **Quality Review for Antibody Products**

**Marjorie Shapiro** (US Food and Drug Administration) gave a perspective on the development of therapeutic mAbs, next generation antibodies and antibody alternatives. She discussed the time line for FDA’s approval of 34 therapeutic and diagnostic mAbs and 5 Fc-fusion protein products between 1986 and 2010, with first approvals of an Fc-fusion protein (etanercept) in 1998, an antibody-drug conjugate (ADC; gemtuzumab ozogamicin) in 2000, and a human mAb derived from phage display (adalimumab) in 2002. Dr. Shapiro noted that more than half (6 of 11) of the antibody therapeutics approved during 2000–04 were treatments for cancer indications, but only two anticancer antibodies (panitumumab, ofatumumab) were among the ten mAbs approved during 2005–2010. Instead, five of these ten recent approvals were treatments for rheumatoid arthritis and other autoimmune disorders.

Dr. Shapiro highlighted the main themes of mAb development in the 21<sup>st</sup> century, which include an emphasis on Fc engineering to reduce or enhance effector function, exploration of mAb fragments and novel mAb constructs such as bispecific mAbs, antibody cocktails and antibody conjugates. A wide variety of formats are now possible, e.g., the antibody portion of conjugates may be whole IgG, Fab, single chain or a single variable domain, while the “payload” may be a drug, radioisotope, cytokine, toxin or peptide. She mentioned that, although gemtuzumab ozogamicin was approved as a drug, i.e., the new drug application (NDA) filed for the product was approved under the Federal Food, Drug and Cosmetic Act, currently the FDA

is determining the regulatory pathway for ADCs on a case-by-case basis and some ADCs will likely be regulated as biologics, i.e., a biologics license application (BLA) filed would be approved under the Public Health Service Act, in the future.

The mechanisms of action (MOA) for mAbs were then discussed by Dr. Shapiro. She reviewed the main elements required to understand binding specificity: (1) map the epitope on the target antigen, (2) examine the reactivity with related antigens and include a histochemistry screen on human/animal tissues, (3) determine cellular localization of the epitope, (4) determine the accessibility of the epitope to the antibody under conditions of the intended use and (5) assess the binding affinity and kinetics, e.g., by surface plasmon resonance and calorimetry. The functionality of the Fc portion should be assessed through demonstration of binding or lack of binding to Fc receptors, complement fixation and C1q binding, and binding to FcRn. Assays such as those for antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and phagocytosis should be done to determine whether the Fc function is related to the MOA. The potential for side effects related to the Fc function, with an emphasis on the potential for cytokine-release syndrome and cell depletion, should also be assessed. In discussing MOA, Dr. Shapiro emphasized some facts recently learned, including that fucose inhibits ADCC,<sup>1,2</sup> but differentially impacts cytotoxicity on different effector cells;<sup>3</sup> the anti-inflammatory properties of IVIG are dependent on sialylation of N-linked Fc glycan;<sup>4</sup> IgG2 mAbs can trigger ADCC with neutrophils and monocytes, but not NK cells, via FcγRIIA;<sup>5</sup> IgG2 has disulfide isoforms;<sup>6-8</sup> IgG4 engages in dynamic Fab exchange;<sup>9</sup> and the presence of non-consensus N-linked glycosylation on CH1 and VL (although the impact is unknown).<sup>10</sup>

The choice of isotype as part of rational design was described as critical by Dr. Shapiro. IgG1 is suitable when effector function is desirable, but IgG2 and IgG4 mAbs should be considered when the objective is to block ligand/receptor interactions or pathogen/receptor interactions, and may be desirable for antibody-drug conjugates that are internalized or use direct/bystander killing as a MOA. She noted that sponsors should provide evidence that Fab arm exchange is not occurring when IgG4 isotypes are used as product candidates. She also stressed that it is important to understand potential differences in potency for charge variants that can arise during production of mAbs. A final point was that patient selection can be a critical factor in the design of clinical trials. Knowledge of biomarkers for intended mAbs and the extent to which Fcγ receptor polymorphism might predict response to immunotherapy would help with selection of patient who will likely benefit from treatment from those who might not respond.

Dr. Shapiro's final comments touched on immunogenicity and affinity. Regarding immunogenicity, she noted that in general immunogenicity assays should be in place and appropriately qualified at the time of initiation of clinical studies, but that for most mAbs it is acceptable to bank samples from early clinical studies and test them all together when an immunogenicity assay is ready. On a case-by-case basis there may be some mAbs that require immunogenicity testing at the initiation of clinical

studies. She discussed the approximate ranges of immunogenicity for various types of mAbs, e.g., murine whole Ig (55–80%, with ibritumomab an exception at 3%), murine Fab and Fab2 (<1–8%), chimeric (<1–13%), humanized (<1–10%), human (<1–4% for mAbs derived from “humanized” mouse, 12% for mAbs derived from phage display administered as monotherapy). Regarding affinity, Dr. Shapiro questioned whether affinities in the picomolar to femtomolar range are really necessary for efficacy, while noting that such high affinity may be desirable for some indications, while very high affinity mAbs might be more likely to bind to normal cells and thus cause an unwanted safety signal.

### Antibody-Drug Conjugates and Radio-immunoconjugates

The session on antibody-drug conjugates (ADCs) and radio-immunoconjugates began with discussion of the development of advanced antibody-based therapeutics in oncology by **Robert Lutz** (ImmunoGen). He noted that ADCs in development today solve some of the challenges of antibody therapy by arming antibodies with cytotoxic effector molecules, improving potency while ensuring the conjugate is inactive in the blood compartment. At least seven ADC utilizing ImmunoGen's maytansinoid technology are in clinical study, including trastuzumab emtansine (T-DM1; Genentech), lorvotuzumab mertansine and IMG388 (ImmunoGen), SAR3419 and SAR 566658 (sanofi-aventis), BIIB015 (Biogen Idec) and BT-062 (Biotest). In addition, at least 9 other ADCs are undergoing clinical development, with two of these, brentuximab vedotin (Seattle Genetics/Takeda) and inotuzumab ozogamicin (Pfizer) in Phase 3 studies.

Dr. Lutz then discussed the four key components of an ADC program: an appropriate and disease-relevant target, the tumor-targeting antibody, the potent cancer cell killing agent, and the engineered linker. The target should be expressed selectively on tumor rather than on normal tissue and should have favorable properties, e.g., density on target cells, internalization rate. He noted targets that are also expressed on normal cells may also be considered if there are relevant toxicity models or appropriate clinical monitoring is available to evaluate potential toxic effects. Another potential issue is that the target on normal cells may act as an ‘antigen sink’ and thus limit exposure of the tumor to the ADC. The antibody itself must be non-immunogenic. Dr. Lutz noted that intrinsic activities such as ADCC are maintained in most ADC strategies, but low potency antibodies can also be appropriate for payload delivery.

With regard to the drug and linker, Dr. Lutz discussed ImmunoGen's maytansinoid agents, which inhibit tubulin polymerization and have potent anti-mitotic activity. The agents thus selectively inhibit proliferating cells. Dr. Lutz mentioned that ImmunoGen uses two classes of maytansinoids that differ in the way they are attached to the antibody, but that the company also has a new class of drug for use in ADCs. The IGNs comprise indolino-benzodiazepine dimers that alkylate and crosslink DNA. IGNs are highly potent and are not substrates for multi-drug resistance protein pumps. He noted that there is a need for

variety in the types of drugs used in ADCs because of the variable sensitivity of different tumor types to the mode of action of the drugs. A variety of strategies to link drugs to the antibody carriers have been used, e.g., selective reduction of intrinsic disulfide bonds, introduction of amino acids for selective chemical modification. The linkers can be designed as cleavable and non-cleavable. ImmunoGen's approach involves modification of intrinsic lysine residues, which allows flexibility in payload optimization. The attachment sites are identifiable through peptide mapping. The modular design of drugs and linkers allows optimization of the therapeutic window for a particular antigen. Dr. Lutz noted that controlling exposure is an important feature of ADC development as the clinical toxicity profiles for maytansinoid ADCs, as well as other current generation ADCs, correlate most closely with conjugate exposures, not free toxophore exposure.

Dr. Lutz briefly reviewed the results for T-DM1 as first-line therapy in HER2-positive metastatic disease that were presented at the 35<sup>th</sup> Congress of the European Society for Medical Oncology conference in Milan, Italy in October 2010. In the trial, 137 women were randomly assigned to treatment with either trastuzumab (8 mg/kg dose, then 6 mg/kg once every three weeks) and docetaxel, or only T-DM1 (3.6 mg/kg once every three weeks until disease progression). The overall response rates were 45% in patients administered T-DM1 and 41% in those administered both trastuzumab and docetaxel after a median follow up of approximately 6 months. Clinically relevant adverse events occurred in 37% of T-DM1 treated patients compared with 75% of those administered both trastuzumab and docetaxel. The three most common adverse events (AE) were nausea, fatigue and pyrexia; incidence of grade 3 or higher AEs was 37% in the T-DM1 arm compared with 75% in the trastuzumab and docetaxel arm.

Dr. Lutz concluded by noting that encouraging clinical results have also been obtained with SAR3419 and lorvotuzumab mertansine. The anti-CD19 SAR3419 is in Phase 1 studies sponsored by sanofi-aventis for the treatment of relapsed/refractory CD19-expressing non-Hodgkin's lymphoma and other B-cell malignancies. The anti-CD56 lorvotuzumab mertansine (IMGN901, BB-10901) has demonstrated impressive single agent efficacy tolerability in dose-escalating trials in Merkel cell carcinoma (MCC) patients. ImmunoGen received orphan medicinal product designation for lorvotuzumab mertansine as a treatment of MCC in both the US and European Union in 2010. The ADC is also in Phase 1 studies of multiple myeloma patients and a Phase 1/2 study of patients with small cell lung cancer.

**Veronique Blanc** (sanofi-aventis) provided practical advice regarding the development of ADCs. She noted that the goal of ADC development is to design a therapeutic that will target very potent cytotoxins to tumor cells. However, ADCs are complex molecules, and the selection of an appropriate combination of target, antibody, drug and linker is difficult because each of these elements has its own selection criteria, but each element interacts and influences the others. Dr. Blanc mentioned that there are 17 ADCs in clinical development, but it is difficult to draw general rules or to define specific criteria for the different elements because most ADCs are in early clinical studies and they

target different antigens and are composed of different drugs and linkers. As a consequence, there is too much variability to make general rules regarding what works and what does not work with the information available to date. Importantly, clinical benefit proof of concept for ADCs has been shown, e.g., results for trastuzumab emtansine and brentuximab vedotin, which are in Phase 3 studies.

The effects of the various elements of ADCs on the safety, efficacy and pharmacokinetics (PK) were then discussed by Dr. Blanc. She noted that the target influences safety in a number of ways. For example, differential expression is critical. If the antigen is expressed on normal and tumor cells at the same level, then a targeted therapeutic would have no safety margin. Dr. Blanc mentioned that skin toxicity was observed in patients treated with the anti-CD44v6 maytansinoid ADC bivatuzumab. Although the antigen was abundantly expressed in tumors, the ADC also bound antigen on skin keratinocytes, which mediated the skin toxicity. She explained that target-associated properties that affect efficacy include density and the rate of internalization and processing. Accurate quantification of the antigen density on cells that are as similar as possible to the tumors encountered in patients is critical for ADC development. Dr. Blanc noted that studies indicate a clear relationship between antigen density and the level of ADC internalized, but the rate of internalization and processing is also important. Low density antigens that internalize and process ADCs quickly may be suitable. The target can also affect PK if it is shed. High levels of shed antigen have been found to increase the rate of antibody clearance, e.g., huC242 and huC242-DM4. Dr. Blanc also emphasized that it is important to understand how the target is modulated by the ADC. She shared preclinical data that showed reductions in the expression of a target that were dependent on the dose of ADC administered, and she suggested that such information may inform decisions regarding dosing schedules in clinical studies.

Dr. Blanc then discussed three points regarding the antibody portion of ADCs. Her first point was that not all antibodies make good ADCs. Studies from Genentech have shown that ADCs comprising the same linker and drug targeting the same antigen and administered at the same dose can behave differently. These results may be due to differences in the capacity of the specific antibodies to be internalized and allow processing. Finding the right antibody is thus still an empirical process. The second point involved efforts to improve the homogeneity of ADCs, which are complex molecules with an average of 3–5 drug molecules per antibody. Dr. Blanc discussed Genentech's ThioMAbs, which have engineered cysteines that are introduced by single point mutations. Formation of a ThioMAB drug conjugate results in a homogeneous ADC with the number of drug molecules defined by the number of engineered cysteines. Such a homogenous ADC may have improved safety, although this must be proven in clinical studies. Her third point was that alternate antibody formats may provide improvement over full-size IgG-based ADC, especially with regard to distribution in tumors. A smaller antibody such as Seattle Genetic's diabody-drug conjugates may hold promise. Seattle Genetics has designed diabody-drug conjugates that have similar activity (3- to 4-fold lower) compared to

IgG-based ADC in a mouse xenograft model despite the faster clearance (25- to 34-fold faster) of the diabody.

Dr. Blanc then discussed differences between cleavable and non-cleavable linkers that may affect safety and efficacy. She presented data on weight loss in mice administered an antibody conjugated to DM4 through either a cleavable or non-cleavable linker. The difference in the highest non-toxic dose was nearly 3-fold, suggesting that the non-cleavable linker could increase the safety margin for an ADC. However, she also presented data on antitumor activity that suggested the cleavable linker may be active against a broader array of tumor types compared to the non-cleavable linker. Taken together, the data for the two types of linkers suggests that a compromise between safety and activity may be required.

The impact of the cytotoxic element on the safety and efficacy of ADCs was Dr. Blanc's final topic. She discussed clinical data for ADCs that included either a DNA-binding drug or a maytansinoid. These ADCs had differences in the maximum tolerated dose of 20- to 80-fold. She noted that no conclusions can be drawn from the data on adverse events. Dr. Blanc emphasized that achieving a sufficient pharmacological index was critical to ADC development, and that ADCs that included both types of drug have had issues with toxicity or efficacy, e.g., gemtuzumab ozogamicin, AVE9633. In the case of AVE9633, there were no dose limiting toxicities at 260 mg/m<sup>2</sup>, but the ADC was not effective despite the fact that all the sites on tumor cells were saturated. Dr. Blanc reiterated the point also made by Dr. Lutz that diversity of mechanism of action is desirable because not all tumors respond to the same drug. Dr. Blanc concluded by noting that the challenge of ADC development is to find the right targets, especially regarding the safety profile and capacity to internalize the drug; select the right cytotoxic agent based on the indication; select the best antibody, likely based on an empirical approach; and find a linker that is adapted to the type of tumor and provides the desired safety profile.

Calicheamicin antibodies and beyond were discussed by **Hans-Peter Gerber** (Pfizer BioTherapeutics). He noted that ADCs combine the best of two worlds by utilizing a targeting system to deliver potent drugs. Clinical proof of concept for ADCs has occurred in the past few years, with impressive results reported for trastuzumab emtansine (T-DM1), brentuximab vedotin (SGN-35) and inotuzumab ozogamicin (CMC-544). The number of ADC clinical programs is expected to substantially increase over the next 3–5 years as more companies become involved in ADC development.

Pfizer's development pipeline focuses on calicheamicin as the "payload" drug. The anti-CD22 IgG4 inotuzumab ozogamicin is in Phase 2 studies as a treatment for non-Hodgkin lymphoma, and a second ADC that targets 5T4, a rapidly-internalized tumor surface antigen that is associated with poor prognosis in colorectal, ovarian and gastric cancer, is undergoing preclinical evaluation. Gemtuzumab ozogamicin (Mylotarg<sup>TM</sup>) was approved by FDA in 2000 for treatment of acute myeloid leukemia, but was withdrawn in June 2010 after no improvement in clinical benefit was observed, and after a greater number of deaths occurred in the group of patients who received the mAb compared with those

receiving chemotherapy alone in a confirmatory, post-approval clinical trial.

Dr. Gerber explained that calicheamicin is an extremely potent drug that is attached to an antibody through an acid-labile, hydrazone linker. After a calicheamicin-containing ADC is internalized in a targeted cell, the drug is released via the activity of a reductive group such as glutathione and causes DNA strand breaks. Calicheamicin-containing ADCs are potent at doses that are 50–100-fold lower compared to those containing tubulin-binding drugs. The extreme potency can potentially pose challenges to achieving an appropriate therapeutic index.

Safety, tolerability and preliminary efficacy data for anti-CD22 inotuzumab ozogamicin as monotherapy in a Phase 1 study and in combination with rituximab in a Phase 1/2 study were presented by Dr. Gerber. In the Phase 1 study of 50 patients, the maximum tolerated dose (MTD) was determined to be 1.8 mg/m<sup>2</sup>, which is approximately 50 µg/kg based on the mAb, administered every four weeks. The half-life was 12–30 h based on data for the first dose, and this increased with dose and cycle. Activity was observed in follicular (68.8% overall response rate, 30% complete response) and diffuse large B-cell lymphoma patients (33% ORR). Adverse events (thrombocytopenia, bone marrow suppression, liver enzyme elevation) were manageable and reversible. The Phase 1/2 study of the inotuzumab ozogamicin/rituximab combination is on-going. The study is designed with two parts: (1) dose-escalating, with 3–6 patients per cohort and (2) two-arm, expanded cohort with inotuzumab ozogamicin administered at the MTD to 30 evaluable follicular B-cell lymphoma patients (arm 1) and 30 evaluable diffuse large B-cell lymphoma patients (arm 2). Preliminary results in non-rituximab refractory patients are encouraging, with overall response rates in the 75–85% range and complete responses in the 50–60% range for both FL and DLBCL patients.

Dr. Gerber then discussed the properties of successful ADCs and the challenges associated with ADC development. He emphasized that successful ADCs show a trend toward longer half-life, higher copy number of the antigen and faster rate of internalization. In particular, the increased antigen copy number and rate of internalization are important because both affect the amount of drug entering the cell. He noted that the location of the release of the drug in the cell is important for success also. Dr. Gerber noted that challenges in ADC development include the lack of "clean" targets, i.e., those with no or limited expression on normal tissues, which ensures that the potent drugs used in ADCs are delivered selectively, target expression levels that are not predictive of pharmacology, liver metabolism and the empirical ADC lead selection process that results from a limited understanding of factors affecting pharmacology and safety. He suggested several strategies to address key ADC limitations, such as use of *in vitro* and *in vivo* biopanning and function first assays to identify tumor selective, fast internalizing ADC targets, use of site-specific conjugation technology to minimize off-target toxicity/liver metabolism of ADCs and sequential use of high throughput screening and rational drug design for payloads and linkers to help optimize the therapeutic index.

**Che-Leung Law** (Seattle Genetics) gave an update on the preclinical and clinical development of auristatin-based ADCs. In his presentation, Dr. Law summarized clinical data to date for brentuximab vedotin (SGN-35), and discussed the preclinical studies and the ongoing Phase 1 clinical study of the anti-CD70 ADC SGN-75. As Dr. Law explained, brentuximab vedotin comprises an anti-CD30 mAb with the payload, anti-tubulin agent monomethyl auristatin E (MMAE), attached via a protease cleavable linker. The ADC targets CD30, which is strongly expressed on the surface of malignant cells in Hodgkin and certain T-cell lymphoma patients. After binding and internalization, the CD30-ADC complex is internalized and traffics to the lysosome where the peptide linker is proteolytically degraded to release the MMAE payload; released MMAE disrupts the cellular microtubule network, induces G<sub>2</sub>/M phase cell cycle arrest, and ultimately leads to apoptosis. Brentuximab vedotin is undergoing evaluation in a Phase 2 pivotal clinical trial in patients with relapsed or refractory Hodgkin lymphoma (NCT00848926), a Phase 3 study in patients at high risk of residual Hodgkin lymphoma following autologous stem cell transplant (NCT01100502), a Phase 2/3 study in patients with progression of Hodgkin lymphoma (NCT01196208), a Phase 2 study in patients with relapsed or refractory systemic anaplastic large cell lymphoma (ALCL; NCT00866047), a Phase 2 study in patients with CD30-positive hematologic malignancies who previously participated in an SGN-35 study (NCT00947856), as well as a number of Phase 1 studies.

Dr. Law reviewed the recently published results of a Phase 1 dose escalation study of brentuximab vedotin.<sup>11</sup> The overall response rate was approximately 39%, and a clear trend toward dose dependency was observed. The maximum tolerated dose (MTD) was 1.8 mg/kg administered every three weeks. Of 12 patients who received the MTD, 50% had an objective response. Dr. Law also noted that encouraging results have been observed in the pivotal study in Hodgkin lymphoma and the Phase 2 study in ALCL. Patients were administered brentuximab vedotin at 1.8 mg/kg every three weeks in both studies. The overall response rate was 75%, and the median duration was more than 6 months, in the Hodgkin lymphoma study. The overall response rate was 86%, and the median duration has not yet been reached, in the ALCL study. In clinical studies to date, the ADC has generally been well-tolerated. Adverse events (AEs) in the Phase 1 study were primarily grade 1 or 2, with the most common being fatigue, pyrexia, diarrhea, nausea, neutropenia and peripheral neuropathy; the safety profile has been similar in other studies. Submission of a BLA based on results from the pivotal trial in relapsed or refractory Hodgkin lymphoma patients is planned for the first half of 2011.

Dr. Law then presented data on the expression profile of CD70 in various tissues. The antigen is restricted to activated B and T cells and mature dendritic cells and is not found on normal tissues outside the hematopoietic system. It binds CD27, a member of the TNF receptor family, to regulate lymphoid proliferation, differentiation and apoptosis. CD70 is abundantly expressed in hematopoietic malignancies such as non-Hodgkin lymphoma and multiple myeloma, but also in solid

tumors, e.g., subsets of clear cell renal carcinoma, pancreatic cancer, lung cancer. Heterogeneity of expression is observed, with patient-to-patient variation. Dr. Law noted that it will therefore be important to understand the relationship between target expression and response in clinical studies, and whether there is a threshold level of expression necessary to achieve a clinical benefit.

The components and mechanism of action of Seattle Genetics' anti-CD70 SGN-75 were explained by Dr. Law. The antibody is a humanized IgG1. The drug portion is the anti-tubulin agent monomethyl auristatin F (MMAF) that is conjugated onto the antibody through a maleimidocaproyl (mc) linker. When the ADC is degraded in the lysosomal compartment, Cys-mc-MMAF is released and kills the cell by disrupting the microtubule network in a manner similar to MMAE. In *in vitro* cytotoxicity studies, SGN-75 has shown potent activity against CD70-expressing cells, e.g., renal cell carcinoma and lymphoma cell lines. The conjugate has also been tested in xenograft models of solid tumors and hematological malignancies and has demonstrated convincing antitumor activity at doses that were  $\leq 3$  mg/kg. Dr. Law noted that a Phase 1 dose-escalation (range 0.3–4.5 mg/kg) study (NCT01015911) to evaluate the safety and tolerability of SGN-75 in patients with CD70-positive relapsed or refractory non-Hodgkin lymphoma or metastatic renal cell carcinoma was initiated in November 2009. Preliminary clinical data suggests the ADC is generally well-tolerated; no MTD has yet been reached.

**Erik Merten** (Bayer Schering Pharma) discussed the technical, regulatory and logistical challenges to bringing radio-immunotherapy (RIT) into clinical practice. RIT combines cancer killing radiation with precise targeting capacity and enables delivery of a high dose of radiation to tumors while normal tissues receive minimal doses. Two RITs, ibritumomab tiuxetan (Zevalin) and tositumomab-I131 (Bexxar) are currently approved by FDA.

Dr. Merten explained that an RIT product must be easily applicable and have a good therapeutic window for use in clinical practice. The manufacturing process must be robust, reproducible and yield a stable product with an appropriate shelf life. In this context, he discussed the development of I-131-L19 small immunoprotein (SIP). L19 is an 80 kDa covalent dimer of a human single-chain antibody fragment with high affinity to extra domain B of fibronectin. The antigen is selectively expressed in solid tumors, lymphomas and metastases. The SIP format has a molecular mass above the renal filtration threshold and shows high total tumor uptake, rapid clearance from circulation, low bone marrow uptake and improved tolerability. Thus, SIP molecules can provide an optimized therapeutic window for RIT compared to single chain and full-size formats.

Use of an appropriate isotope and labeling method are important aspects of RIT development. Dr. Merten explained that I-131 was selected because the isotope emits high energy beta radiation, has a half-life (8.04 days) appropriate for radiotherapy and can be introduced via direct iodination. Direct iodination is a high yield reaction providing product with medium-to-high specific activity, no precursor or preliminary chemistry is needed and the reaction is selective for tyrosine residues. Disadvantages

include the possibility of oxidative damage to proteins and the need for effective purification methods.

Dr. Merten discussed radiolysis as a key challenge for scale up. A protein damaged by radiolysis may have decreased or no binding affinity to its target, thereby causing a loss of efficacy and reduced therapeutic window. Radiolysis also reduces shelf life, which may result in loss of flexibility in manufacturing, shipment and clinical use. Therefore, formulation of RITs should include suitable scavenging agents such as ascorbic acid, gentisic acid, maltose, inositol, methionine or human serum albumin. These agents have high anti-oxidizing potential, good tolerability, low toxicity and are available in appropriate quality and amounts for RIT formulation.

Supply concepts for the commercial phase need to be considered early in the development of an RIT because they determine critical technical development goals. Dr. Merten thus discussed the use of a decentralized vs. central labeling approach for supply of the radiolabeled product. He noted that the decentralized approach is used for yttrium-90 labeled ibritumomab tiuxetan, i.e., the product is compounded as a radiopharmaceutical at hospitals, whereas tositumomab-I131 is centrally labeled, i.e., the radiolabeled product is produced by a contract manufacturing organization (CMO) for distribution to hospitals. He explained that each approach has advantages and disadvantages. The decentralized approach provides more flexibility in patient scheduling due to the flexibility of producing the “hot” product at the hospitals, but infrastructure and authorization to use radioactive material is needed at all sites, availability of sterile isotope is required and there is substantial effort involved in the site set up. Central labeling is less complex to set up, there is more control of the product quality and the radiolabeling, filling, and product packaging and distribution are done by a CMO. However, formulation and stability of the product are more challenging, distribution may require frozen shipments and a suitable CMO must be identified.

Dr. Merten noted that I-131-L19SIP will be commercialized using the central manufacturing concept. The first results using the process have been encouraging. Scale up has provided material of >1 Ci in >90% radiochemical yield and with a radiochemical purity of >>90%. The immunoreactivity was >90% and the radiolabeled material was stable for a minimum of five days. The goal is to develop a manufacturing process for batch sizes at activity level of 10 Ci or greater, identify appropriate packaging, and design a worldwide supply chain concept based on shipment of the “hot” I-131-L19SIP product. In conclusion, Dr. Merten stated that I-131 radiolabeled proteins are suitable to give broad access to RIT in clinical practice.

The uses of radiolabeled antibodies for tumor imaging and as anticancer agents were discussed by **Jacques Barbet** (University of Nantes) in a thorough and well-referenced presentation. He emphasized that RIT has evolved over the course of more than 20 years and that numerous improvements have been made, such as use of new stable chelates, humanized antibodies and pre-targeting methods. Dr. Barbet noted that William H. Beierwaltes was a pioneer in the use of nuclear medicine because he was the first to administer radiolabeled (I-131) antibodies to a

melanoma patient and he invented meta-iodobenzyl-guanidine (MIBG). Early work with radiolabeled antibodies emphasized imaging (immunoscintigraphy) with isotopes of iodine; development of chelating agents enabled use of other radio-labels.<sup>12</sup> Immunoscintigraphy has largely been replaced by 18F-deoxyglucose positron emission tomography (18F-FDG PET) for detection of cancer, and the focus for use of radiolabeled antibodies has shifted to development of therapies.

The multiple modes of action of RIT were then discussed by Dr. Barbet. The effects of RIT result from both radiobiological (e.g., direct radiation of bound cell, cross-fire irradiation) and immunological (e.g., ADCC, CDC, apoptosis) mechanisms.<sup>13</sup> Two radiolabeled antibodies are currently approved for treatment of cancer: ibritumomab tiuxetan (Zevalin<sup>®</sup>), which is an anti-CD20 murine IgG1 mAb conjugated to the yttrium-90 or indium-111 chelator tiuxetan, and tositumomab (Bexxar<sup>®</sup>), which is an anti-CD20 murine IgG2a mAb linked to iodine-131. Differences in clinical outcomes have been demonstrated in comparisons of (90)Y ibritumomab tiuxetan and rituximab, which also targets CD20 on B cells, with statistically and clinically significant higher overall response rate and complete responses observed in non-Hodgkin lymphoma patients treated with (90)Y ibritumomab tiuxetan compared with those who were administered rituximab.<sup>14,15</sup>

Dr. Barbet noted that better efficacy has been shown when RIT is given for minimal disease<sup>14,16-18</sup> and that RIT consolidation therapy after initial tumor reduction may maximize treatment efficacy and significantly improve progression-free survival. In consolidation therapy, RIT is given to treat minimal residual disease that exists after initial treatment of patients with immuno-chemotherapy that is intended to reduce tumor burden. In a Phase 3 study of patients with advanced-stage follicular lymphoma, consolidation of first remission with (90)Y-ibritumomab tiuxetan was shown to prolong progression-free survival by two years and result in high rates for conversion of partial responses to complete responses compared to patients who received no treatment other than the first-line therapy.<sup>19</sup>

Dr. Barbet then discussed improvements in radio-immunotherapy based on use of better radionuclides and methods such as pretargeting.<sup>20</sup> In addition to the well-studied beta/gamma emitting iodine and yttrium isotopes, researchers are also investigating alternate isotopes such as the alpha-emitters actinium-225, bismuth-213 or astatine-211. Dr. Barbet explained that  $\alpha$  particle radiation has advantages because of its limited range and high linear energy transfer, which may make  $\alpha$ -emitters better suited for the treatment of residual disease in cancer patients.<sup>21</sup>

As discussed by Dr. Barbet, pretargeting methods involve administration of an unlabeled, bifunctional (anti-tumor and anti-chelate) antibody or other cell-targeting agent followed by administration of a radionuclide construct that binds to the pre-localized antibody. This approach was pioneered by David A. Goodwin and colleagues.<sup>22-25</sup> An example of a pair of agents used in the method include a bispecific antibody that localizes to tumor cells and then binds a radiolabeled hapten-peptide that is subsequently administered.<sup>26-28</sup> Streptavidin-antibody constructs paired with biotin-radionuclide conjugates have also been used in



pretargeting approaches to the treatment of cancer. Clinical studies of pretargeting radio-immunotherapy have produced encouraging results.<sup>29-31</sup>

In conclusion, Dr. Barbet remarked that targeted radionuclide therapy is a workable therapeutic option against disseminated tumors, and specified that radio-immunotherapy is efficient in lymphoma. In contrast, nuclear medicine therapy is proving its efficacy against small tumor masses in solid tumors. He noted that the therapy has been tested mostly as a single therapeutic agent injected only once. As a consequence, repeated injections and combined therapy should be more thoroughly investigated. Dr. Barbet also reiterated the point that better radionuclides must be tested, including the alpha particle emitters that are ideally suited to treat diseases where tumor cells are isolated. Because development and advancement of the field requires highly multidisciplinary approaches, Dr. Barbet called for greater cooperation between academia and industry.

### Bispecific and Recombinant Oligoclonal Antibodies

The afternoon session was opened by **Scott Glaser** (Biogen Idec) who discussed advances in the preclinical development of IgG-like bispecific antibodies. He noted that next generation antibodies are designed to improve clinical outcomes, e.g., reduce immunogenicity, provide improved binding/access to target, increase potency by more effectively engaging immune effector functions, target two or more biological pathways or engage multiple cell types. Bispecific antibodies (BsAb) are not a new format, but manufacturability and protein stability posed substantial hurdles that took several decades to overcome. A number of second generation designs are using protein engineering strategies to address manufacturing issues. Dr. Glaser explained that Biogen Idec has been developing tetravalent IgG-like BsAbs<sup>32-35</sup> that bind one antigen via Fab and a second antigen via single chain variable fragments (scFv) attached to the Fc while retaining key antibody features such as IgG-like PK, an option for immune effector function, adaptability to current mAb manufacturing processes, and acceptable product quality.

Dr. Glaser noted that scFvs, which are key building blocks of IgG-like bispecific antibodies, often do not have sufficient intrinsic stability to permit scale-up production of molecules with acceptable biopharmaceutical properties. CHO cells produce heterogeneous mixtures of products, with monomer present as approximately 60% and soluble aggregates as 40% of the product. The monomer fraction is also unstable and will eventually degrade and aggregate during storage. Biogen Idec has applied a number of methods for designing stabilized scFvs, including statistical analyses, structure-guided design and knowledge-based design, to yield information regarding which scFv positions to target for mutagenesis. They synthesized mutagenic oligonucleotides, constructed focused plasmid libraries, transformed *E. coli* and then screened the libraries for mutants that demonstrated elevated thermal stability in a thermal challenge screen. An iterative stability screening process yielded stability engineered scFvs that met their goals ( $T_m$  of at least 65° C) for production of stable BsAbs.

Based on their experience with stabilization of scFvs, the wild-type variable light (VL) domain has typically shown greater stability than variable heavy (VH) domain and first focusing on stabilizing VH has been productive. However, Dr. Glaser described a BsAb designed to target two soluble ligands, designated as M1 and M2, in which the scFv VL hits destabilized VH to some extent. They resolved the problem by determining the  $T_m$  values of individual VL designs by differential scanning calorimetry (DSC), finding hits and combining those with the best VH combinations to yield a stable scFv. This led them to a new library work flow, which included DSC on wild-type scFv while assuming VH is the least stable domain, screening of the top 10–20 VH libraries in a thermal challenge assay, iterative combination of VH mutations, construction of VL libraries in the context of most stable VH and finally iterative combination of VL mutations. He noted that splitting the iterations into two steps increases speed due to the factorial nature of possible combinations. To date, seven scFvs have been successfully stabilized using their design tools, which have proven robust enough to manually make high-scoring mutants to rescue misbehaving scFvs.

Dr. Glaser noted that Biogen Idec's BsAb can be produced in CHO cell lines that provide titers in the 2–4 g/L range and that production can be scaled to a 1,000 L bioreactor. Platform purification that provides an acceptable product quality can be performed, e.g., main peak 97.9% by size exclusion chromatography (SEC) analysis. The purified BsAb is stable for a minimum of 5 months at a concentration of 10 mg/mL in phosphate buffered saline. In some examples, the bispecific antibody protein has been concentrated to over 100 mg/mL in research formulations without inducing aggregates or protein loss. Stability studies performed with 10, 50 and 100 mg/mL solutions indicate that BsAb are amenable to standard antibody formulation.

In concluding his presentation, Dr. Glaser discussed characteristics of the M1 x M2 BsAb. Biacore analysis indicated that the BsAb binds both M1 (target of scFv) and M2 (target of Fab) simultaneously, but the magnitude of the M1 binding signal suggested a different stoichiometry of binding to the BsAb compared to an anti-M1 mAb. Specifically, the stoichiometry of M1 binding to anti-M1 mAb decreases as the density of mAb increased, but the bispecific antibody was relatively insensitive to this effect. The length of the linker between the scFv and the Fc was observed to have an effect on BsAb activity, e.g., 1:1 binding of a M1 or M2 trimer to a mAb required a long, flexible linker. The behavior of the reverse BsAb (C-terminal anti-M2 scFv) suggested that this effect is not unique to the original BsAb. These results imply that valency, mobility and distance between binding sites are important considerations for optimizing BsAb activity. In experiments in mice, the M1 x M2 BsAb was found to simultaneously inhibit early and late phase activation of signaling pathway by M1 and M2 mediators. The M1 x M2 BsAb is thus functional in vivo and capable of neutralizing the two mediators.

**Tariq Ghayur** (Abbott Laboratories) discussed Abbott's dual variable domain (DVD)-Ig<sup>TM</sup> technology platform. The DVD-Ig<sup>TM</sup> format combines the antigen binding domains of two mAbs into a single entity by adding a binding domain to each Fab

arm.<sup>36</sup> It preserves the dual functional specificities of the parental mAbs, but behaves like conventional mAbs in many aspects. DVD-Ig<sup>TM</sup> molecules can be made to a variety of target pairs (e.g., two soluble ligands, soluble/cell surface antigens, two cell surface antigens) and using different variable domains (e.g., two human, human/humanized, human/mouse). Dr. Ghayur described several examples of DVD-Ig<sup>TM</sup> molecules that have been tested in preclinical animal models, including a DVD-Ig<sup>TM</sup> construct targeting IL-12/IL-18 that inhibited *Staphylococcus aureus* Cowan I (SAC)-induced IFN $\gamma$  in a HuPBMC-SCID mouse model and a DVD-Ig<sup>TM</sup> construct targeting IL-1 $\alpha$ /IL-1 $\beta$  that inhibited disease progression in a collagen-induced rheumatoid arthritis model.<sup>37</sup> Dr. Ghayur also showed in vitro re-directed toxicity data for several CD3 $\times$  DVD-Ig<sup>TM</sup> constructs (IC<sub>50</sub> in pM), and mentioned that two of these constructs (CD3/CD20 and CD3/EGFR) tested in vivo showed tumor growth inhibition activity in xenograft animal models.

The features to consider in making a DVD-Ig<sup>TM</sup> drug were then discussed in detail by Dr. Ghayur. Specifically, four key questions were addressed: (1) can the two variable domains (VD) retain function, e.g., affinity, potency, specificity? (2) can DVD-Ig<sup>TM</sup> constructs with desired physicochemical and drug-like properties be identified early in discovery? (3) do DVD-Ig<sup>TM</sup> possess desired PK properties? and (4) can DVD-Ig<sup>TM</sup> be expressed and manufactured in CHO cells? Dr. Ghayur explained that, in general, protein expression correlates with protein stability and physicochemical properties, and expression profiles have been used as a tool to identify stable VH/VL combinations for mAbs. In a similar fashion, expression profiles may allow selection of stable variable domain combinations/orientations in DVD-Ig<sup>TM</sup> constructs. He emphasized use of an 'anchor' concept, i.e., keeping one domain constant while altering domain combinations and orientations and the length of the heavy chain and light chain linkers.

Dr. Ghayur discussed the stepwise approach to building DVD-Ig<sup>TM</sup> generate hundreds of DVD-Ig<sup>TM</sup> molecules in a short time period; establishment of processes to fully characterize multiple DVD-Ig<sup>TM</sup> in parallel; and features that determine the desired drug-like properties (DLP) and PK for a DVD-Ig<sup>TM</sup> molecule. Initially, over a thousand DVD-Ig<sup>TM</sup> constructs were made, expressed, purified and preliminary functional characterization of both VDs by ELISA, Biacore, FACS or bioassays was performed. The contribution of VD combination, VD orientation and linker lengths to mammalian cell (HEK293) expression was evaluated and described. Dr. Ghayur then described detailed biophysical/chemical and DLP analysis, PK in rats (half-life typically in the 3–15 day range), and CHO cell expression and MTX amplification (100 nM) for 20–26 DVD-Ig<sup>TM</sup> molecules. Selected DVD-Ig<sup>TM</sup> were then additionally amplified; yields of ~1 g/L or more have been achieved. Dr. Ghayur provided the identity of some of the assays used for biophysical/biochemical evaluation of DVD-Ig<sup>TM</sup> constructs, e.g., intact and reduced LC-MS analysis; peptide mapping by LC-MS, SDS-PAGE under reducing and non-reducing conditions, Western blot analysis, DSC, universal buffer platform assays at pH 4, 6 and 8; assessment of freeze/thaw stability, sedimentation assay by SV-AUC; FTIR and near

UV spectroscopy; capillary zone electrophoresis, analytical HIC chromatography and size exclusion chromatography—multi-angle laser light scattering (SEC MALLS).

Dr. Ghayur then described how their high throughput processes can be applied to a program. As an example, he showed data from a factorial study involving 96 possible DVD-Ig<sup>TM</sup> composed of combinations of three anti-VEGF VDs paired with four anti-Delta-like 4 (DLL4) VDs in two orientations (inside and outside positions) via four linkers (long and short linkers each for VH and VL). Using the anchor concept, the anti-DLL4 VD or anti-VEGF VD was held constant in the inside position, while the linkers and various sequences for the anti-VEGF or anti-DLL4 domain were varied. Of the 96 constructs, 24 constructs with various VD combinations, VD orientations and linker lengths were selected and tested in vitro for functional activity. From this analysis, three DVD-Ig<sup>TM</sup> with different features were selected for further characterization.

The final topic of Dr. Ghayur's presentation was the external (independent) validation of the DVD-Ig<sup>TM</sup> format. In this study, a DVD-Ig<sup>TM</sup> derived from anti-VEGF-A bevacizumab and anti-OPN hu1A12 effectively bound and inhibited both targets.<sup>38</sup> Biacore analysis indicated that the K<sub>d</sub>s were 9.43 and 1.64 nM for OPN and VEGF, respectively, which were similar to the K<sub>d</sub>s of parental mAbs. In addition, VEGF/OPN DVD-Ig<sup>TM</sup> effectively suppressed HCCLM3 tumor growth and spontaneous lung metastasis in nude mice when dosed sc at 20 mg/kg, and was more effective than either bevacizumab or hu1A12 alone in the two animal models studied.

HESylation<sup>®</sup> of new scaffolds to improve drug characteristics was discussed by **Frank Feller** (Fresenius Kabi). The process involves coupling hydroxyethyl starch (HES) derivatives to drug substances in order to modify drug properties such as half-life. HES is a poly-disperse amylopectin polymer that is isolated from corn (waxy maize); HES products are marketed as plasma expanders and have a long history of safe use (e.g., intravenous doses up to 210 g per day can be administered to adults without side effects). Allergic reactions are known to occur in only a very small percentage (0.058%) of patients, and immunogenicity was demonstrated to be very low.<sup>39</sup>

The HES polymer is modified by acid hydrolysis to achieve the desired molecular weight (MW) and glucose residues are hydroxyethylated (molar substitution, MS) to control the rate of degradation caused by alpha-amylase. The MW and the MS can be tailored to achieve the required pharmacokinetic properties and these parameters also affect the degradation of a HESylated<sup>®</sup> therapeutic agent. Clearance is decreased with increasing MW of the HES derivative, while the degree of molar substitution directly interferes with degradation caused by alpha-amylase.

Mr. Feller explained that HESylation<sup>®</sup> enables prolongation of the circulation half-life of therapeutic agents by increasing the stability of the molecules, as well as by reducing renal clearance, resulting in increased biological activity. HESylation<sup>®</sup> also reduces immunogenicity or allergenicity and reduces viscosity (up to 4-fold compared to PEG). He concluded by describing two examples of drugs that demonstrated

improved pharmacodynamics or PK after HESylation<sup>®</sup>. In mice, HESylated<sup>®</sup> erythropoietin was shown to substantially increase hematocrit compared with the same dose of unHESylated<sup>®</sup> product and the hyperglycosylated erythropoietin darbepoetin alfa (Aranesp<sup>®</sup>; Amgen). The percent change of hematocrit was shown to increase with increasing MW of three HESylated<sup>®</sup> erythropoietins (HES 30, 60, 100 kDa). In dogs, the HES100-erythropoietin demonstrated a thrice amplified half-life compared to darbepoetin alfa. In rabbits, HESylated<sup>®</sup> interferon alpha showed higher in vitro activity, longer half-life and earlier onset of antiviral effect compared to peginterferon alfa-2a (Pegasys<sup>®</sup>; Genentech).

### Beyond Antibodies: Domain Antibodies and New Scaffolds

The final session of the meeting began with a presentation from **Ruud de Wildt** (Domantis), who provided an update on domain antibodies (dAbs) that comprise the pipeline and technology of Domantis. GlaxoSmithKline (GSK) acquired Domantis in 2007, then formed the biopharm units in 2008 and the biopharm discovery units in 2009. The units are responsible for defined areas of research and they function on a three year funding model based on specific business plans. Non-vaccine biopharmaceuticals currently comprise approximately 20% of GSK's overall clinical pipeline.

Dr. de Wildt described the properties of domain antibodies (dAbs) and highlighted the advantages of dAbs compared to full-size mAbs. DAbs are composed of human VH or VL and thus are the smallest functional binding units of antibodies. The molecules are derived from phage display libraries and bind to either protein A or protein L for detection, immobilization and purification. DAbs are soluble, stable and heat resistant, and they can be made to target a wide variety of antigens, including cell surface receptors. A comparison of dAbs with conventional human VH-VL structure has shown that the frameworks and complementarity-determining regions (CDR) have fully human conformations. At 11–13 kDa, dAbs are approximately one-tenth the size of IgG1. They can be designed to be either single or dual specific, as well as either mono- or bi-valent; they can also be derivatized, e.g., by conjugation to toxins or drugs. The half-life can be adjusted as desired and effector functions can be added.

Dr. de Wildt explained that dAbs can be delivered via a number of routes, e.g., intravenous, subcutaneous, inhaled, ocular, dermal, and he then discussed the pulmonary route in detail. The lungs are well-suited for local delivery because of their large surface area (>100 m<sup>2</sup>), highly permeable membrane and lack of mucociliary clearance. The small size of dAbs allows substantially higher doses to be delivered locally (approximately 12 times the equivalent mAb dose), efficient tissue penetration, and relatively slow clearance from the lung (half-life approx 4 h in mice). The molecules are also resistant to the proteases that exist in diseased lung, and can withstand the shear and thermal stress of nebulization. Lead dAb candidates have been developed that are thermodynamically stable (T<sub>m</sub> >65° C), maintain activity after 14 days at 50° C, resist degradation by proteases such as trypsin,

elastase and leucoszyme, and remain stable after nebulization at 20 mg/mL. There are currently three human dAb-based molecules in clinical studies (anti-inflammatory dAb, dAb targeted multicomponent vaccine, anti-TNF dAb-Fc fusion ART621).

The validation of TNFR1, also known as the p55 receptor, as a target in pulmonary disease was then discussed by Dr. de Wildt. Studies of TNF receptor pathways have identified divergent functions of TNF and suggested that selective inhibition of TNFR1 signaling may be more beneficial than total TNF blockade. For example, TNFR1 knock-out mice were protected from ventilator-induced lung injury (VILI) whereas TNFR2 knock-out mice died more quickly than wild-type mice.<sup>40</sup> In a murine model of tobacco smoke-induced lung inflammation, daily intranasal administration 1 mg/kg of an anti-TNFR1 dAb demonstrated a greater percent inhibition of response to smoke compared with a 10 mg/kg dose of PEGylated anti-TNFR1 dAb intraperitoneally (ip) administered once every two days and twice daily 10 mg/kg doses of PDE4 inhibitor given orally. The PEGylated and native dAbs had half maximal inhibitory concentrations (IC<sub>50</sub>) of approximately 1 nM. Dr. de Wildt explained that the duration of action of the dAb targeting TNFR1 is greater than 6 h, as determined in a mouse study of the dAb delivered intranasally at 0.3 mg/kg at various times prior to intranasal TNF challenge. This suggests that the dAb may be suitable as a therapeutic with daily dosing. He additionally described experiments that suggested that selective blockade of TNFR1 with the dAb attenuates VILI, whereas total blockade of TNF with an anti-TNF mAb does not attenuate VILI.

The in vitro/in vivo profile of an anti-human TNFR1 lead dAb was the final topic presented by Dr. de Wildt. The ability of dAbs to stimulate IL8 release from human lung fibroblast cells was examined, and no agonist activity was observed using monomeric dAbs tested at concentrations up to 16 μM, whereas the conventional anti-TNFR1 control mAb 225 showed agonist activity. The effect of the dAb on neutrophil cell influx in a cynomolgus lipopolysaccharide (LPS) challenge model was assessed. The nebulized dAb was administered 1 h prior to LPS challenge. Medium and high doses of dAb significantly inhibited LPS-induced bronchoalveolar lavage (BAL) neutrophil influx at 6 and 24 h (p < 0.005). The inhaled dAb also inhibited inflammatory mediators such as IL6, MIP1b and IL8 when tested in the cynomolgus LPS challenge model. In a PK study of inhaled vs. intravenous administration of similar doses of dAb in cynomolgus monkeys, the lung/plasma ratios indicated lung retention and low systemic bioavailability (<1%). The observed PK profile may support once or twice daily dosing in man.

An overview of modified single domain shark antibodies as therapeutic interventions in disease was presented by **Michael Foley** (AdAlta). He discussed the current trend toward development of next generation antibodies and engineered scaffolds that may have advantages as therapeutics compared to conventional full size IgG molecules. AdAlta is developing two of these types of products: Ig new antigen receptors (IgNARs),<sup>41</sup> which are antibodies found in cartilaginous fish such as sharks, rays, skates and ratfish, and i-bodies, which are single domain human proteins that mimic IgNARs. AdAlta's IgNARS have been shown

to be thermally stable, as well as stable to digestive enzymes and urea, and no immunogenicity has yet been detected in rabbits and mice.

Shark antibodies have no CDR2 and a long CDR3, which can affect the fine specificities of the antibodies. The average CDR3 loop length in sharks is 16,<sup>42</sup> whereas the loop length averaging 12–13 in humans, 9–10 in mouse,<sup>43</sup> and 14 in camel. AdAlta's library construction starts with blood taken from a wobbegong shark. The lymphocyte fraction is isolated and an mRNA preparation is made from the lymphocytes. cDNA corresponding to mRNA from antibody-producing cells are then used to produce libraries that contain  $6 \times 10^9$  antibodies that have random combinations of amino acid sequences. The sequence of the naïve shark DNA can be modified in the CDR1 and CDR3 regions, and the loop length of the CDR3 can be altered to be in the range of 10–20 amino acids. Targets may have a wide diversity and include antibodies, receptors, mitochondrial receptors, microbial transmembrane proteins, bacterial surface proteins, viral coat proteins and parasite proteins.

Dr. Foley then discussed specific binders isolated from an IgNAR library, including IgNARs targeting the *Plasmodium falciparum* apical membrane antigen 1 (AMA1). This antigen is involved in the invasion of red blood cells by the parasite, and has a hydrophobic trough that may serve as a binding surface for the elongated CDR3 of IgNARs. The 12Y-2 IgNAR variable domain<sup>44,45</sup> was selected from a library of individual variable domains. The clone bound AMA1 with a  $K_d$  of  $2.41 \times 10^{-7}$  M and was affinity-matured by error-prone PCR, resulting in several mutants with enhanced affinity over 12Y-2 that were also shown to be potent inhibitors of invasion of *Plasmodium falciparum* strain 3D7. Comparison of the epitope of IgNARs targeting AMA1 indicates that it overlaps with the epitope of the anti-AMA1 mAb 1F9 and that the long CDR3 loop penetrates the trough in AMA1.

In a similar fashion, IgNARs targeting insulin-like growth factor-1 receptor (IGF-1R) have been identified and characterized. The molecules can be purified from *E. coli* periplasm in yields up to 2–3 mg/L of purified protein. Dr. Foley explained that the anti-IGF-1R IgNAR AD0027 has been shown to have antagonist activity and to inhibit MCF-7 cancer cell growth with an  $IC_{50}$  of 10.4  $\mu$ M. Affinity maturation of AD0027 resulted in identification of a mutant with improved binding ( $K_d = 42$  nM compared with  $K_d = 75$  nM for AD0027).

Dr. Foley mentioned that IgNARs have humanization potential because their structure is very similar to human adhesion molecules such as the neural cell adhesion molecule (NCAM), which are part of the immunoglobulin domain structural (I-SET) family. AdAlta's i-body libraries are based on a human protein scaffold that has structural homology of >95% with the IgNARs. i-bodies are humanized single domains with both CDR1 and CDR3, with the CDR3 loop ranging from 10–20 amino acids in length. AdAlta has screened an i-body library on 5G8, a monoclonal antibody to malaria, and the malaria protein AMA1. The expression yields were 1.4 mg/L and 0.4 mg/L for the 5G8 and AMA1 i-bodies, respectively. The appropriate specificity of each was verified via ELISA. In concluding, Dr. Foley

noted that AdAlta's internal pipeline is currently at the discovery phase, with optimization of lead candidates in three disease areas (cancer, asthma/inflammation, HIV infection) expected in 2011.

Novel insights into IgNAR V-domain structure and function were provided by Brian Fennell (Pfizer). He explained that full-size IgNARs are homodimers of two polypeptide chains each comprising a variable (VNAR) and five constant domains (CNARs). The VNARs, which are the focus of development efforts, contain a short CDR1 loop, HV2, which is an area of natural amino acid diversity that replaces the classical CDR2 loop, and a long CDR3 loop. IgNARs have Type I and II subtypes based on the presence or absence of non-canonical cysteine residues in the VNAR Fw and CDR regions, and share structural features with some vertebrate Ig domains, including TCR  $V\alpha$  chain and IgG  $V\kappa$  chain. Interest in developing VNARs stems from the size of the paratope, which has only 2–3 loops yet has an antigen-binding interface that is similar in size (in angstroms) compared with classical dual-domain antibodies, the ability to target clefts and advantageous physicochemical properties, e.g., tolerance to high levels of salt and urea, thermostability.

Dr. Fennell discussed Pfizer's efforts to better understand the structural features that define the qualities of the VNAR domain through a structure/function study of a Type I VNAR. The study involved identification of areas of the molecule that can tolerate mutation(s), i.e., VNAR hotspots; investigations into the importance of the cysteine residues to VNAR structure/function; affinity maturation of the anti-hen egg-white lysozyme (HEL) shark VNAR 5A7<sup>46</sup> using ribosome display technology; and determination of VNAR-specific and VNAR/Ig VL/TCR  $V\alpha$  overlapping hallmark residues, i.e., residues crucial to the structure/functioning of the protein. Their 'molecular scanning' approach included generation of a ribosome-displayed 5A7 mutant library with mutations across the entire VNAR domain, and selection of a library on the HEL antigen over three rounds with iterative mutagenesis between rounds. The library inputs and outputs were sequenced at the DNA level and they screened for HEL binding using a number of functional assays, e.g., ELISA, Biacore. A heat map/scanning map of 5A7 was then constructed based on results of sequencing and functional assays.

They identified 14 hotspots, defined as an area of the NAR protein that tolerated amino acid substitutions greater than five times, that were located mostly in the solvent exposed parts of the protein; a high level of mutational plasticity was observed across the VNAR domain. However, the 5A7 paratope was found to be non-plastic, with only conserved substitutions tolerated, e.g., Y29H, A88G, A95G, which suggested that it was unlikely that 5A7 affinity-matured variants would come from further maturation of CDR1/3. Dr. Fennell presented data for 5A7 variants that indicated that mutation in the Fw1 region appeared to result in an improved off-rate and mutation in the HV4 region appeared to result in an improved on-rate. They then explored combinations of amino acid mutations and identified one triple mutant (A1D, S61R, G62R) that showed a 20-fold gain in affinity compared with 5A7 ( $K_d$  of 0.46 nM vs. 9.33 nM). Dr. Fennell noted that a structural model of 5A7 containing beneficial mutations indicated that Asp1 formed a salt bridge with R112 and K116 of

HEL, which resulted in an improved off-rate, Arg61 formed a weak ionic interaction with D101 of HEL, which resulted in an improved on-rate and Arg 62 may also contact D101 of HEL or increase VNAR solubility, thereby leading to increased stability.

The group also investigated the importance of cysteines to VNAR structure/function. Four of six variants in which wild-type cysteines were mutated and nine clones in which native amino acids were replaced with cysteines lost HEL-binding. HEL binding was retained with the variants G15C (Fw1 region), G42C (Fw2 region) and S61C (HV4 region), suggesting that these might be novel sites for covalent labeling of VNAR domains. Dr. Fennel concluded by describing the set of experiments designed to identify hallmark residues, i.e., residues crucial to the structure/functioning of the protein that may have to be retained during humanization. The group constructed a 5A7 heat map, identified framework residues that did not tolerate amino acid mutation, and conducted bioinformatic analysis of suspected VNAR hallmark residues that included a comparison with unique spiny/spotted shark clones, nurse shark clones, and Ig VL and TCR V $\alpha$ , the latter of which defined residues that overlap between v-domain classes. A total of 21 residues located predominantly to the core of the NAR were classified as overlapping with VNAR/IgVL/TCR hallmarks, and three residues (L18, T34 and E57) were identified as VNAR specific hallmarks. The study findings should aid future VNAR engineering and optimization studies towards development of the proteins as viable therapeutics.

**Laurent Audoly** (Pieris) closed the meeting with a discussion of the proof of concept (POC) trials for new scaffolds that have VEGF and c-Met as targets. He first noted that, in general, there is a need for targeted molecules with improved pharmacological and biophysical properties compared to those of antibodies, including access to greater target space and efficacy, more flexible formatting (e.g., inhaled), and reduced cost of goods. Anticalins<sup>®</sup>, which are engineered lipocalins, are in development by Pieris as targeted therapeutics. The 12 known human lipocalins endogenously bind, store and transport a broad spectrum of molecules; they are monomeric and very stable proteins that are approximately 18–20 kDa. The natural binding pocket of wild type can be redesigned to generate highly specific hapten and peptide binding Anticalin<sup>®</sup> molecules. Lipocalins also have CDR-like loops that can be modified to generate highly potent protein binding Anticalin<sup>®</sup> molecules. Combinatorial libraries up to  $1 \times 10^{11}$  are produced by diversification at key amino acid positions. A platformed phage display based-screening process results in the identification of candidates with pM affinity that can be produced in *E. coli* and modified as desired, e.g., PEGylation or fusion to protein, to extend the half-life in plasma.

Dr. Audoly explained that the screening for Anticalins<sup>®</sup> is done with the same host and in the same final format from discovery to identification of drug candidate to manufacturing. The cycle time from discovery to in vivo POC is approximately 18 months, with the overall time from discovery to IND filing of three years. Pieris has one candidate in Phase 1 clinical studies (anti-VEGF PRS-050), two candidates at the in vivo POC stage (anti-cMET PRS-110, PRS-080 targeting an undisclosed

antigen) and two projects at the anticalin selection stage (inhaled anti-IL4Ra PRS-060, and a bispecific “Duocalin” PRS-190).

Data on PRS-050, a VEGF targeting Anticalin<sup>®</sup> for oncology was then presented by Dr. Audoly. The molecule is specific for all major human VEGF-A splice forms (K<sub>d</sub> = 1 nM) and has comparable affinity and efficacy to competitors in preclinical models. The binding mode is antagonistic, with an IC<sub>50</sub> of 12 nM in competition ELISA; the molecule blocks VEGF interaction with VEGF-R1 and -R2. Half-life extension was achieved via site-directed mono-PEGylation with branched 40 k-PEG. The molecule shows cross-reactivity towards murine and cynomolgus monkey VEGF with similar potency. PK in mice, rat and cynomolgus monkeys suggested that the molecule would have a five to six day half-life in humans. PRS-050 inhibited the extravasation of dye in an assay of VEGF-induced enhanced vascular permeability in guinea pigs and also inhibited angiogenesis when dosed at 9.3 mg/kg daily ip in a Matrigel plug angiogenesis assay. In an A673 rhabdomyosarcoma tumor xenograft model, PRS-050 inhibited tumor growth in a dose (2.6, 7.7 or 15.4 mg/kg dosed ip daily) and time (7.7 mg/kg dosed ip daily or every other day) dependent manner. Dr. Audoly also noted that published reports have described an unexpected increase in thromboembolic events in clinical trials of anti-VEGF bevacizumab<sup>47</sup> and that a possible cause is platelet activation induced by bevacizumab-VEGF-heparin immune complexes. As of today, it is also important to note that this increase in thromboembolic events is controversial as this has not been consistently measured. Nonetheless, studies in Fc $\gamma$ RIIIa transgenic mice have shown that PRS-050 does not form immune complexes and does not cause significant thrombocytopenia in a preclinical model suggesting a possible path forward for differentiation. From a manufacturing “line of sight”, a robust GMP process has been developed. PRS-050 is produced in *E. coli*, undergoes two-step purification via chromatography, which is followed by PEGylation, another chromatography step and then sterile filtration and fill/finish. The first administration to humans with solid tumors occurred in June 2010 and preliminary human single dose PK data suggest the half-life is approximately six days.

The final topic discussed by Dr. Audoly was the development of PRS-110, a monovalent c-Met antagonist. cMet is implicated in tumor cell proliferation, metastasis and angiogenesis, and is the only receptor identified to date for hepatic growth factor/scatter factor (HGF). Dysregulation of c-MET or HGF expression has been correlated with a variety of human malignancies. At least four therapeutic proteins targeting either c-MET or HGF are in clinical studies: anti-c-MET one-armed antibody-like molecule ortuzumab (Genentech), anti-HGF rilotumumab (Amgen), anti-HGF TAK701 (Takeda) and anti-HGF AV299 (AVEO). Dr. Audoly presented data for PRS-110 indicating that the molecule is highly specific with low nM affinity against c-Met and displays in vivo efficacy. Anti-tumor activity was observed with PRS-110 dosed at 7.5 mg/kg as a single agent, and enhanced activity was observed when the agent was combined with temozolomide. PRS-110 can be produced with a fermentation yield of 8.9 g/L, and a 36% yield after 2-step purification. The PEG conversion is 88% and final purity is 95% by SDS-PAGE and 96% by SEC-HPLC.

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