7th Annual European Antibody Congress 2011
November 29–December 1, 2011, Geneva, Switzerland

Alexey A. Lugovskoy,[1,]* Janice M. Reichert[2,]* and Alain Beck[3,]*

[1]Merrimack Pharmaceuticals Inc.; Cambridge, MA USA; [2]Landes Bioscience; Austin, TX USA; [3]Centre d'Immunologie Pierre Fabre; Saint-Julien en Genevois, France

The 7th European Antibody Congress (EAC), organized by Terrapin Ltd., was again held in Geneva, Switzerland, following on the tradition established with the 4th EAC. The 2011 version of the EAC was attended by nearly 250 delegates who learned of the latest advances and trends in the global development of antibody-based therapeutics. The first day focused on advances in understanding structure-function relationships, choosing the best format, glycoengineering biobetter antibodies, improving the efficacy and drugability of mAbs and epitope mapping. On the second day, the discovery of novel targets for mAb therapy, clinical pipeline updates, use of antibody combinations to address resistance, generation and identification of mAbs against new targets and biosimilar mAb development were discussed. Antibody-drug conjugates, domain antibodies and new scaffolds and bispecific antibodies were the topics of the third day. In total, nearly 50 speakers provided updates of programs related to antibody research and development on-going in the academic, government and commercial sectors.

Note

Summaries were prepared from PDFs of the presentations provided by speakers after the meeting. In the cases when a speaker was not able to share their presentation, detailed summaries are not included, although the speaker's name, affiliation and topic appear in the report.

November 29, 2011: Day 1
Alexey Lugovskoy

The 7th European Antibody Congress was opened by the Chairman, **Alain Beck** (Pierre Fabre), who is also Associate Editor of *mAbs*. He welcomed the attendees and gave an overview of the agenda. He dedicated the first day of the congress to antibody structure-function relationship studies, glycoengineered antibodies, biobetters and next generation monoclonal antibodies (mAbs), improving efficacy and developability, and methods for antibody analysis.

Dr. Beck gave the first talk, which was entitled "Biosimilar, biobetter and next generation antibodies." He remarked that there is a substantial amount of confusion regarding the terms used to describe the copies of original biological medicinal products. In addition, the counterfeit medicines are occasionally confused with biosimilars[1] and European Medicines Agency (EMA) biosimilar guidance is commonly misinterpreted. Dr. Beck introduced the definitions of biosimilar, biobetter and next generation antibodies.[2] A biosimilar is a generic version of an "innovator" antibody with the same amino-acid sequence, but produced from a different cell clone and in a distinct manufacturing process that result in differences in glycosylation and other microvariations. A biobetter is an antibody with a very close amino acid sequence, but with an optimized glycosylation profile or engineered Fc domain to increase the serum half-life. All other follow-on antibodies with different amino acid sequences should be classified as next generation antibodies. Dr. Beck presented a case study of an antibody[3] that was intended as a biosimilar of trastuzumab. The innovator's trastuzumab is heterogeneously glycosylated,[4,5] but the follow-on product has two amino acid differences attributable to a different Fc allotype and therefore cannot be considered a biosimilar per EMA guidance.[6]

Dr. Beck presented several case studies of biobetter antibodies: glycoengineered trastuzumab,[7] afucosylated trastuzumab,[5] a CHO-produced cetuximab[8] and a Glycotope cetuximab (CetuGEX) derivative. The latter two biobetters do not to carry Gal-α-1,3-gal epitope, which triggers adverse immunological reactions in a subset of patients. Dr. Beck also highlighted the work on biobetter versions of bevacizumab and cetuximab engineered to have a longer half-life in human plasma[9] that translates into greater in vivo efficacy in animal models. The significance of structure-function relationships in antibodies is well-understood, and these insights are being used to increase antibody homogeneity, mitigate the chemistry, manufacture and control liabilities, and to improve overall developability of next generation antibodies.

In the final part of his talk, Dr. Beck reviewed several examples of next generation antibodies in clinical development. Typically, next generation antibodies possess characteristics that can give them superior efficacy. In the case of anti-respiratory syncytial virus (RSV) antibodies palivizumab and motavizumab, the latter binds to the same epitope of RSV with 70-fold higher affinity.[10]

*Correspondence to: Alexey A. Lugovskoy, Janice M. Reichert and Alain Beck;
Email: alugovskoy@merrimackpharma.com,
janice.reichert@landesbioscience.com and alain.beck@pierre-fabre.com

This results in 20-fold improvement in RSV neutralization in vitro, reduces pulmonary RSV titers in cotton rats to 100-fold lower levels and 50% reduction in the incidence of RSV-specific infections in Phase 3 clinical study.[11] There are three clinical-stage next-generation antibodies follow-ons to rituximab: ocrelizumab,[12] ofatumumab,[13] and obinutuzumab.[14] While ocrelizumab is a humanized version of rituximab, obinutuzumab and ofatumumab are human antibodies that target different epitopes and possess distinct mechanisms of action. Pertuzumab binds to different ErbB2 epitope than trastuzumab and works mostly by blocking ErbB2 heterodimerization with ErbB3 and ErbB4. Different mechanisms of action of pertuzumab and trastuzumab result in synergistic activity of their combination that is currently being investigated in the Phase clinical trials. The next generation anti-EGFR antibodies are perhaps the largest group. Following the success of cetuximab, necitumumab, panitumumab, nimotuzumab, zalutumumab, all of which target the same EGFR epitope and matuzumab, which targets a distinct epitope, have been developed. Dr. Beck noted that, to date, the superior in vitro and in vivo properties of these next generation antibodies have not translated into commercial success and, particularly in oncology, the original innovator products generate the most sales.

Claudio Rhyner (Swiss Institute of Asthma and Allergy Research) discussed kinetics on cells in the context of bridging the gap between traditional biosensor and cell based assays. He gave an example of how the Attana Cell 200 QCM system, the biosensor that measures molecular interactions label free directly on cell surfaces, has proved to be useful in discovering novel targeting vaccines that generate specific antibody responses in mice.

Dongxing Zha (GlycoFi Inc.,/Merck and Co.,) gave a talk entitled "Optimized glyco-engineered IgGs produced in yeast: Glycoengineered Pichia produced anti-HER2 is comparable to trastuzumab in preclinical study." He reminded the attendees on the role N-linked glycosylation in the stability and pharmacokinetics of antibodies, as well as in antibody-dependent cell-mediated cytotoxicity (ADCC). Antibodies produced in wild-type yeast carry high-mannose sugars and are cleared quickly in cynomolgus monkeys through mannose-receptor mediated uptake. Glycofi's glycoengineered Pichia produces antibodies with low mannose content that do suffer from this limitation. Glycofi yeast strain collection contains 22 isoforms. These strains allow high-throughput transition from fermentation at 0.5 L scale, to 1,200 L in the Glycofi antibody production platform.

Dr. Zha presented a case study of a trastuzumab biobetter produced in glycoengineered Pichia. This molecule, which is naturally afucosyl, has a single mannose O-linked glycan on CH1. The molecule is homogenous by SDS-PAGE and size exclusion chromatography (SEC) and shows lower glycan heterogeneity than that known for different batches of trastuzumab.[4] The Glycofi biobetter trastuzumab shows similar binding to SKBR3 human breast cancer cells as measured by fluorescence-activated cell sorting. This translates to similar inhibition of BT474.m1 cancer cell line growth and comparable efficacy in BT474.m1 tumor xenograft model. The Glycofi biobetter trastuzumab binds to Fcγ RIIIa 10-fold tighter and shows enhanced in vitro ADCC. It has comparable pharmacokinetics (PK) in C57BL6 mice and in cynomolgus monkeys, and shows no increase in the incidence of anti-drug antibodies. The bioprocess has been successfully scaled-up from 30 L to 1,200 L with the final product yield of about 0.6 g/L. Dr. Zha concluded that glycoengineered Pichia offers a unique platform for the production of novel biologics.

Steffen Goletz (Glycotope) discussed biobetter antibodies in oncology in clinical development. He introduced GlycoExpress™ Technology and its collection of glycoengineered human cell lines. Over a thousand enzymes control glycosylation in humans. Glycotope has selected human cell lines and pursued rigorous optimization of sialylation, branching, galactosylation and fucosylation. The resultant cell lines show high productivity of 1–7 g/L, yielding to 4–5.5 g per run of purified cetuximab biobetter (CetuGEX) after upstream and downstream processes. It is a high-density system yielding 8–500 million cells/ml. It uses proprietary medium and can be scaled up to 9,000 L fermentation. This technology has been used in more than 15 successful case studies with antibody products. One of them is trasGEX, a glyco-optimized trastuzumab biobetter. It shows 10–140-fold increase in ADCC and shows efficacy in low ErbB2 MCF-7 cancer cell line that is insensitive to trastuzumab. As a cetuximab biobetter, CetuGEX also has higher ADCC, up to 2-fold longer half-life in cynomolgus monkey, and no high-mannose structures that represent non-mature carbohydrates. This molecule is currently being investigated in Phase I clinical studies. In conclusion, Dr. Goletz remarked that its administration does not result in skin rash in the patients.

Marco Thomann (Roche) discussed state-of-the-art analytical methods for mAbs characterization. He described how mass spectrometry techniques could be used to pursue analysis of post-translational modifications of mAbs, and presented different analysis methods for oligosaccharide analysis, high throughput glycan analysis, disulfide analysis and degradation analysis. He also introduced the software for peptide mapping analysis toolbox for glycoanalytics that assist users in user guided selection and assignment of mass spectroscopy peaks.

Catherine Evans (Bruker Daltonics) gave a talk entitled "Biopharmaceutical Characterization—mass spectrometric solutions for challenging analysis," which provided a didactic overview of state of art mass spectrometry methods for antibody characterization. In the last five years, over 200 publications have reported research on antibody characterization by mass spectrometry. This technique is well suited to intact mass analysis, peptide mapping, amino acid sequence confirmation and post-translational modification and degradation product analysis. Dr. Evans emphasized that different technologies, different instruments and high-sophistication software are required to maximize the precision of such analyses. Dr. Evans presented several case studies on successful applications of quadrupole time-of-flight (Q-TOF) mass spectrometry and BioPharma Compass™ software to glycoform analysis, subunit analysis, clone comparison, peptide sequencing and quantification of impurities in antibodies.

Dr. Evans remarked that deamidation analysis presents a separate set of challenges because of the isobaric nature of aspartate and isoaspartate species. This challenge could be addressed by electron transfer dissociation (ETD) mass spectrometry that

differentiates between isobaric species.[15] She gave an overview of biopharmaceutical characterization techniques with matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI TOF/TOF) mass spectrometry and presented a study on automated glycan detection and identification using GlycoQuest™ software package where all 11 glycoforms of an antibody were successfully detected and quantified relative to each other on the basis of signal intensity. Dr. Evans also highlighted the use of MALDI TOF/TOF for N- and C-terminal confirmation via top down sequencing. The amino acid sequence confirmation can be achieved in less than one minute using minimal amounts of material. The method does not require proteolytic digestion because the fragmentation is induced by a laser.

Jim Freeth (Retrogenix) gave a talk entitled "Function-led antibody drug discovery: Solving the target deconvolution bottleneck." He contrasted function-led "hypothesis-free" and target-led "hypothesis-driven" drug discovery[16] and remarked that there is a lot of competition in the target-led arena. The function-led approach was quite successful for small molecules[17] and opportunities for function-led antibody drug discovery exist. Retrogenix technology assists researchers in solving the target deconvolution bottleneck by presenting full-length human plasma membrane proteins expressed in natural human cell environment for functional analysis. In brief, the technology works as follows: the expression vectors that encode a protein of interest are spotted on a glass slide; human 293 cells are placed on the slide in a confluent monolayer; these cells uptake the spotted plasmid and overexpress the encoded membrane proteins. This functional array can be probed with an antibody of interest and the binding event can be detected using standard techniques. Use of proprietary transfection and expression conditions, as well as optimized spot density and size, results in tight, spatially separated transfected cell clusters and yields fewer than 10% intra- and inter-experimental variability.

Retrogenix has constructed a set of over 2,500 full-length, unfused human plasma membrane proteins derived from 2,054 unique genes. This set will soon be extended to over 3,000 membrane proteins, which will ensure the complete coverage of the proteome. Dr. Freeth presented several case studies on the analysis of antibodies and one Fc-fusion protein directed against transmembrane targets. The affinities of these antibodies ranged from 9 to 340 nM; nonetheless, in all the cases the primary targets were found. Additionally, one non-G-protein coupled receptor (GPCR) secondary target was identified. Dr. Freeth highlighted the importance of pre-screening with non-transfected mammalian cells because, in non-prescreened conditions, human Fc receptors are also detected as targets.

Robert Burns (4-Antibody) discussed 4-Antibody's core technology for antibody discovery and optimization, which allows fast generation of antibody hits and improved efficiency in conversion of antibody hits to development-ready leads. The technology is centered on the innovative use of Retrocyte Display® where mouse pre-B cell line is transformed with retroviral vectors encoding for light and heavy chains of human antibodies yielding human IgG antibody-producing B lymphocyte library. This is screened against labeled antigen by fluorescence-activated cell sorting or magnetic-activated cell sorting. This cycle can be conducted iteratively until the single-cell sorted cell clones with required affinity are isolated. Antigen-specific antibody genes are then recovered and the sequences are determined. Using this approach, 4-Antibody can convert any non-human antibody into a human antibody, or discover novel human antibodies in 12–16 weeks. Dr. Burns concluded his talk by presenting a case study of de novo antibody discovery against an undisclosed antigen where 11 antibodies meeting optimal functional characteristics were identified.

Bernhard Helk (Novartis) delivered a talk entitled "Developability Index: A Rapid in silico tool for the Screening of Antibody Aggregation Propensity." He explained a principle of spatial-aggregation-propensity (SAP) technology[18,19] and highlighted its use in developability ranking, design of stable proteins, predicting binding regions for disease targets and identifying sites for payload conjugation. Dr. Helk described the discovery of developability index that combines hydrophobicity and electrostatics into a single parameter for the ranking of mAbs. Novartis's empirical aggregation propensity score, which was extensively trained on antibody stability (measured as accumulation of percent aggregates 40°C at 100 mg/mL concentration in 20 mM histidine pH 6.5), differs from SAP score in antibody complementarity determining regions (CDR) in situations where the net antibody charge is variable. Therefore, both computer SAP CDR score and antibody net charge were included in the developability index formula and the weighting coefficients were trained on the 25°C and 40°C stability data obtained for 11 antibodies. The parameterized development index was prospectively validated on the set of five mAbs and showed good predictive power. As expected, the molecules with high SAP scores aggregate slower at low pH due to their higher net charge. In comparison, the molecules with low SAP scores were stable at all the pH testing conditions. SAP technology can be used to engineer stable proteins. When SAP-predicted suboptimal hydrophobic amino acids were substituted for lysines in a mAb, the aggregation stability in a stress test conducted for 12 or 24 h at 58°C was improved as judged by SEC results. Because the mutagenized sites were located in the Fc region of an antibody, they did not interfere with antigen binding. SAP technology can also be used to predict protein binding regions[20] and to improve the determination of sites for payload conjugation.[21] In the former example, regions of high SAP score overlap with protein binding regions and, in the latter example, payload conjugation sites should be designed in partially solvent-exposed areas with low SAP scores.

Christian Klein (Roche Glycart AG) discussed how the epitope characterization and crystal structure of GA101 have provided insight into its mechanism of action. GA101 (obinutuzumab) is a type II CD20 antibody that displays high ADCC, low complement-dependent cytotoxicity (CDC), lack of partitioning to lipid rafts and partial antigen binding capacity. These properties are distinct from those of type I antibodies. Rituximab, a type I antibody, partitions to lipid rafts, induces potent CDC and displays full antigen binding capacity. GA101 was glycoengineered to have low fucose content resulting in high ADCC; it also features elbow hinge modification that is requires to induce

apoptosis directly.[22] GA101 induces time-dependent cell death in a panel of non-Hodgkin lymphoma cell lines by invoking actin-dependent lysosome- and cathepsin-mediated cell death.

Dr. Klein noted that, interestingly, GA101 binds to the same loop as rituximab but targets it differently. The GA101 binding site is shifted to the C-terminus with asparagine 171 being critical to rituximab binding, but not to GA101 binding.[14] The crystal structure of GA101 bound to CD20-derived cyclic peptide reveals that GA101 interacts about 10% more of the CD20 surface and has a more open elbow hinge angle compared with rituximab. The GA101 binding pose is rotated 90° around its middle axis and tilted approximately 70° toward the carboxyl terminus of the CD20 peptide relatively to that of rituximab. These structural data allowed researchers in Glycart to propose a model for the binding of type I and type II anti-CD20 antibodies to CD20 tetramers. According to this model, type II antibodies crosslink intra subunits of CD20 tetramer, whereas type I antibodies bind across CD20 tetramers. In cell culture, fluorescently-labeled rituximab and GA101 separate into different cell membrane domains, suggesting that GA101 recognizes specific CD20 subpopulations. This hypothesis is supported by the results of electron tomography studies, where one "closed" conformation of anti-CD20 tetramer unique to GA101 was identified. Consistently, GA101 treatment triggered distinctly higher magnitude changes in mRNA profiles of lymphoma lines compared with rituximab.

An additional difference in the mechanism of action between type I and type II antibodies lays in the ability of type I antibodies to modulate CD20 membrane levels through internalization. This phenomenon is FcγRIIb mediated and, consistently, FcgRIIb expression levels predict progression free survival in mantle cell lymphoma patients treated with rituximab regimens.[23] GA101 is a glycoengineered antibody that lacks core fucose.[24,25] This deletion relieves the steric glycan hindrance in the antibody Fc receptor complex[26] and increases antibody affinity to FcγRIIIa receptor without affecting its binding to FcγRIIb, thus enhancing ADCC. GA101 exhibits up to 100-fold higher ADCC than rituximab and ofatumumab,[22] displays superior B cell depletion in B cell-chronic lymphocytic leukemia patient-derived whole blood compared with rituximab and induces complete tumor remission in SU-DHL4 xenograft model. This remission is independent of GA101 glycan status, as non-glycoengineered version of GA-101 induces comparable effect. When administered following rituximab treatment in SU-DLBCL xenografts, GA101 causes sustained tumorostasis. Importantly, this effect was achieved at physiologically relevant exposures that matched clinical efficacious trough levels. Dr. Klein concluded his talk by summarizing GA101 mechanism of action. GA101 causes enhanced direct cell death,[22,27] enhanced antibody-dependent cell-mediated cytotoxicity,[22,26,28] reduced CDC and decreased CD20 internalization.[23,29] Four Phase 3 clinical trials of GA101 are currently ongoing.

Bing Kuang (Pfizer) discussed a holistic approach for free and total measurement of therapeutic antibody and target. He highlighted the importance of accurate measurement of free and total drug concentrations in both PK and pharmacodynamic (PD) analytical assays. Detailed understanding of quantitative pharmacology is essential to provide PK- or PD-guided dosing regimens. If the target is a non-shedding cell surface receptor, the bound drug is quickly removed from the site of action and all circulating drug can be considered to be in its free form. In this case, only free drug assay is sufficient. The situation is more complex in the cases of soluble target or shedding cell surface target, as bound drug circulates in multiple complexes until their elimination. In this case, both free and bound drug assays are required. Dr. Kuang noted that there are several popular analytical assay formats for therapeutic mAb quantitation, including antigen capture ELISA, bridging ELISA, anti-idiotype capture ELISA, generic anti-human IgG ELISA and competitive antigen biding assay. In all of these assays, antibody-target equilibrium can be shifted during measurement in accordance with the law of mass action.

Dr. Kuang presented an example of the use of antigen-specific capture ELISA to measure the concentration of anti-amyloid β antibody where the use of capture antigen altered the free fraction of anti-amyloid β antibody in dose-dependent fashion. In a related example, a cetuximab antigen-capture ELISA was found to be sensitive to the concentration of capture antigen to larger extent than to the concentration of soluble EGFR receptor. In these cases, the antigen-capture ELISA assay was clearly not measuring the free concentration of antibody. In the case of daclizumab, the bridging assay to measure the concentration was used.[30] In theory, this assay would measure only the unbound antibody; however, minimal interference was observed at antibody-to-target molar ratios of close to one. This assay interference was due to soluble IL-2R and was eventually decreased by sample dilution. Dilution should be utilized with caution, as the effect on the measurement can be pronounced at low antibody-to-target ratios when all complexes may dissociate. On the contrary, if antibody is in 10-fold excess, free fraction will never be less than 90% regardless of antibody affinity and total drug assay may be acceptable. Dr. Kuang concluded by emphasizing the need for careful development of PK and PD assays. He gave practical guidance on development of such assays. Researchers should pay close attention to: (a) total concentration of antigen and the target, (b) antibody/antigen affinity; (c) molar ratio of total antibody; (d) target and antibody concentration; (e) sensitivity of their assays; (f) adequate reference standards; (g) dissociation rates of antibody/antigen complex; and (h) rigorous drug and ligand interference testing.

Saileta Prabhu (Genentech) gave a talk entitled "Effects of molecular properties on antibody pharmacokinetics: recent lessons." She reviewed molecular, delivery and host characteristics as well as formulation effects that determine the PK properties of mAbs. She explained how antibody PK could be influenced by net charge, structure, binding affinity, antibody subclass and molecular size differences. Dr. Prabhu concluded that a thorough understanding of the PK of IgGs is beneficial in informing their development plans.

Ton Logtenberg (Merus BV) discussed MeMo®, a state-of-the-art single light-chain transgenic mouse for the generation of therapeutic human monoclonal antibodies. The MeMo® mouse

harbors a single rearranged human light chain and rearranged human heavy chain locus comprising all D and J genes and a cohort of V genes that are selected for structural diversity, stability and frequent use in natural immune repertoires. Murine heavy chain constant regions and control elements are retained to promote robust antibody responses that provide rapid access to a large collection of high affinity antibodies for direct functional analysis. The single light chain antibody format enables the expression of two or more human antibodies in a single cell without formation of non-functional contaminants. This expanded the capability of MeMo® platform to the production of bispecific antibodies or mixtures of antibodies. These innovative formats retain the biophysical characteristics of component antibodies and production levels are comparable to those of conventional mAbs.

Patrick Schindler (Novartis) gave a presentation describing the successes and pitfalls of epitope mapping. He introduced the concept of linear and discontinuous epitopes and remarked that the latter are typically interrogated via multiple technologies including modeling, mutagenesis, affinity measurements, X-ray crystallography, hydrogen-deuterium exchange mass spectroscopy and atomic mass spectroscopy. He presented two case studies on mapping linear and discontinuous epitopes.

Jeffrey Luo (Centocor Research and Development Inc.,) discussed how to obtain desired antibody epitopes by innovative screening and selection strategies. He presented structural, modeling and binding analysis of three antibodies against Toll-like receptor 3,[31] that led to hypotheses on their distinct mechanisms of action.

Fabrizio Gannotta (ProGenosis) presented a talk entitled "Identification of epitope region and key amino-acid determination using BHP technology as a BioTool." Fine description and understanding of structure-activity relationships can be difficult to attain for interactions involving large molecules. This is because epitopes are often nonlinear and are formed by residues that are sequentially discontinuous, but come close together in three-dimensional space. The hybrid β-lactamase display methodology developed by ProGenosis utilizes the insertions of the pieces of target proteins in the β-lactamase gene. Such insertions enhance target solubility and improve the folding of its domains. Antibiotic selection can be applied to select bacteria that produce the soluble β-lactamase inserted fragment enabling the proofreading of protein folding. This technology was utilized to elucidate a complex epitope on the extracellular domain of CD22, which serves as a specific marker on the surface of malignant B cells. The ProGenosis group showed that the interaction between CD22 and the antibody involves at least three discontinuous regions of CD22. Three key paratope amino acids were confirmed by site-directed mutagenesis and the results were consistent with the structural model of the closed conformation of CD22.[32]

David Lowe (MedImmune) discussed techniques used to reveal pharmacologically interesting epitopes. Antibody epitopes play key roles in their mechanism of action and specificity. Dr. Lowe gave an overview of epitope-mapping techniques and highlighted the advantages of X-ray crystallography for unambiguous epitope determination. He gave an example of the engineering of an anti-interleukin (IL)-15 antibody that directly competed with

IL-1 receptor by recognizing a 21 amino acid epitope. The parental antibody did not cross-react to mouse antigen, and its binding to cynomolgus monkey antigen was reduced 3-fold. An analysis of available X-ray structure allowed the MedImmune group to understand the amino acid determinants of species cross-reactivity and to design an engineering strategy to overcome this liability.

Dr. Lowe presented an interesting case study on the discovery of anti-Intercellular Adhesion Molecule 1 (ICAM-1) antibody for treatment of chronic obstructive pulmonary disease (COPD). Infection by human rhinovirus (HRV) is a major cause of upper and lower respiratory tract disease worldwide and COPD peaks coincide with peaks in human rhinovirus infection. On the molecular level, HRV uses two main routes of viral entry and utilizes ICAM-1 as a main entry receptor. The model of HRV-ICAM-1 complex[33] suggests that the HRV site on ICAM-1 is adjacent to the LFA-1 site. This presents a formidable challenge, as blockade of LFA-1 and Mac-1 can trigger side effects and should be avoided. Multiple ICAM-1 constructs were designed for an antibody generation campaign focusing on the areas where sequence identity between human and cynomolgus monkey ICAM-1 was the highest. Following antibody lead identification by phage display on multiple constructs, high-throughput assay triage with human and cynomolgus monkey ICAM-1 was performed. This triage included HRV and LFA-1 biochemical assays, cell binding assays and HRV infection blockade. The antibodies that met these criteria were tested in HRV infectivity assay and analyzed for cytostatic killing of HeLa cells. Importantly, all of the identified clones block most prevalent HRV-16 and HRV-14 serotypes. Dr. Lowe highlighted two groups of isolated antibodies: the first group comprises ICM10064 and ICM10088, which bind to domain 1 of ICAM-1 and do not cross-react to ICAM-1 from mice and cynomolgus monkeys. The second group comprises ICM10098 and ICM10103, which bind between domains 1 and 2 to the epitope that has the highest similarity to cynomolgus sequence. These antibodies cross-react to pharmacologically and toxicologically-relevant species and therefore, hold higher promise for further development.

The final presentation of the day was delivered by **Sarah Fredriksson** (Genovis). She discussed antibody characterization using unique enzymes in combination with mass spectrometry analysis. She first gave an overview of two unique enzymes from the pathogen *Streptococcus pyrogenes*. EndoS (termed IgG ZERO) specifically and rapidly cleaves N-linked glycans from antibodies, leaving one N-acetyl-D-glucosamine and one fucose. This enzyme cleaves all IgG subclasses in 30 min under non-denaturing conditions. Enzyme IdeS (termed FABRICATOR) is a unique cysteine protease that cleaves GG amino acid motif separating Fab2' and Fc antibody fragments. This proteolysis reaction is specific, complete and takes only five minutes. Dr. Fredriksson highlighted a study utilizing these enzymes for rapid liquid chromatography-mass spectrometry (LC-MS) screening for IgG Fc modifications and allelic variants in blood.[34] She recommended the broader use of these enzymes to streamline mass spectroscopy and structural analysis of antibodies.

References

1. Weise M, Bielsky MC, De Smet K, Ehmann F, Ekman N, Narayanan G, et al. Biosimilars-why terminology matters. Nat Biotechnol 2011; 29:690-3; PMID:21822237; http://dx.doi.org/10.1038/nbt.1936.

2. Beck A, Wurch T, Bailly C, Corvaia N. Strategies and challenges for the next generation of therapeutic antibodies. Nat Rev Immunol 2010; 10:345-52; PMID:20414207; http://dx.doi.org/10.1038/nri2747.

3. Xie H, Chakraborty A, Ahn J, Yu YQ, Dakshinamoorthy DP, Gilar M, et al. Rapid comparison of a candidate biosimilar to an innovator monoclonal antibody with advanced liquid chromatography and mass spectrometry technologies. mAbs 2010; 2:379-94.

4. Damen CW, Chen W, Chakraborty AB, van Oosterhout M, Mazzeo JR, Gebler JC, et al. Electrospray ionization quadrupole ion-mobility time-of-flight mass spectrometry as a tool to distinguish the lot-to-lot heterogeneity in N-glycosylation profile of the therapeutic monoclonal antibody trastuzumab. J Am Soc Mass Spectrom 2009; 20:2021-33; PMID:19744865; http://dx.doi.org/10.1016/j.jasms.2009.07.017.

5. Junttila TT, Parsons K, Olsson C, Lu Y, Xin Y, Theriault J, et al. Superior in vivo efficacy of afucosylated trastuzumab in the treatment of HER2-amplified breast cancer. Cancer Res 2010; 70:4481-9; PMID:20484044; http://dx.doi.org/10.1158/0008-5472.CAN-09-3704.

6. Reichert JM, Beck A, Iyer H. European Medicines Agency workshop on biosimilar monoclonal antibodies: July 2, 2009, London UK. MAbs 2009; 1:394-416; PMID:20065643; http://dx.doi.org/10.4161/mabs.1.5.9630.

7. Zhang N, Liu L, Dumitru CD, Cummings NR, Cukan M, Jiang Y, et al. Glycoengineered Pichia produced anti-HER2 is comparable to trastuzumab in preclinical study. mAbs 2011; 3:289-98; PMID:21487242; http://dx.doi.org/10.4161/mabs.3.3.15532.

8. Wang C, He X, Zhou B, Li J, Li B, Qian W, et al. Phase 1 study of anti-epidermal growth factor receptor monoclonal antibody in patients with solid tumors. mAbs 2011; 3:67-75; PMID:21051930; http://dx.doi.org/10.4161/mabs.3.1.14021.

9. Zalevsky J, Chamberlain AK, Horton HM, Karki S, Leung IW, Sproule TJ, et al. Enhanced antibody half-life improves in vivo activity. Nat Biotechnol 2010; 28:157-9; PMID:20081867; http://dx.doi.org/10.1038/nbt.1601.

10. Wu H, Pfarr DS, Johnson S, Brewah YA, Woods RM, Patel NK, et al. Development of motavizumab, an ultra-potent antibody for the prevention of respiratory syncytial virus infection in the upper and lower respiratory tract. J Mol Biol 2007; 368:652-65; PMID:17362988; http://dx.doi.org/10.1016/j.jmb.2007.02.024.

11. Wu H, Pfarr DS, Losonsky GA, Kiener PA. Immunoprophylaxis of RSV infection: advancing from RSV-IGIV to palivizumab and motavizumab. Curr Top Microbiol Immunol 2008; 317:103-23; PMID:17990791; http://dx.doi.org/10.1007/978-3-540-72146-8_4.

12. Hutas G. Ocrelizumab, a humanized monoclonal antibody against CD20 for inflammatory disorders and B-cell malignancies. Curr Opin Investig Drugs 2008; 9:1206-15; PMID:18951300.

13. Robak T. Ofatumumab, a human monoclonal antibody for lymphoid malignancies and autoimmune disorders. Curr Opin Mol Ther 2008; 10:294-309; PMID:18535937.

14. Niederfellner G, Lammens A, Mundigl O, Georges GJ, Schaefer W, Schwaiger M, et al. Epitope characterization and crystal structure of GA101 provide insights into the molecular basis for type I/II distinction of CD20 antibodies. Blood 2011; 118:358-67; PMID:21444918; http://dx.doi.org/10.1182/blood-2010-09-305847.

15. Mukherjee R, Adhikary L, Khedkar A, Iyer H. Probing deamidation in therapeutic immunoglobulin gamma (IgG1) by 'bottom-up' mass spectrometry with electron transfer dissociation. Rapid Commun Mass Spectrom 2010; 24:879-84; PMID:20196189; http://dx.doi.org/10.1002/rcm.4464.

16. Terstappen GC, Schlüpen C, Raggiaschi R, Gaviraghi G. Target deconvolution strategies in drug discovery. Nat Rev Drug Discov 2007; 6:891-903; PMID:17917669; http://dx.doi.org/10.1038/nrd2410.

17. Swinney DC, Anthony J. How were new medicines discovered? Nat Rev Drug Discov 2011; 10:507-19; PMID:21701501; http://dx.doi.org/10.1038/nrd3480.

18. Chennamsetty N, Helk B, Voynov V, Kayser V, Trout BL. Aggregation-prone motifs in human immunoglobulin G. J Mol Biol 2009; 391:404-13; PMID:19527731; http://dx.doi.org/10.1016/j.jmb.2009.06.028.

19. Chennamsetty N, Voynov V, Kayser V, Helk B, Trout BL. Prediction of aggregation prone regions of therapeutic proteins. J Phys Chem B 2010; 114:6614-24; PMID:20411962; http://dx.doi.org/10.1021/jp911706q.

20. Chennamsetty N, Voynov V, Kayser V, Helk B, Trout BL. Prediction of protein binding regions. Proteins 2011; 79:888-97; PMID:21287620; http://dx.doi.org/10.1002/prot.22926.

21. Voynov V, Chennamsetty N, Kayser V, Wallny HJ, Helk B, Trout BL. Design and application of antibody cysteine variants. Bioconjug Chem 2010; 21:385-92; PMID:20092294; http://dx.doi.org/10.1021/bc900509s.

22. Mössner E, Brünker P, Moser S, Püntener U, Schmidt C, Herter S, et al. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. Blood 2010; 115:4393-402; PMID:20194898; http://dx.doi.org/10.1182/blood-2009-06-225979.

23. Lim SH, Vaughan AT, Ashton-Key M, Williams EL, Dixon SV, Chan HT, et al. Fcgamma receptor IIb on target B cells promotes rituximab internalization and reduces clinical efficacy. Blood 2011; 118:2530-40; PMID:21768293; http://dx.doi.org/10.1182/blood-2011-01-330357.

24. Ferrara C, Stuart F, Sondermann P, Brünker P, Umaña P. The carbohydrate at FcgammaRIIIa Asn-162. An element required for high affinity binding to non-fucosylated IgG glycoforms. J Biol Chem 2006; 281:5032-6; PMID:16330541; http://dx.doi.org/10.1074/jbc.M510171200.

25. Umaña P, Jean-Mairet J, Moudry R, Amstutz H, Bailey JE. Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity. Nat Biotechnol 1999; 17:176-80; PMID:10052355; http://dx.doi.org/10.1038/6179.

26. Ferrara C, Grau S, Jäger C, Sondermann P, Brünker P, Waldhauer I, et al. Unique carbohydrate-carbohydrate interactions are required for high affinity binding between FcgammaRIII and antibodies lacking core fucose. Proc Natl Acad Sci USA 2011; 108:12669-74; PMID:21768335; http://dx.doi.org/10.1073/pnas.1108455108.

27. Alduaij W, Ivanov A, Honeychurch J, Cheadle EJ, Potluri S, Lim SH, et al. Novel type II anti-CD20 monoclonal antibody (GA101) evokes homotypic adhesion and actin-dependent, lysosome-mediated cell death in B-cell malignancies. Blood 2011; 117:4519-29; PMID:21378274; http://dx.doi.org/10.1182/blood-2010-07-296913.

28. Patz M, Isaeva P, Forcob N, Müller B, Frenzel LP, Wendtner CM, et al. Comparison of the in vitro effects of the anti-CD20 antibodies rituximab and GA101 on chronic lymphocytic leukaemia cells. Br J Haematol 2011; 152:295-306; PMID:21155758; http://dx.doi.org/10.1111/j.1365-2141.2010.08428.x.

29. Beers SA, Chan CH, French RR, Cragg MS, Glennie MJ. CD20 as a target for therapeutic type I and II monoclonal antibodies. Semin Hematol 2010; 47:107-14; PMID:20350657; http://dx.doi.org/10.1053/j.seminhematol.2010.01.001.

30. Fayer BE, Soni PP, Binger MH, Mould DR, Satoh H. Determination of humanized anti-Tac in human serum by a sandwich enzyme linked immunosorbent assay. J Immunol Methods 1995; 186:47-54; PMID:7561147; http://dx.doi.org/10.1016/0022-1759(95)00131-S.

31. Leonard JN, Ghirlando R, Askins J, Bell JK, Margulies DH, Davies DR, et al. The TLR3 signaling complex forms by cooperative receptor dimerization. Proc Natl Acad Sci USA 2008; 105:258-63; PMID:18172197; http://dx.doi.org/10.1073/pnas.0710779105.

32. Bannister D, Popovic B, Sridharan S, Giannotta F, Filée P, Yilmaz N, et al. Epitope mapping and key amino acid identification of anti-CD22 immunotoxin CAT-8015 using hybrid β-lactamase display. Protein Eng Des Sel 2011; 24:351-60; PMID:21159620; http://dx.doi.org/10.1093/protein/gzq114.

33. Kolatkar PR, Bella J, Olson NH, Bator CM, Baker TS, Rossmann MG. Structural studies of two rhinovirus serotypes complexed with fragments of their cellular receptor. EMBO J 1999; 18:6249-59; PMID:10562537; http://dx.doi.org/10.1093/emboj/18.22.6249.

34. Goetze AM, Zhang Z, Liu L, Jacobsen FW, Flynn GC. Rapid LC-MS screening for IgG Fc modifications and allelic variants in blood. Mol Immunol 2011; 49:338-52; PMID:21945018; http://dx.doi.org/10.1016/j.molimm.2011.09.002.

November 30, 2011: Day 2 Morning Session
Janice M. Reichert

The moderator of the morning session was the ever-gracious **Paul Parren** (Genmab), who introduced the agenda and theme of the session, which was the discovery of novel targets for mAb therapy. The first speaker, **Anat Cohen-Dayag** (Compugen), discussed a novel systemic approach for the discovery of new antibody targets, and presented data demonstrating the potential of two in silico predicted proteins, CGEN-928 and CGEN-15001T, as new targets for mAbs therapeutics. Dr. Cohen-Dayag explained that the membrane protein CGEN-928 previously had no known function, but that Compugen's Monoclonal Antibody Targets Discovery Platform predicted an involvement in multiple myeloma. Analysis of expression profiles done by Compugen has shown that CGEN-928 is highly expressed in multiple myeloma samples vs. normal tissue, including late stage multiple myeloma and drug resistant and aggressive primary tumor cell lines. A second membrane protein, CGEN-15001T, was previously uncharacterized, but was predicted by Compugen to belong to the B7/CD28 family, which is implicated in immune system regulation. Dr. Cohen-Dayag presented data showing that CGEN-15001T is expressed in prostate cancer tissues and immune cells within the tumor. She also presented data supporting the therapeutic potential and mechanism of action of CGEN-15001, the extracellular domain of CGEN-15001T fused to an Fc, for immune-related conditions such as multiple sclerosis and rheumatoid arthritis.

The state of the art of antibody therapeutics development was described by **Janice Reichert** (Tufts University), who is also the Editor-in-Chief of *mAbs*. Professor Reichert commented that there were multiple reasons for the success of antibodies as therapeutic agents, including advances in antibody design and engineering; manufacturing; understanding of their modes of action; and target biology, although a critical factor was the advancement of technologies that afford human antibodies (e.g., transgenic mouse, display technologies). She briefly described a data set of nearly 700 clinically-evaluated antibodies that she has maintained since 2001, and stated that the data show that that the number of new antibody therapeutics entering clinical study has nearly tripled since the late 1990s. Approximately half of these are for cancer, with 27% and 22% for immunological disorders and all other disorders, respectively. She noted that the US approval success rate for the anticancer mAbs is now about half that of the mAbs for immunological disorders (13% vs. 26%). Professor Reichert then discussed the current clinical pipeline of antibodies, with a focus on antibody-drug conjugates (ADCs), bispecific antibodies, engineered antibodies and antibody fragments/domains.[1,2] Her 'score card' for the anticancer mAb pipeline indicated that only 51% of this pipeline comprises unmodified mAbs, with 15% ADCs, 6% bispecifc mAbs and 10% each engineered antibodies and antibody fragments/domains. In contrast, ~90% of mAbs for non-cancer indications are unmodified. Professor Reichert concluded by remarking that, if historical success rate values for antibodies are steady, the increase in number of pipeline antibodies suggests that an increase in marketing approvals should occur; however, it remains to be seen whether the historical rates will be the same as those for the modified antibodies currently in the pipeline.

Alexey Lugovskoy (Merrimack Pharmaceuticals, Inc.,) presented a systems biology approach to the design of optimal antibody-like therapeutic molecules. He first reviewed reasons to develop multi-specific antibodies, e.g., many patients do not respond adequately to monotherapy, tumors rely on multiple or redundant pathways for proliferation, then noted that mechanistic models can help in the identification of optimal molecular properties such as affinity, avidity and format.[3] However, engineering bispecific antibodies with robust pharmaceutical properties can be challenging.

The focus of the presentation was a case study of the design of a potent and manufacturable bispecific antibody targeting two receptor tyrosine kinases. The specific targets, referred to herein as growth factor receptor (GFR)1 and GFR2, were identified via analysis of a complex signaling network. The format for the bispecific antibody was selected based on experiments that indicated that a bivalent bispecific would be superior to an IgG mixture for signaling inhibition and a tetravalent bispecific would be superior to a bivalent bispecific with regard to IC_{50} over a broad range of receptor numbers. A tetravalent 'proof-of-concept' (POC) IgG that included anti-GFR1 Fabs and anti-GFR2 scFvs was prepared, but proved to be unstable compared with IgG. Diverse variants (50–100 in total) were assessed for characteristics such as thermostability, aggregation and pH stability and affinity. One lead and 2–4 diverse back-ups were selected based on drug properties such as potency, PK and manufacturability.

Rational engineering approaches were used to affinity mature and stabilize both the anti-GFR1 and -2 arms; inverted orientation of the specificities was also explored in parallel. Optimization of the arms involved use of a focused yeast display strategy that included mutations that lead to a stable germline framework. The optimization was based on sequence consensus, structural analysis, mutation of sites conferring potential CMC liabilities (e.g., unpaired cysteines or irregular disulfides, glycosylation, methionine oxidation, domain stability and chain pairing, asparagine deamidation) and NNS/doped oligo substitutions in CDR sites. A thermal challenge assay wherein scFvs fused to yeast were heated to 55°C for 5 min was used to enrich for stable scFvs. All scFvs that showed over 5-fold improvement in K_d on yeast surface were produced as soluble scFvs and characterized using micro-scale techniques that required <100 μg of protein in total. Characterization included binding to soluble receptors, signaling inhibition, cook and bind ELISA, serum stability and differential scanning fluorescence. The affinity matured scFvs showed improved binding to soluble receptors (EC_{50} as low as 0.6 nM compared with EC_{50} of 6.1 for wild-type scFv), and increased signaling inhibition in BxPC3 cells. Overall, triage of the scFv arms was rapid (less than four weeks).

Dr. Lugovskoy explained that the re-engineered tetravalent bispecific was then constructed and evaluated vs. the POC version. The re-engineered bispecific proved to be more potent, more thermostable (Tm = 68°C compared with 61°C for the POC version), more stable in mouse serum and less prone to aggregation, e.g., the re-engineered bispecific remained stable as a monomer

for over 60 d at 20 mg/mL, 4°C. In concluding, Dr. Lugovskoy indicated that computational simulations can help identify optimal targets and molecular properties such as formats, affinity and avidity, and that the integrated approach that he described in the case study should be generally applicable to improving the potency and manufacturability of other multi-specific molecules in one design cycle.

Hervé Perron (GeNeuro) described a novel antibody therapy for multiple sclerosis. The target is an endogenous human multiple sclerosis-associated retro-viral envelope (MSRV-Env) protein implicated in the pathogenesis of MS.[4] Dr. Perron explained that MSRV can replicate in the case of co-infection with common viruses and exert pro-inflammatory effects via the Env protein, e.g., activation of inflammatory cascade via toll-like receptor 4. The Env protein can induce direct and indirect neurotoxicity, and effects on neural regeneration. Human peripheral blood mononuclear cells (PBMC) exposed to MSRV-Env release pro-inflammatory cytokines such as INFγ, IL-6, IL-12p40. Regarding the association with MS, Dr. Perron noted that MSRV-Env mRNA expression is selectively upregulated in the brain tissue of MS patients; the RNA accumulates in the MS plaques in the brain of MS patients, but not in normal brain or in brains of patients with other neurological disorders; and Env protein is observed in the MS plaques in post-mortem MS brain tissue sections. In addition, MSRV-Env is associated with a risk of progression of MS and risk of conversion to secondary progressive MS.

Dr. Perron then presented data for GNbAC1, a humanized, hinge-stabilized IgG4 targeting MRSV-Env. The mAb was selected using a TLR-4 signaling screening method and the preclinical proof of concept was established in an experimental allergic encephalitis (EAE) model. GNbAC1 was shown to decrease clinical score and stabilize weight of treated SCID mice in the EAE model; all treated mice survived. Non-treated mice showed continuous progression of clinical signs, no weight gain and all died. Dr. Perron concluded by noting that a Phase 1 study authorized by Swissmedic in 2011 is complete and a Phase 2 study is planned for the first quarter of 2012.

Sensitivity and resistance to epidermal growth factor receptor (EGFR) inhibitors were discussed by **Thomas Valerius** (Christian-Albrechts University), who focused on approaches to enhance the efficacy of anti-EGFR antibodies. Professor Valerius noted that there are ~500 kinases in the human genome, with 90 of these tyrosine kinases (TKs) and 43 tyrosine-like kinases. A number of drugs that inhibit the membrane TK EGFR are marketed, e.g., erlotinib (Tarceva®), gefitinib (Iressa®) and second generation molecules are in development. He then introduced data on biomarker analyses in EGFR-directed therapy and its affect on patient selection. A number of published studies have revealed that activating mutations in EGFR affect responsiveness to EGFR-targeted drugs.[5-8] These mutations include G719S, L858R, L861Q, which are located within the kinase domain of EGFR.[6] Professor Valerius then discussed EGFR-targeting antibodies, including two marketed products, Cetuximab (Erbitux®) and panitumumab (Vectibix®) and zalutumumab. Clinical evidence for cetuximab, which was first approved in 2004 and panitumumab,

which was first approved in 2006, indicates that mutations in the GTPase KRAS predict resistance in colorectal cancer patients treated with these antibodies. The American Society of Clinical Oncology recommends that patients with metastatic colorectal carcinoma should currently not receive anti-EGFR antibody therapy if KRAS mutation in codon 12 or 13 is detected.[9] Professor Valerius noted that mutations are not the only factor to consider. For example, expression levels of EGFR have recently been reported to be relevant in responsiveness to cetuximab in non-small cell lung cancer patients, with high expression predicting a survival benefit.[10]

Professor Valerius then discussed the effector mechanisms of EGFR antibodies and approaches to enhance their efficacy,[11] focusing on complement-dependent cytotoxicity (CDC) and antibody dependent cell-mediated cytotoxicity (ADCC). He and his collaborators have shown that Fc engineered mAbs they produced against EGFRvIII enhance tumor cell-specific CDC, particularly in combination with zalutumumab.[12] Furthermore, EGF-R mAb variants with increased affinity for the natural killer cell-expressed FcγRIIIa triggered significantly enhanced mononuclear cell mediated killing of KRAS-mutated tumor cells compared with wild-type mAbs.[13] Professor Valerius also presented results showing distinctions between the EGFR-targeting zalutumumab and panitumumab (IgG1 and IgG2 mAbs, respectively) in effector cell recruitment.[14] In particular, he emphasized the importance of IgG isotype selection in the development of antibody therapeutics, but he also noted that IgA2 should be considered. Study results indicate that IgG1 constructs trigger tumor cell lysis primarily by mononuclear cells and complement, while IgA1 and IgA2 can mediate lysis by polymorphonuclear neutrophils.[15] Professor Valerius' group can produce IgA in CHO-K1 cells under serum free conditions in mg amounts per week per flask.[16] Monomeric IgA was obtained by a combination of affinity chromatography-(CaptureSelect anti-kappa-light chain matrix) and size exclusion chromatography (Superdex200 26 x 60 gel filtration), while purified dimeric IgA was obtained by inclusion of an immobilized metal ion affinity chromatography step. For monomeric IgA, ADCC by IgA2 was found to be enhanced compared with IgG1 and IgA1; however, dimeric IgA was found to additionally recruit Fab-mediated effector functions more effectively compared with monomeric IgA or IgG1 mAbs.[17]

In concluding, Professor Valerius stated that study results have shown that EGFR mutations affect response to small molecule tyrosine kinase inhibitors, but appear to be irrelevant for mAbs. KRAS mutations negatively affect mAb therapy, but the affects can be overcome. EGFR immunohistochemical testing of NSCLC patients for selection of treatment regimens that include small molecule tyrosine kinase inhibitors is controversial, but may become relevant for selection of mAb therapies. In addition, myeloid cell recruitment has been found to be suboptimal with IgG1, possible with IgG2 and improved with IgA.

Klaus Schwamborn (Pepscan Therapeutics) presented a new peptide-based strategy for the generation of mAbs against G-protein coupled receptors (GPCRs) that involves protein

mimicry and Pepscan Therapeutics's CLIPS technology. The acronym CLIPS stands for 'Chemically LInked Peptides onto Scaffolds' and the technology involves the constraining the conformations of peptides.[18] Key features of CLIPS chemistry are the mild conditions, high speed and yields, versatility (e.g., ability to generate single, double or triple loops, compatibility with biological systems). In addition, no catalyst or side chain protection is required. The CLIPS technology can be used to identify functional mimics of targets. Applied to epitope mapping, the technology was used to reveal a unique CD20 binding site for ofatumumab (Arzerra®) compared with that of rituximab (Rituxan®),[19] and a difference in CD20 binding of obinutuzumab (GA101) compared with that of rituximab.[20]

Dr. Schwamborn discussed the opportunities for development of antibodies targeting GPCRs and noted that there are no GPCR-targeting antibodies on the market to date. It is challenging to generate anti-GPCR antibodies because GPCRs can lose structure when isolated from the cellular membrane in which they are embedded and immunization with cells or membrane fragments is often unsuccessful due to the high background of irrelevant epitopes. Pepscan's solution is to generate synthetic immunogens that mimic extracellular parts of GPCR. This approach makes GPCRs accessible for antibody drug discovery, allows tailoring to specific domains and is compatible with an antibody generation platform. Three levels of immunogen design address different aspects of protein mimicry: (1) structure; (2) extracellular domain presentation; (3) specific characteristics of individual GPCR. Besides rational design, immunogens can also be selected based on their capability of binding to ligand. He then presented data from studies of CXCR4, CXCR7 and an undisclosed immunogen as targets. In the case of CXCR4 as the immunogen, anti-CXCR4 Mab210 was generated, and it was shown by flow cytometry to bind to native overexpressed and endogenous GPCR. MAb210 also compared favorably compared with the positive control 12G5 in three different cell-based assays (cAMP detection, chemotaxis, aequorin assay). In the case of CXCR7, phage display screening led to generation of a number of Fabs that were functional in β-arrestin and internalization assays. In his final example of target X, Dr. Schwanborn described use of an alternate panning approach that involves both peptide and cells. The Pepscan mAb generated using the approach was shown to bind the target and have functionality that compared favorably with the benchmarking antibody.

Dr. Schwamborn concluded by remarking that CLIPS technology for epitope mapping is validated, and that the synthetic mimicry technology has been successfully used for raising antibodies against GPCRs. Results of assessments of binding and in vitro functionality to different targets indicate that the technology is a generic approach for the generation of antibodies to GPCRs. Generation of an antibody response tailored to specific domains of any given GPCR is possible. Dr. Schwamborn noted that an alternate concept with immunogens and cells is a very promising approach to raising antibodies against GPCRs, and that a new generation of immunogens is now showing outstanding first results. The next steps include expansion into discovery

and development of therapeutic antibodies against other relevant targets, including ion channels.

November 30, 2011: Day 2, Stream A
Janice M. Reichert

Following the morning session, participants then elected to attend either the Stream A session, which was moderated by **Alexey Lugovskoy** (Merrimack Pharmaceuticals, Inc.,) and focused on the generation and identification of mAbs, or the Stream B session, which was moderated by **David Lee** (Abbott Bioresearch Center) and focused on aspects of mAb production and scale-up relating to quality, comparability and the safety of originator and biosimilar products.

In Stream A, the first speaker, **Majid Mehtali** (Vivalis), described the VIVA SCREEN platform, which is a high throughput single B lymphocyte screening technology for the rapid discovery and production of fully human antigen-specific monoclonal antibodies. Dr. Mehtali explained that B lymphocytes, which come from donors accessed through a partnership with the French Blood Transfusion Center, are isolated, activated and expanded in vitro. Binding and functional screening assays are used to identify B cells producing antibodies with therapeutic potential. HTP single cell screening on microarrays allows isolation of individual B cells of interest. VH and VL antibody regions derived from the cells can then be cloned into expression vectors for production. Dr. Mehtali also briefly described Vivalis' EB66 cell line, which is derived from duck embryonic stem cells and can be used to produce genetically engineered mAbs at yields beyond 1 g/L as suspension cells grown in serum-free culture media.[21]

Jean-François Haeuw (Pierre Fabre) discussed the tetraspanin CD151 as a target for antibody-based cancer immunotherapy. The tetraspanin family includes 35 members in mammals. The proteins have four transmembrane domains, with two extracellular loops EC1 and EC2. A number of the tetraspanins, including CD37, CD151, CD9, CD63 and CD81, are targets of antibody-based therapeutics at various stages of development. The most advanced antibody-based therapeutics that target tetraspanins are TRU-016 and IMGN529. The anti-CD37 TRU-016 (Emergent Biosolutions/Abbott) is undergoing evaluation n a Phase 1/2 study of patients with relapsed chronic lymphocytic leukemia (NCT01188681) and a Phase 1/2 study (NCT01317901) of patients with relapsed indolent lymphoma. The investigational new drug application for IMGN529 (Immunogen), an antibody-drug conjugate targeting CD37, was filed in September 2011 and is now active.

In focusing on CD151, Dr. Haeuw explained this tetraspanin is overexpressed on cancer cells and functionally linked to cancer metastasis. CD151 interacts directly with integrin α3β1, which mediates rapid tumor cell migration and integrin α6β4, which mediates stable cell attachment and organizes the integrins in tetraspanin-enriched microdomains.[22,23] Anti-CD151 mAbs have the potential to function at various cancer stages, including early tumor growth as well as metastasis.[24] Dr. Haeuw described the generation and characterization of anti-CD151 mAbs using

a classical mouse hybridoma approach. mAb m214B2 was selected for further study based on its anti-tumoral activity in a PC3 xenograft model. Effects on metastasis were evaluated in an orthotopic lung cancer model (A549), an ectopic squamous cell lung carcinoma model (EBC-1) and an orthotopic prostate cancer model (PC3); m214B2 was able to inhibit metastasis compared with control in these models. The effects on tumoral growth were assessed in xenograft models of prostate carcinoma (PC3), epidermoid carcinoma (A431) and lung carcinoma (NCI-H441); m214B2 inhibited tumor growth compared with control in all three models. m214B2 binds to the EC2 loop of CD151 and has a K_d of 6.38 ± 0.45 nM measured by binding of I-125 mAb to PC3 cells.

Dr. Haeuw then explained that m214B2 and another anti-CD151 mAb were engineered as chimeric IgG1 forms, but these versions had reduced in vivo anti-tumoral activity. Chimeric IgG1, 2 and 4 forms of mAb 214B2 were generated and evaluated in an in vitro cell-to-cell interaction assay using PC3 cells, and only the IgG2 form was able to increase cell-to-cell interactions. Dr. Haeuw noted that the loss of activity could have been due to differences between the isotypes in the hinge region sequences. Modification of the hinge region by shortening the upper hinge and introducing an extra Cys residue was done to rigidify the IgG1 and IgG4 forms. An increase of cell-to-cell interactions was induced in the IgG1 and IgG4 forms after the hinge region engineering. The chimeric IgG2 form showed strong inhibition of cell migration and anti-tumoral activity comparable to that of m214B2 in the PC3 xenograft model. Dr. Haeuw concluded by noting that the next steps are humanization of the anti-CD151 mAb, in vivo evaluation in other models, assessment of effector functions and toxicity studies.

A case study of mAbs with multi-intervention points in the development of cancer was described by **Hélène Haegel** (Transgene). The target in this case is CD115, which is also known in the literature as the colony-stimulating factor 1 receptor, c-fms and macrophage colony-stimulating factor receptor.[25] CD115 is a tyrosine kinase receptor of type III subfamily that has 5 Ig-like extracellular domains and an intracellular kinase domain. The molecular weight is ~150 kDa, it is heavily glycosylated and the ligands are CSF-1 and IL-34. CD115 is expressed on mononuclear phagocytes and is involved in the proliferation of myeloid progenitors in bone marrow. Dr. Haegel explained that CD115 is expressed in a variety of human tumors, with CSF-1 and CD115 frequently co-expressed at late or metastatic stages. Tumor cell invasion is controlled by an autocrine CSF-1/CD115 loop along with a paracrine CSF-1/EGF loop with macrophages. In addition, tumor-associated macrophages (TAMs) express CD115 and their differentiation is CD115-dependent. TAMs are responsible for the angiogenic switch that leads to tumor progression and they promote migration of tumor cells, thereby promoting their metastasis. Differentiation of osteoclasts is CD115-dependent; CSF-1-expressing bone metastases induce osteoclast differentiation, leading to bone damage. Thus, the rationale for targeting CD115 in cancer included the intended elimination of tumor cells, but also the inhibition of host cells involved in cancer progression.

Data from preliminary in vivo studies with rat anti-mouse CD115 mAb (AFS98), and in vitro studies with the newly generated mouse (CXIIG6 IgG2) and chimeric (CXIIG6 IgG1) anti-human CD115 mAbs was described by Dr. Haegel. Using IMGT®, humanization was done by substitution of murine residues outside the CDR with high solvent accessibility by closest human germline sequences. Affinity for CD115 as measured by SPR was assessed for the 30 best VH/VL combinations, leading to selection of three possible clinical candidates. TG3003 was then selected as the final candidate based on highest homology to human, best affinity (K_d) and lowest in silico immunogenicity. The K_d of TG3003 is in the sub-nM range as measured on EL4-CD115 cells and no cross-reactivity with other cell surface receptors from the type III tyrosine kinase family (e.g., VEGFR, PDGFR, Flt-3, SCF) was observed.

Dr. Haegel presented data showing: (1) TG3003 inhibits human osteoclast differentiation from CD34+ precursors and osteoclast activity; (2) inhibition of FcγR1 surface expression in human macrophages differentiated in the presence of TG3003; (3) Fc-dependent induction of a CD86^{bright} SSC^{low} macrophage population by TG3003; (4) TG3003 induces interleukin (IL)-12 secretion by human macrophages; (5) TG3003 suppresses MCP-1/CCL2 and IL-6 secretion by human macrophages; (6) TG3003 polarizes macrophages toward M1-type (tumor suppressing); (7) only weak toxicity of TG3003 toward human monocytes in vitro; (8) human PBMCs kill EL4-hCD115 target cells by TG3003-mediated ADCC. Dr. Haegel concluded by emphasizing the potential multiple therapeutic intervention points of TG3003, and she noted that the mAb represents a promising candidate for the treatment of solid tumors associated with bone metastases.

Lois A. Lampson (Harvard Medical School) presented her perspectives on the role of the blood-brain barrier (BBB) in the mechanism of action of mAbs for neuro-oncology indications. She focused on the properties and challenges presented by brain tumors; the properties and relevance of the BBB; and how and where mAb therapeutics might be expected to work in brain tumor patients.[26] In discussing brain tumors, Dr. Lampson noted that brain tumors can originate in the brain or they can be blood-borne metastases from other sites. The source of the tumor matters because the biology of the tumor will reflect the cell of origin. For example, high grade glioma, including glioblastoma multiforme, are derived from brain glia, primary CNS lymphoma is usually a B cell lymphoma and brain metastases may develop from lung cancer, breast cancer, melanoma or many other tumor types. Dr. Lampson emphasized that the tumor origin has important consequences for its control, i.e., metastases in the brain must be controlled but the originating tumor in the body must also be controlled, whereas control of a primary brain tumor can be limited to the brain.

The differing biologies of microscopic tumor vs. a tumor mass were discussed by Dr. Lampson. She noted that micro-tumors are infiltrative, residual or disseminated primary tumor that spread through tissue or are carried in cerebrospinal fluid, and that the two sizes of tumors present different challenges for treatment. For example, the environment around micro-tumor is like normal

brain, whereas the midst of a tumor mass could not be expected to have a normal brain environment. Dr. Lampson emphasized that immune regulation varies within the brain, e.g., site-to-site variation in activation of microglia and local neurochemicals affect immune regulation.[27,28] She then discussed the attributes of the BBB, which has physical (e.g., tight junctions) and biochemical (e.g., degradative enzymes) components that will block passive entry of mAbs but not metabolically active cells. A critical point, however, is that the BBB is not static. It is altered at tumor sites, and may thus allow entry of mAbs, and the BBB changes as tumor grows or responds to therapy.

In discussing mAbs as treatments for brain tumors, Dr. Lampson stressed that the possible mechanisms of action are varied. MAbs can attack the tumor directly by targeting a tumor-associated antigen or indirectly by affecting tumor vessels or modulating an immune response. In the case of indirect modes of action, the mAb does not need to pass the BBB. Dr. Lampson explained that a direct attack does require a mAb to act in brain, but there might be reasons besides BBB properties that would explain treatment failure. For example, the tumor could be too large or have developed resistance, or the mechanism of action might not be efficient. Failure to cross the BBB is more likely to be an explanation for treatment failure in the case of micro-tumors, which are less likely to cause breakdown of the BBB because of their small size. Dr. Lampson suggested that if the BBB is blocking access to a mAb treatment, then multiple responses are possible. The BBB could be opened or bypassed; the mAb treatment could be continued to allow time for the BBB to change in response to the tumor and, possibly, for an immune response to be stimulated; or treatment could be initiated with a mAb with a different mechanism of action, e.g., one that works indirectly. Dr. Lampson concluded by stating that developing therapies for brain tumors is challenging. Animal models are often poor mimics of the human condition and clinical trial results can be difficult to interpret, but this situation is the same for all types of new therapeutics.

Bodo Brocks (Morphosys) discussed making therapeutic antibodies "fit for pipeline," which is a concept that supports smooth chemistry, manufacturing and controls (CMC) development of therapeutic antibodies. "Fit for Pipeline" covers the life cycle of therapeutic antibody products from library generation[29] to GMP manufacturing. Dr. Brocks illustrated the concept through examples from lead candidate selection and early development of MorphoSys' proprietary discovery platform. Integral parts of this concept are early stability assessment (ESA) followed by down-stream stress simulation (DSS). During ESA, observations made during antibody production and data from stability indicating routine tests are compiled and used to assess productivity, processability and stability of the IgG molecules. As a supportive ranking factor, apparent melting temperatures are estimated by differential scanning fluorimetry.[30] Following ESA, a set of final lead candidates is subjected to DSS, comprising various well-defined stress conditions such as pH, temperature and agitation, indicative for typical downstream process situations. The DSS samples are then extensively characterized by a wide panel of physico-chemical tests, including methods that can detect a wide spectrum of aggregate types. As an example, dynamic light scattering (DLS) data from stressed and native IgG were shown.

Dr. Brocks then presented a case study dealing with the characterization of a highly concentrated liquid formulation (HCLF) product. After identification of most critical manufacturing steps, potential impact on the product quality was addressed by relevant physico-chemical and biochemical tests. Interestingly, concentration of the product to 150 mg/mL had no significant impact on aggregation as characterized by HP-SEC, AUC and DLS. UPLC-ESI-MS based peptide mapping identified a slightly increased oxidation of Met428, which, however, had no detectible affect on FcRn binding. In concluding, Dr. Brocks noted that ESA and DSS are considered helpful strategies to significantly reduce the risks of drawbacks during CMC process development as well as storage, transportation and clinical use of therapeutic antibodies. Favorable stability and solubility are particularly important for development of HCLF products and should thus be assessed as early as possible in the drug discovery process.

Study results for 515H7, a new anticancer mAb that blocks SDF-1/CXCR4 signaling, were presented by **Christine Klinguer-Hamour** (Pierre Fabre). She explained that CXCR4 receptor is a GPCR family member and its ligand is stromal cell-derived factor-1 (SDF-1), which is also a natural agonist ligand for CXCR7 receptor. CXCR4 is involved in organogenesis, hematopoiesis and immune response,[31] as well as metastasis of a variety of tumors.[32] Dr. Klinguer-Hamour described generation of an anti-CXCR4 mAb derived from hybridoma and in vitro study results for the mAb m515H7. In an SDF-1-induced GTPγS binding inhibition assay (NIH3T3-CXCR4 membranes), the IC_{50} was 2.5 nM. mAb m515H7 induced a significant inhibition of Ca^{2+} release in all cell lines tested (CHO-CXCR4, MDA-MB-231, U937), inhibited (>80%) cAMP modulation upon stimulation by SDF-1 and inhibited SDF-1-induced β-arrestin 2 recruitment (95%). In addition, m515H7 induced CXCR4 homodimer conformational changes in the presence or absence of SDF-1, induced CXCR4/CXCR2 heterodimer conformational changes in the presence or absence of SDF-1 and induced 80% inhibition of U937 cell migration.

Dr. Klinguer-Hamour presented data from the evaluation of m515H7 in xenograft models. In the Karpas 299 T cell NHL model, female Nod/SCID mice received s.c. tumor and then were treated starting at day 5 with twice a week 1 mg/dose i.p. injections following a loading dose of 2 mg/dose. Animals treated with m515H7 showed 63% inhibition of tumor growth vs. control at day 33. In the Ramos B lymphoma model, following the same protocol in female SCID mice, animals treated with m515H7 showed a 95% inhibition of tumor growth vs. control at day 25. In the MDA-MB-231 breast cancer model, following the same protocol, animals treated with m515H7 showed a 60% inhibition of tumor growth vs. control at day 40. Dr. Klinguer-Hamour then presented data for a humanized IgG1 version of the mAb, hz515H7, which was shown to efficiently inhibit SDF-1-induced signaling pathways in vitro and inhibit SDF-1-induced U937 cell migration (~80% ± 12%). An experiment in the Ramos xenograft model with once a week i.p. injections ranging from 1.25 to 40 mg/kg and a loading dose of twice the maintenance dose

is on-going. Data from day 26 indicates a reduction in tumor volume vs. control that is dose-dependent. Dr. Klinguer-Hamour concluded by remarking that the data suggests that hz515H7 is a promising agent for cancer therapy.

Robert Friesen (Crucell) discussed immunotherapy and prophylaxis against influenza A Groups I and II. He first presented an informative video depicting the steps of influenza infection of cells and the mechanism by which CR8020, a human IgG1 mAb, blocks viral infection. The video, Monoclonal antibodies against flu, can be viewed on YouTube. Dr. Friesen noted that CR8020 has broad neutralizing activity against most Group II viruses. CR8020 binds a unique, highly conserved site in the hemagglutinin stem that is close to the viral membrane, inhibits conformational changes in hemagglutinin and blocks proteolytic activation.[33] He discussed the possibility that CR8020 combined CR6261, which neutralizes influenza Group I virus, may be an effective therapy for certain patients populations, e.g., elderly people hospitalized with influenza.

Cisbio's solutions for biotherapeutics were reviewed by **Stéphane Martinez** (Cisbio Bioassays). She first explained that HTRF® (homogeneous time resolved fluorescence) is a technology based on a combination of fluorescence resonance energy transfer chemistry and the use of fluorophores with long emission half-lives and discussed how the technology can be used for full antibody characterization, including biochemical assays, cellular binding and functional characterization. Dr. Martinez presented data derived from a new cellular CD16a (FcγRIIIa) assay that measures the affinity between CD16a and the Fc of antibodies. The assay can be used to study kinetics, evaluate affinities of different IgG subclasses, and estimate levels of fucosylation. The data she presented showed affinity differences between antibodies that were 4% to 80% afucosylated, and that the CD16a binding and ADCC functional assays were well-correlated.

Michael Rudolf (Kenta Biotech) discussed human mAbs as adjunct therapies in infectious disease. He first introduced Kenta's current pipeline of five human mAbs, which include four in development as treatments for *Pseudomonas aeruginosa* (mAbs KBPA101 and KBPA104), *Staphylococcus aureus* (mAb KBSA301), or *Acinetobacter baumannii* (mAb KBAB401), and one in development for the prevention of respiratory syncytial virus (RSV) infection (mAb KBRV201). He noted that Kenta's pipeline is derived from their MabIgX® technology, which allows generation of human mAbs of all isotypes and does not require genetic engineering. The human B cell source is blood from convalescent donors; fusion with LA55 cells yields the hybridoma cell lines that are also the production cell lines.

Dr. Rudolf then discussed study results for KBPA101 and KBPA104, which are IgMs that target *P. aeruginosa*. KBPA101 has been evaluated in a Phase 2a study as a treatment for hospital-acquired pneumonia and ventilator-acquired pneumonia caused by *P. aeruginosa*. Patients treated with KBPA101 achieved an effective clearance of pneumonia and had a 100% survival rate. KBPA101 seemed to be safe and well-tolerated and no immunogenicity was observed. KBPA104 is in preclinical development and Dr. Rudolf discussed differences observed in the characteristics of the antibody produced in hybridoma vs. CHO cells. Using the hybridoma system, a cell line was generated by fusion of a lymphoblastoid cell line with the heteromyeloma LA55 to produce KBPA104. Using the recombinant system, a cell line was generated by transfection of three vectors for heavy, light and human J-chain into CHO (Polymune Scientific) to produce rKBPA104. The hybridoma-derived IgM and recombinant CHO-derived IgM had comparable in vitro and in vivo effector functions, but different PK and serum half life (~24 h for KBPA104 and ~15 h for rKBPA104), and the recombinant IgM was found to have higher immunogenic potential.

Dr. Rudolf also provided an update for KBSA301, which is a human IgG1κ that targets *Staphylococcus aureus* α toxin with high affinity (K_d = 1.4 ± 0.1 nM). KBSA301 was derived from a hybridoma of a patient with polymicrobial bacteremia. The in vivo functionality of KBSA301 was evaluated in a prophylactic mouse lung challenge model. Prophylactic administration of KBSA301 resulted in dose dependent protection against methicillin-sensitive *Staphylococcus aureus*, hospital acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and community-associated MRSA strains, and lead to a marked reduction of lung bacterial load. Administration of KBSA301 was also found to mediate protection in a therapeutic *S. aureus* pneumonia model with a therapeutic window of 4–12 h post-infection. Dr. Rudolf concluded by noting that the process development of KBSA301 has been completed and clinical grade material is available for first-in-human studies in pneumonia patients planned for the first quarter of 2012.

References

1. Reichert JM. Which are the antibodies to watch in 2012? mAbs 2012; 4:1-3; http://dx.doi.org/10.4161/mabs.4.1.18719.

2. Reichert JM. Antibody-based therapeutics to watch in 2011. mAbs 2011; 3:76-99; PMID:21051951; http://dx.doi.org/10.4161/mabs.3.1.13895.

3. Fitzgerald J, Lugovskoy A. Rational engineering of antibody therapeutics targeting multiple oncogene pathways. MAbs 2011; 3:299-309; PMID:21393992; http://dx.doi.org/10.4161/mabs.3.3.15299.

4. Perron H, Lang A. The human endogenous retrovirus link between genes and environment in multiple sclerosis and in multifactorial diseases associating neuroinflammation. Clin Rev Allergy Immunol 2010; 39:51-61; PMID:19697163; http://dx.doi.org/10.1007/s12016-009-8170-x.

5. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350:2129-39; PMID:15118073; http://dx.doi.org/10.1056/NEJMoa040938.

6. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004; 304:1497-500; PMID:15118125; http://dx.doi.org/10.1126/science.1099314.

7. Han SW, Kim TY, Hwang PG, Jeong S, Kim J, Choi IS, et al. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. J Clin Oncol 2005; 23:2493-501; PMID:15710947; http://dx.doi.org/10.1200/JCO.2005.01.388.

8. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci USA 2004; 101:13306-11; PMID:15329413; http://dx.doi.org/10.1073/pnas.0405220101.

9. Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol 2009; 27:2091-6; PMID:19188670; http://dx.doi.org/10.1200/JCO.2009.21.9170.

10. Pirker R, Pereira JR, von Pawel J, Krzakowski M, Ramlau R, Park K, et al. EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the phase 3 FLEX study. Lancet Oncol 2012; 13:33-42; PMID:22056021.

11. Peipp M, Dechant M, Valerius T. Sensitivity and resistance to EGF-R inhibitors: approaches to enhance the efficacy of EGF-R antibodies. MAbs 2009; 1:590-9; PMID:20068398; http://dx.doi.org/10.4161/mabs.1.6.10051.

12. Klausz K, Berger S, Lammerts van Bueren JJ, Derer S, Lohse S, Dechant M, et al. Complement-mediated tumor-specific cell lysis by antibody combinations targeting epidermal growth factor receptor (EGFR) and its variant III (EGFRvIII). Cancer Sci 2011; 102:1761-8; PMID:21718386; http://dx.doi.org/10.1111/j.1349-7006.2011.02019.x.

13. Schlaeth M, Berger S, Derer S, Klausz K, Lohse S, Dechant M, et al. Fc-engineered EGF-R antibodies mediate improved antibody-dependent cellular cytotoxicity (ADCC) against KRAS-mutated tumor cells. Cancer Sci 2010; 101:1080-8; PMID:20331636; http://dx.doi.org/10.1111/j.1349-7006.2010.01505.x.

14. Schneider-Merck T, Lammerts van Bueren JJ, Berger S, Rossen K, van Berkel PH, Derer S, et al. Human IgG2 antibodies against epidermal growth factor receptor effectively trigger antibody-dependent cellular cytotoxicity but, in contrast to IgG1, only by cells of myeloid lineage. J Immunol 2010; 184:512-20; PMID:19949082; http://dx.doi.org/10.4049/jimmunol.0900847.

15. Dechant M, Vidarsson G, Stockmeyer B, Repp R, Glennie MJ, Gramatzki M, et al. Chimeric IgA antibodies against HLA class II effectively trigger lymphoma cell killing. Blood 2002; 100:4574-80; PMID:12393717; http://dx.doi.org/10.1182/blood-2002-03-0687.

16. Beyer T, Lohse S, Berger S, Peipp M, Valerius T, Dechant M. Serum-free production and purification of chimeric IgA antibodies. J Immunol Methods 2009; 346:26-37; PMID:19427867; http://dx.doi.org/10.1016/j.jim.2009.05.002.

17. Lohse S, Derer S, Beyer T, Klausz K, Peipp M, Leusen JH, et al. Recombinant dimeric IgA antibodies against the epidermal growth factor receptor mediate effective tumor cell killing. J Immunol 2011; 186:3770-8; PMID:21317397; http://dx.doi.org/10.4049/jimmunol.1003082.

18. Timmerman P, Beld J, Puijk WC, Meloen RH. Rapid and quantitative cyclization of multiple peptide loops onto synthetic scaffolds for structural mimicry of protein surfaces. Chembiochem 2005; 6:821-4; PMID:15812852; http://dx.doi.org/10.1002/cbic.200400374.

19. Teeling JL, Mackus WJ, Wiegman LJ, van den Brakel JH, Beers SA, French RR, et al. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol 2006; 177:362-71; PMID:16785532.

20. Niederfellner G, Lammens A, Mundigl O, Georges GJ, Schaefer W, Schwaiger M, et al. Epitope characterization and crystal structure of GA101 provide insights into the molecular basis for type I/II distinction of CD20 antibodies. Blood 2011; 118:358-67; PMID:21444918; http://dx.doi.org/10.1182/blood-2010-09-305847.

21. Olivier S, Jacoby M, Brillon C, Bouletreau S, Mollet T, Nerriere O, et al. EB66 cell line, a duck embryonic stem cell-derived substrate for the industrial production of therapeutic monoclonal antibodies with enhanced ADCC activity. MAbs 2010; 2:405-15; PMID:20562528.

22. Stipp CS. Laminin-binding integrins and their tetraspanin partners as potential antimetastatic targets. Expert Rev Mol Med 2010; 12:3; PMID:20078909; http://dx.doi.org/10.1017/S1462399409001355.

23. Zevian S, Winterwood NE, Stipp CS. Structure-function analysis of tetraspanin CD151 reveals distinct requirements for tumor cell behaviors mediated by α3β1 versus α6β4 integrin. J Biol Chem 2011; 286:7496-506; PMID:21193415; http://dx.doi.org/10.1074/jbc.M110.173583.

24. Haeuw JF, Goetsch L, Bailly C, Corvaia N. Tetraspanin CD151 as a target for antibody-based cancer immunotherapy. Biochem Soc Trans 2011; 39:553-8; PMID:21428938; http://dx.doi.org/10.1042/BST0390553.

25. Hume DA, Macdonald KP. Therapeutic applications of macrophage colony-stimulating factor (CSF-1) and antagonists of CSF-1 receptor (CSF-1R) signaling. Blood 2011; In press; PMID:22186992; http://dx.doi.org/10.1182/blood-2011-09-379214.

26. Lampson LA. Monoclonal antibodies in neuro-oncology: Getting past the blood-brain barrier. MAbs 2011; 3:153-60; PMID:21150307; http://dx.doi.org/10.4161/mabs.3.2.14239.

27. McCluskey LP, Lampson LA. Local neurochemicals and site-specific immune regulation in the CNS. J Neuropathol Exp Neurol 2000; 59:177-87; PMID:10744056.

28. McCluskey LP, Lampson LA. Local immune regulation in the central nervous system by substance P vs. glutamate. J Neuroimmunol 2001; 116:136-46; PMID:11438168; http://dx.doi.org/10.1016/S0165-5728(01)00295-8.

29. Prassler J, Thiel S, Pracht C, Polzer A, Peters S, Bauer M, et al. HuCAL PLATINUM, a synthetic Fab library optimized for sequence diversity and superior performance in mammalian expression systems. J Mol Biol 2011; 413:261-78; PMID:21856311; http://dx.doi.org/10.1016/j.jmb.2011.08.012.

30. Ericsson UB, Hallberg BM, Detitta GT, Dekker N, Nordlund P. Thermofluor-based high-throughput stability optimization of proteins for structural studies. Anal Biochem 2006; 357:289-98; PMID:16962548; http://dx.doi.org/10.1016/j.ab.2006.07.027.

31. Busillo JM, Benovic JL. Regulation of CXCR4 signaling. Biochim Biophys Acta 2007; 1768:952-63.

32. Zlotnik A, Burkhardt AM, Homey B. Homeostatic chemokine receptors and organ-specific metastasis. Nat Rev Immunol 2011; 11:597-606; PMID:21866172; http://dx.doi.org/10.1038/nri3049.

33. Ekiert DC, Friesen RH, Bhabha G, Kwaks T, Jongeneelen M, Yu W, et al. A highly conserved neutralizing epitope on group 2 influenza A viruses. Science 2011; 333:843-50; PMID:21737702; http://dx.doi.org/10.1126/science.1204839.

David Lee (Abbott Bioresearch Center) moderated the Stream B session, which focused on mAb production and scale-up with regard to the quality, comparability and safety of originator and biosimilar products, as well as the safety and immunotoxicity assessment of immunomodulatory mAbs. First, the efficient production of antibodies using QMCF Technology, a stable episomal expression platform in mammalian cells was discussed by **Mart Ustav** (Icosagen Cell Factory). The QMCF plasmids, which contain a mouse polyomavirus (Py) DNA replication origin and Epstein-Barr virus (EBV) EBNA-1 protein binding site as nuclear retention element, are stably propagated in QMCF cell lines that are based on CHO, HEK293 and U2OS cell lines. Generation of expression cell banks can be accomplished within one week.

Dr. Lee then switched to his role as a speaker and presented a novel comparability technique of protein therapeutics involving bottom-up liquid chromatography coupled to mass spectrometry (LC-MS) with stable isotope-tagged reference standards (SITRS).[1] Comparability studies are routinely used in the biopharmaceutical industry to evaluate differences among manufacturing processes and stability studies of protein therapeutics. Low resolution chromatographic and electrophoretic methods facilitate quantitation, but do not always yield detailed insights into the effect of the manufacturing change or environmental stress. Conversely, mass spectrometry can provide high resolution information on the molecule, but conventional methods are not very quantitative. As illustrated by Dr. Lee, this gap was fulfilled recently by the use of a SITRS, i.e., a version of the antibody to compare that is uniformly labeled with $^{13}C_6$-arginine and $^{13}C_6$-lysine. The SITRS served as an internal control that is trypsin-digested and analyzed by liquid chromatography with the analyte sample. The ratio of the ion intensities of each unlabeled and labeled peptide pair was then compared with that of another sample. A comparison of these ratios provided a readily accessible way to highlight small differences among samples.

As an illustration, Dr. Lee presented a case study of a mAb spiked with varying amounts of the same antibody bearing point mutations. Peptides containing the mutations were readily identified and quantified at concentrations as low as 2% relative to unmodified peptides. The method was shown to be robust, reproducible and produced a linear response for every peptide that was monitored. Interestingly, the method was also successfully used to distinguish between two batches of a mAb that was produced in two different cell lines (CHO and HEK293, respectively) while two batches produced from the same cell line were found to be highly comparable. Last but not least, he showed that the use of the SITRS method in the comparison of two stressed mAb samples enabled the identification of sites susceptible to deamidation and oxidation, as well as their quantitation. The experimental results indicated that use of a SITRS in a peptide mapping experiment with mass spectrometry detection enabled sensitive and quantitative comparability studies of proteins at high resolution. This method will likely find broad utility in the analysis of therapeutic antibodies and other protein biotechnology products, including biosimilars, biobetters and next generation antibodies.[2,3]

Martin Schiestl (Sandoz) discussed acceptable changes in quality attributes of glycosylated biopharmaceuticals.[4,5] Quality attributes of antibodies and other recombinant glycoproteins may change during the product life cycle. Dr. Schiestl presented case studies involving evaluation of structure variation in three major marketed biologics, Aranesp® (darbepoetin alfa), Rituxan®/Mabthera® (rituximab) and Enbrel® (etanercept). Glycosylated proteins are complex molecules and even a well-controlled product such as erythropoietin, may consist of several hundred isoforms having the same amino acid sequence but different glycan composition. When making these products, the manufacturer has to deliver consistent product quality to guarantee an acceptable safety profile and reproducible clinical performance. Current analytical methods allow the detection of even small changes in quality attributes and can therefore enable sensitive monitoring of the batch-to-batch consistency and variability of the manufacturing process. The purpose of the study presented by Dr. Schiestl was to provide more transparency, and to contribute to the ongoing debate about acceptable changes in quality attributes.[6] Identifying such variations in quality attributes is expected to help biotech companies in their development efforts and also the scientific and medical community in understanding these complex products.[7,8]

Dr. Schiestl explained that rituximab batches with expiry dates from September 2007 to October 2011 were, for example, characterized using glycan mapping, cation exchange chromatography (CEX) and antibody-dependent cellular cytotoxicity (ADCC) in vitro bioactivity assays. In 2008, an abrupt change in the quality profile became apparent for batches with expiry dates in 2010 or later. The most obvious difference was found in the amount of the C-terminal lysine and N-terminal glutamine variants when analyzed by cation exchange chromatography. The post-change batches contained a much smaller amount of these basic variants whose relative amounts are reduced from ~30–50% to ~10%. Another physicochemical difference was detected in the glycan map for unfucosylated G_0 glycans. The abundance of this glycoform was only ~1%, but it had a substantial effect on ADCC potency, which is an essential part of the clinical mode of action. MAbs having only unfucosylated glycans are known to exert much higher ADCC potency than fucosylated versions. In summary, by analyzing the quality profiles of three glycosylated recombinant therapeutic proteins sourced in the European Union, factual examples of acceptable variations for products that have remained on the market with unchanged labels became publically available.

Barbara Maertens (Qiagen) presented data generated using accelerated screening of antibody fragments produced by cell-free expression. Antibody fragments represent an alternative to the use of full-length mAbs as therapeutics. Qiagen developed a high-throughput process to screen for potential drug candidates by using cell-free protein expression in a modified *E. coli* lysate. The screening system involves the use of PCR products as templates to enable the production of hundreds of proteins in a 2-d procedure. The amount of protein generated is sufficient

for multiple activity assays. A variety of Fab and scFv fragments have been evaluated using this system. The expressed fragments were shown to be intact and displaying correct disulfide bonding and Fab heterodimer formation. They were also demonstrated to be functional by antigen-binding and enzyme inhibition assays. Dr. Maertens explained that the system provides substantial cost and time savings compared with traditional expression screening based on cellular protein production. Due to their small size and reduced complexity, antibody fragments are easier to develop and can be produced in expression systems, such as yeast and *E. coli*, that are low-cost compared with mammalian cells. Antibody fragments can be selected from synthetic libraries, by in vitro display technologies and the selected proteins may be used as drugs as illustrated by the success of ranibizumab (Lucentis®) and certolizumab pegol (Cimzia®). Various types of antibody fragments have been generated that show comparable or even better selectivity and affinity than antibodies obtained from animal immunization.

The conventional way to produce and screen variants of antibody fragments obtained from, for example, a phage display procedure, is to amplify individual coding regions by PCR, clone the genes into an expression vector, verify the clone by sequencing, transfect the vectors into animal cells, cultivate the cells and isolate the expressed proteins from cell culture for subsequent analysis. This is a time-consuming and costly procedure. Qiagen has developed a process for expression screening of antibody fragments on an industrial scale. They modified existing cell-free lysate systems by supplementing the cell-free lysates with chaperone-mixes and by optimizing other parameters of the protein synthesis reaction. The result was a system that enables the formation of disulfide bridges and provides functionally active disulfide-bonded proteins. Cell-free expression can be used to overcome the disadvantages associated with cloning and cellular expression of large numbers of antibody fragments. The generated templates are added to a cell-free lysate derived from *E. coli* or insect cells to produce proteins rapidly (~several-hours procedure). The lysates containing the overexpressed proteins can be directly used in activity assays or affinity-purified for specific applications. Dr. Maertens and colleagues have also evaluated the newly developed cell-lysates with several single-chain variable (scFv) and antigen-binding (Fab) antibody fragments.

Dimiter Dimitrov (National Cancer Institute) reported the story of the discovery and the development of therapeutic antibodies against Hendra and Nipah viruses.[9] Hendra virus (HeV) is a recently emerged zoonotic paramyxovirus that can cause severe and often fatal disease in horses and humans. HeV is categorized as a biosafety level four agent, which has made the development of animal models and testing of potential therapeutics and vaccines very challenging. Dr. Dimitrov explained that infection of African green monkeys (AGMs) with HeV was recently demonstrated, and the resulting disease mirrored fatal HeV infection in humans. The monkeys manifested multisystemic vasculitis symptoms with widespread virus replication in vascular tissues and severe pathologic manifestations in the lung, spleen and brain. Dr. Dimitrov and colleagues demonstrated that m102.4, a HeV-neutralizing human mAb, can protect AGMs from disease

after infection with HeV. Fourteen AGMs were challenged intratracheally with a lethal dose of HeV and 12 subjects were then infused twice with a 100 mg dose of m102.4 beginning at either 10, 24 or 72 h after infection and again ~48 h later. The presence of viral RNA, infectious virus and HeV-specific immune responses demonstrated that all subjects were infected after challenge. All 12 AGMs that received m102.4 survived infection, whereas the untreated control subjects succumbed to disease on day 8 after infection. Animals in the 72 h treatment group exhibited neurological signs of disease, but all animals started to recover by day 16 after infection. These results represented successful post-exposure in vivo efficacy by an investigational drug against HeV and highlight the potential impact a human mAb can have on human disease. Dr. Dimitrov summarized his talk by indicating that m102.4 was the first mAb administered to humans as a therapeutic of a rare viral disease. One unusual feature of this achievement is that this antibody was identified and characterized in an academic setting, and the large scale GMP production was supported by governmental funds to private companies. The batches are stocked and will be used on a compassionate basis in Australia in case of henipavirus outbreaks. This is the first mAb approved as a therapeutic against a rare viral disease that was successfully developed in academic settings.

The focus of the session then changed to the safety and immunotoxicity assessment of immunomodulatory mAbs. **Jeroen Lammerts van Bueren** (GenMab) presented experimental data for the binding of IgE anti-galactose-1,3 α-galactose antibodies from allergic patients to a panel of therapeutic antibodies.[10] As an introduction, he highlighted the structure of non-human α-Gal glycan found on therapeutic antibodies currently on the market, discussed the interaction between IgE against α-Gal and antibody therapeutics and explained the underlying mechanisms. Dr. Lammerts van Bueren recalled that most of the therapeutic mAbs and related biologics are produced in eukaryotic cells to allow proper glycosylation, which is often critical for function (such as ADCC and CDC for mAbs). The first mAbs were produced in the mouse-derived cell lines SP2/0 and NS0, which produce proteins containing a mixture of human (such as G_0F, G_1F, G_2F) and non-human glycan structures (such as galactose-α-1,3-galactose (α-Gal) and *N*-glycolylneuramic acid (NGNA)). The non-human forms can adversely affect safety and half-life. For example, cetuximab (Erbitux®) is produced in SP2/0 cells and administration of the product has been associated with α-Gal-specific, IgE-mediated anaphylactic responses. In addition, patients with IgE antibodies to α-Gal may experience allergic reactions to red meat. The quantity of α-Gal required to induce hypersensitivity reactions is not known. However, it is clear that binding of IgE antibodies against α-Gal is an important trigger in this pathology.

Dr. Lammerts van Bueren and colleagues studied whether IgE antibodies against α-Gal can interact with α-Gal moieties on six representative therapeutic mAbs produced in three different mammalian production systems: SP2/0 (cetuximab and infliximab), NS0 (basiliximab and palivizumab) and CHO (panitumumab and zalutumumab). First, they quantified the amount of α-Gal present on these mAbs. Antibody glycans

were enzymatically released using *N*-glycanase F and characterized using high-performance anion exchange chromatography with pulsed amperometric detection (HPEAC-PAD) analysis. Under native conditions, *N*-glycanase F only releases glycans from the Fc domain (linked to asparagine 297) and therefore the enzyme treatment was also performed in the presence of hexadecyltrimethylammonium bromide (CTAB) detergent to release glycans at other potential sites. Sodium dodecyl sulfate PAGE (SDS-PAGE) analyses of *N*-glycanase F treated mAbs demonstrated a clear shift in molecular weight of the heavy chain of ~2 kDa, indicating the release of the glycans. For cetuximab only, an additional weight shift was observed for the heavy chain when enzyme treatment was performed in the presence of CTAB, supporting previous evidence that cetuximab harbors additional glycans outside the Fc domain.

Of the six intact mAbs tested, only cetuximab binds α-Gal-specific IgE antibodies as present in the serum of red-meat allergy patients and this binding is restricted to α-Gal moieties in its Fab domains. Neither cetuximab nor infliximab Fc domains bound such IgE antibodies, even though they contained detectable α-Gal moieties. Dr. Lammerts van Bueren proposed three observations to explain the absence of binding: (1) α-Gal in the Fc domain is protected by shielding as indicated by the emergence of binding after glycan exposure by means of proteolytic degradation of the amino acid back bone; (2) an increased affinity of α-Gal IgE antibodies for bi α-galactosylated biantennary complex glycans present in the cetuximab Fab domain, compared with mono-α-galactosylated glycans detected in Fc domains; and (3) the presence of α-galactosylated glycans in both Fab arms of cetuximab provides a more avid binding surface for IgE, potentially by allowing bivalent IgE binding. Taken together, the presented data suggested that therapeutic mAbs that are only glycosylated in their Fc domains and produced in rodent cell lines do not bind α-Gal-specific IgE antibodies.

References

1. Manuilov AV, Radziejewski CH, Lee DH. Comparability analysis of protein therapeutics by bottom-up LC-MS with stable isotope-tagged reference standards. MAbs 2011; 3:387-95; PMID:21654206; http://dx.doi.org/10.4161/mabs.3.4.16237.

2. Beck A. Biosimilar, biobetter and next generation therapeutic antibodies. MAbs 2011; 3:107-10; PMID:21285536; http://dx.doi.org/10.4161/mabs.3.2.14785.

3. Reichert JM. Next generation and biosimilar monoclonal antibodies: essential considerations towards regulatory acceptance in Europe. February 3–4, 2011, Freiburg, Germany. MAbs 2011; 3:223-40; PMID:21487235; http://dx.doi.org/10.4161/mabs.3.3.15475.

4. Schiestl M, Stangler T, Torella C, Cepeljnik T, Toll H, Grau R. Acceptable changes in quality attributes of glycosylated biopharmaceuticals. Nat Biotechnol 2011; 29:310-2; PMID:21478841; http://dx.doi.org/10.1038/nbt.1839.

5. Schiestl M. A biosimilar industry view on the implementation of the WHO guidelines on evaluating similar biotherapeutic products. Biologicals 2011; 39:297-9; PMID:21784654; http://dx.doi.org/10.1016/j.biologicals.2011.06.014.

6. McCamish M, Woollett G. Worldwide experience with biosimilar development. MAbs 2011; 3:209-17; PMID:21441787; http://dx.doi.org/10.4161/mabs.3.2.15005.

7. Beck A, Wurch T, Reichert JM. 6th Annual European Antibody Congress 2010: November 29–December 1, 2010, Geneva, Switzerland. MAbs 2011; 3:111-32; PMID:21441785; http://dx.doi.org/10.4161/mabs.3.2.14788.

8. Reichert JM, Beck A, Iyer H. European Medicines Agency workshop on biosimilar monoclonal antibodies: July 2, 2009, London UK. MAbs 2009; 1:394-416; PMID:20065643; http://dx.doi.org/10.4161/mabs.1.5.9630.

9. Bossart KN, Geisbert TW, Feldmann H, Zhu Z, Feldmann F, Geisbert JB, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. Sci Transl Med 19 October 2011; 3:105.

10. Lammerts van Bueren JJ, Rispens T, Verploegen S, van der Palen-Merkus T, Stapel S, Workman LJ, et al. Anti-galactose-α-1,3-galactose IgE from allergic patients does not bind α-galactosylated glycans on intact therapeutic antibody Fc domains. Nat Biotechnol 2011; 29:574-6; PMID:21747378; http://dx.doi.org/10.1038/nbt.1912.

December 1, 2011: Day 3
Alain Beck

The third and final day of the European Antibody Congress was moderated by **Clive Wood** (Bayer HealthCare AG), and was dedicated to discussion of antibody-drug conjugates (ADCs), alternative protein scaffolds and bispecific antibodies. **Hans-Peter Gerber** (Pfizer) was the first speaker to present technological and clinical updates on ADCs. In most cases, administration of function blocking, naked antibodies as single agents to patients with solid tumors results in only minimal anti-tumor responses when tested in early stage clinical studies.[1,2] As a consequence, naked antibodies are administered most successfully in combination with chemotherapy because of their favorable safety profiles, circumventing additional toxicity signals in the combination setting.[3] The limited activity of naked antibodies as single agents targeting carcinomas has stimulated the pursuit of improved therapeutic strategies to enhance the anti-tumor effects of antibodies, including the development of ADCs. These three-component drugs (antibody, linker, cytotoxic payload) are designed to minimize the systemic toxicity of the free drug and to augment the antitumor activity of the targeting mAb.

Dr. Gerber noted that novel research concepts to study ADC targets, targeting vehicles, linkers and payloads have transformational potential for ADC development. One of the most promising areas of future ADC research is, for example, the identification of novel targets with optimal internalization kinetics and intracellular trafficking properties.[4] Development of technologies enabling the screening of a large repertoire of targeting vehicles to select for compound with optimal characteristics may represent an important future trend in ADC development. Recent improvements in drug-linker conjugations, in particular site-specific conjugation methods, resulted in a significant reduction in the off-target toxicity and potentially increased the therapeutic indexes of ADCs.[5]

Dr. Gerber then gave an update on inotuzumab ozogamicin, an ADC composed of G544, an IgG4 that specifically recognizes human CD22, and the derivative of calicheamicin.[6] The majority (>90%) of non-Hodgkin lymphomas (NHLs) are of B cell origin, with CD22 being expressed in 60% to 90% of B lymphoid

malignancies. CD22 has many of the ideal properties for an ADC target. Unconjugated G544, having no effector function, has no anti-tumor activity; however, conjugation with the cytotoxic payload confers potent dose-dependent cytotoxicity in in vitro and in vivo animal tumor models. Inotuzumab ozogamicin was effective against human B cell lymphomas resistant to rituximab in murine models, and its combination with rituximab showed additive efficacy. Downregulation of CD20 and upregulation of complement inhibitory factors play a central role in acquired resistance to rituximab. CD55 is a regulator of complement-dependent cytotoxicity in malignant B cells, and its expression correlates with resistance to complement-dependent cytotoxicity, which is one of the mechanisms of action of rituximab. In Daudi and Raji cells, the effect of rituximab significantly increased within 12 h after incubation with inotuzumab ozogamicin. The levels of CD22 and CD55 were significantly reduced. Similar results were obtained in cells from patients. Inotuzumab ozogamicin showed significant activity against CD22 leukemia, and it is now being evaluated in patients with CD22 acute lymphoblastic leukemia (ALL).

Dr. Gerber also detailed the development of an ADC targeting the oncofetal antigen 5T4, which is expressed on tumor initiating cells (TICs) in a variety of solid tumors. 5T4, also known as trophoblast glycoprotein (TPBG) is an N-glycosylated, transmembrane, 72 kDa glycoprotein expressed by many malignant tumors including colorectal, ovarian and gastric cancers and has very limited expression in normal tissue. Dr. Gerber noted that TICs are a subset of tumors cells, and they are more aggressive, proliferate indefinitely and can initiate tumor growth. Failure to kill TICs may be a major reason for resistance and recurrence with current cancer therapies. Targeting TICs may reduce recurrence, extend remission and lead to cures. The anti-5T4 ADC A1-mcMMAF was selected as the lead molecule for development based on a payload-linker license obtained from Seattle Genetics. Excellent efficacy was demonstrated in several animal models. In addition, the compound demonstrated a good safety profile in non-human primate that warrants further development.

Ulrich Brinkmann (Roche) gave a talk on bispecific digoxigenin-binding antibodies used for targeted payload delivery involving generation and optimization of hapten-binding bispecific antibodies. He described the complexation and targeted delivery of haptenylated payloads, as well as targeted payload delivery in vitro and in vivo. Bispecific antibodies simultaneously bind two different antigens and can be applied to block two targets on cell surfaces to improve therapeutic efficacy.[7] Recognition of two targets may also increase targeting specificity toward tissues or tumors that express both antigens. Bispecifics that bind tumor-associated antigens and effector cells (e.g., by binding CD3) can also be used in immunotherapy to activate effector cells at tumors. Bispecific antibodies are also applicable for payload delivery. One option to achieve targeted delivery is conjugation of haptens to the payload, and subsequent complexion with hapten-binding bispecific antibodies. Complexion via antibody-antigen interaction avoids chemical modification of antibodies, and thereby reduces risks of inactivating the targeting entity or generating immunogenic sites within the protein. A conjugation step for attachment of hapten to payload is still needed, but this procedure can be performed by standard technologies.

Bispecific antibodies that bind cell-surface targets as well as digoxigenin (Dig),[8] were generated for targeted payload delivery. The targeting moieties are IgGs that bind the tumor antigens HER2, IGF1R, CD22 or LeY. A Dig-binding single-chain Fv was attached in disulfide-stabilized form to the C termini of CH3 domains of targeting antibodies. Bispecific molecules were expressed in mammalian cells and purified in the same manner as unmodified IgGs. These molecules were shown to be stable without aggregation propensity, and to retain binding specificity and affinity to cell-surface antigens and Dig. Digoxigeninylated payloads were generated that retain full functionality and can be complexed to bispecific antibodies in a defined 2:1 ratio. Payloads included small compounds (Dig-Cy5, Dig-Doxorubicin) and proteins (Dig-GFP). Complexed payloads are targeted by the bispecifics to cancer cells. These complexes are stable in serum and can be applied for targeted delivery. Because Dig bispecifics also effectively capture digoxigeninylated compounds under physiological conditions, separate administration of uncharged Dig bispecifics followed by application of Dig payload was shown to be sufficient to achieve antibody-mediated targeting both in vitro and in vivo.

Continuing with the topic of ADCs, **Adeela Kamal** (MedImmune) discussed strategies for efficient ADC target discovery and lead selection, and **Robert Lutz** (ImmunoGen) provided an overview of development of advanced antibody-based therapeutics in oncology. **Vincent de Groot** (Synthon) then presented data on combinations of DNA-damaging duocarmycins and suitable linker technologies as an alternative payload technology. Synthon acquired Syntarga and its ADC technology in June 2011, and has entered into a number of new research collaborations with biopharmaceutical and biotechnology companies. ADCs undergoing evaluation by the company's collaborators comprise the newest Synthon Linker-Drug (L-D) chemistries linked to collaborator antibodies. Synthon is leveraging its proprietary technologies and expertise to generate and commercialize, alone and with partners, a portfolio of next-generation ADC products.

Dr. de Groot explained that Synthon's duocarmycins are DNA alkylating agents that bind in the minor groove; these drugs are potent as free drugs and as ADCs in vitro against multi-drug resistant cell lines. The drug is not toxic in vivo as free drug at molar levels much higher than ADC efficacious doses. Thus, even if unintentionally released from stable linker, toxicity may be low. The linkers have demonstrated high stability in human plasma for all DNA alkylator-linked L-Ds and DNA alkylator-linked L-Ds are more stable than DNA binder-linked L-Ds. The aim for the company is to translate from 'linker-drug discovery' to ADC product, to select the best linker-drug and target/mAb combination, and to advance their first ADC to the clinic. In the studies done to date, Synthon's ADCs have been safe at high dose and highly efficacious at low dose. Dr. de Groot presented results of preclinical development, including drug potencies, ADC and payload stabilities in plasma, cleavage kinetics and in vivo therapeutic window aspects for ADCs directed against HER2.

Addressing the topic of 'Beside antibodies: domain antibodies and new scaffolds', **Hilda Revets** (Ablynx) discussed single domain antibody development, which involves the tailoring of Nanobodies® for clinical indications. **Andrew Nixon** (Dyax) then gave an update on Dyax's pipeline and technology, and presented the ecallantide (DX-88) approval success story. Ecallantide was developed as part of their lead program targeting human plasma kallikrein for the treatment of hereditary angioedema (HAE), a genetic disorder caused by low or dysfunctional levels of C1 esterase inhibitor. It was selected from a phage display library of rationally designed variants of the first Kunitz domain of human tissue factor pathway inhibitor (TFPI), and produced in *Pichia pastoris* as a non-glycosylated recombinant peptide of approximately 7 kDa. Ecallantide inhibits the proteolytic activity of human plasma kallikrein with a K_i of 25 pM and a high selectivity vs. other proteases. The product was evaluated in four Phase 1, and three Phase 2 clinical trials that included 134 HAE patients. Two Phase 3 trials (EDEMA3 and 4) that included a total of 143 patients were completed and provided the basis for FDA approval of ecallantide (Kalbitor®) on November 30, 2009. Ecallantide is also undergoing a Phase 2 trial in patients having coronary artery bypass grafting, following successful completion of a Phase 1/2 dose escalation study in this indication. **Laurent Audoly** (Pieris AG) concluded this section of the meeting by discussing the path to clinical development of Pieris' Anticalins, as well as other new scaffolds.

Following the networking lunch, the focus of the meeting turned to bispecific antibodies.[7,9] **Christian Klein** (Roche) described a general approach to correctly assemble two heavy and two light chains, derived from two existing antibodies, to form human bivalent bispecific IgG antibodies[10] without use of artificial linkers. Based on the knobs-into-holes technology that enables heterodimerization of the heavy chains, correct association of the light chains and their cognate heavy chains is achieved by exchange of heavy-chain and light-chain domains within the antigen binding fragment (Fab) of one half of the bispecific antibody.[11] This "crossover" retains the antigen-binding affinity but makes the two arms so different that light-chain mispairing can no longer occur. Applying the three possible "CrossMab" formats, Dr. Klein and colleagues generated bispecific antibodies against angiopoietin-2 (Ang-2) and vascular endothelial growth factor A (VEGF-A) and showed that they can be produced by standard techniques, exhibit stabilities comparable to natural antibodies, and bind both targets simultaneously with unaltered affinity. Because of its superior side-product profile, CrossMabCH1-CL was selected for in vivo profiling and showed potent anti-angiogenic and anti-tumoral activity. Because the crossover approach leaves the antigen-binding regions of the parental antibodies intact, the method provides a way to convert any given pair of antibodies (e.g., those with proven clinical efficacy) into an almost natural bispecific IgG antibody. Also, because the Fc part of these molecules is left untouched, Fc-mediated effector functions and properties, such as complement activation, neonatal Fc receptor interaction or FcγRIIIa interaction required for ADCC, are retained.

Apart from pharmacoeconomic advantages and convenience, bispecific antibodies offer several advantages over the therapeutic application of monospecific antibodies or their combination. These advantages include the recruitment of effector cells, reduction of systemic toxicities by targeting to disease sites and modulation of internalization properties, and the synergistic effects that might be seen when cell-surface receptors are targeted. The use of bispecific antibodies may help target several pathways simultaneously to avoid escape and resistance mechanisms, and ultimately may establish combination therapy for biologics in the clinical reality. The principle of using a bispecific antibody directed against two soluble ligands was demonstrated. Interestingly, bispecific CrossMabs can also be generated for other targets, such as cell-surface receptors or combinations of targets, allowing various therapeutic applications in oncology, inflammation, virology and metabolic diseases.

As summarized by Dr. Klein, CrossMab domain exchange technology is suitable to solve the challenge of correct light chain assembly in bispecific antibodies. In addition, the knobs-into-holes technology with stabilizing disulfide bridges is suitable to ensure correct heavy chain assembly in CrossMabs. Classical and generic 1 plus 1 bivalent bispecific formats based on existing antibodies are independent of epitope without requirement for identification of a common light chain. The resulting molecules contain knobs-into-holes mutations and novel fusion points, but do not require additional linkers or connectors. As a result, there is reduced risk for immune complexes compared with tetravalent bispecific antibodies. Stability and PK properties of conventional IgGs are retained, as are FcR interaction and effector function of IgGs. Technical feasibility was also demonstrated by large scale IgG-like production in CHO cells, and by the management of side-product formation. The overall CrossMab approach was also successfully applied to additional examples that contribute to confirmation of the technical feasibility.

The development of dual-variable domain (DVD)-Ig technology was discussed by **Tariq Ghayur** (Abbott Laboratories). In particular, Dr. Ghayer provided an update on the preclinical evaluation of multiple distinct DVD-Ig molecules. **Kristen Bower** (CovX Research, Pfizer) then discussed the CovX-Bodies platform. Bispecific antibodies are regarded as promising therapeutic agents due to their ability to simultaneously bind two different antigens. Several bispecific antibody derivatives have been developed, but their utility so far has been limited because of problems with stability and manufacturing complexity. Dr. Bower reported a versatile technology, based on a scaffold antibody and pharmacophore peptide heterodimers, that enables rapid generation and chemical optimization of bispecific antibodies, and results in generation of molecules termed bispecific CovX-Bodies.[12-14] The concept is to use two different peptides that are joined together using a branched azetidinone linker and fused to the scaffold antibody under mild conditions in a site-specific manner. Whereas the pharmacophores are responsible for functional activities, the antibody scaffold allows long half-life and Ig-like behavior. The pharmacophores can be chemically optimized or replaced with other pharmacophores to generate optimized or unique bispecific antibodies.

As a prototype, Dr. Bower and colleagues developed a bispecific antibody that binds both VEGF and angiopoietin-2 simultaneously, inhibits their function, shows efficacy in tumor xenograft studies, and greatly augments the anti-tumor effects of standard chemotherapy.[15] Dr. Bower also reported the use of a technology based on the aldolase catalytic antibodies that facilitates rapid generation and optimization of unique bispecific CovX-Bodies. A bispecific CovX-Body contains two different pharmacophores covalently bound to the nucleophilic heavy chain lysine at position 93 (according to Kabat numbering), which is located deep in the hydrophobic binding pockets on each of the two Fab arms of the scaffold antibody, was also described.[16,17]

References

1. Sapra P, Hooper AT, O'Donnell CJ, Gerber HP. Investigational antibody drug conjugates for solid tumors. Expert Opin Investig Drugs 2011; 20:1131-49; PMID:21599617; http://dx.doi.org/10.1517/13543784.2011.582866.

2. Beck A, Haeuw JF, Wurch T, Goetsch L, Bailly C, Corvaïa N. The next generation of antibody-drug conjugates comes of age. Discov Med 2010; 10:329-39; PMID:21034674.

3. Gerber HP. Emerging immunotherapies targeting CD30 in Hodgkin's lymphoma. Biochem Pharmacol 2010; 79:1544-52; PMID:20096666; http://dx.doi.org/10.1016/j.bcp.2010.01.015.

4. Gerber HP, Senter PD, Grewal IS. Antibody drug-conjugates targeting the tumor vasculature: Current and future developments. MAbs 2009; 1:247-53; PMID:20069754; http://dx.doi.org/10.4161/mabs.1.3.8515.

5. Lacy SE, Bond CJ, Benjamin D, Gerber HP, Dimitrov DS, Reichert JM. Americas Antibody Congress: September 21–23, 2009, Washington DC. mAbs 2009; 1:523-30.

6. Beck A, Senter P, Chari R. World Antibody Drug Conjugate Summit Europe: February 21–23, 2011; Frankfurt, Germany. MAbs 2011; 3:331-7; PMID:21691144; http://dx.doi.org/10.4161/mabs.3.4.16612.

7. Beck A, Reichert JM. The amazing, multipurpose antibody. MAbs 2011; 3:221-2; PMID:21487245; http://dx.doi.org/10.4161/mabs.3.3.15625.

8. Metz S, Haas AK, Daub K, Croasdale R, Stracke J, Lau W, et al. Bispecific digoxigenin-binding antibodies for targeted payload delivery. Proc Natl Acad Sci USA 2011; 108:8194-9; PMID:21536919; http://dx.doi.org/10.1073/pnas.1018565108.

9. Reichert JM. Bispecific antibodies and ADCs: Once and future kings? MAbs 2011; 3:329-30; PMID:21654205; http://dx.doi.org/10.4161/mabs.3.4.16589.

10. Beck A, Wurch T, Bailly C, Corvaia N. Strategies and challenges for the next generation of therapeutic antibodies. Nat Rev Immunol 2010; 10:345-52; PMID:20414207; http://dx.doi.org/10.1038/nri2747.

11. Schaefer W, Regula JT, Bähner M, Schanzer J, Croasdale R, Dürr H, et al. Immunoglobulin domain crossover as a generic approach for the production of bispecific IgG antibodies. Proc Natl Acad Sci USA 2011; 108:11187-92; PMID:21690412; http://dx.doi.org/10.1073/pnas.1019002108.

12. Li L, Leedom TA, Do J, Huang H, Lai J, Johnson K, et al. Antitumor efficacy of a thrombospondin 1 mimetic CovX-body. Transl Oncol 2011; 4:249-57; PMID:21804921.

13. De Palma M, Naldini L. Angiopoietin-2 TIEs up macrophages in tumor angiogenesis. Clin Cancer Res 2011; 17:5226-32; PMID:21576085; http://dx.doi.org/10.1158/1078-0432.CCR-10-0171.

14. Bower KE, Lam SN, Oates BD, Del Rosario JR, Corner E, Osothprarop TF, et al. Evolution of potent and stable placental-growth-factor-1-targeting CovX-bodies from phage display peptide discovery. J Med Chem 2011; 54:1256-65; PMID:21280651; http://dx.doi.org/10.1021/jm101226k.

15. Huang H, Lai JY, Do J, Liu D, Li L, Del Rosario J, et al. Specifically targeting angiopoietin-2 inhibits angiogenesis, Tie2-expressing monocyte infiltration and tumor growth. Clin Cancer Res 2011; 17:1001-11; PMID:21233403; http://dx.doi.org/10.1158/1078-0432.CCR-10-2317.

16. Doppalapudi VR, Huang J, Liu D, Jin P, Liu B, Li L, et al. Chemical generation of bispecific antibodies. Proc Natl Acad Sci USA 2010; 107:22611-6; PMID:21149738; http://dx.doi.org/10.1073/pnas.1016478108.

17. Murphy RE, Kinhikar AG, Shields MJ, Del Rosario J, Preston R, Levin N, et al. Combined use of immunoassay and two-dimensional liquid chromatography mass spectrometry for the detection and identification of metabolites from biotherapeutic pharmacokinetic samples. J Pharm Biomed Anal 2010; 53:221-7; PMID:20547023; http://dx.doi.org/10.1016/j.jpba.2010.04.028.