

## Meeting Report

# 4<sup>th</sup> European Antibody Congress 2008

December 1–3, 2008, Geneva, Switzerland

The Fourth European Antibody meeting, organized by Terrapin Ltd., was held in Geneva, a center of the European biopharmaceutical industry. Merck-Serono, NovImmune, Pierre Fabre and Therapeomic are located nearby, as are R&D centers of Boehringer-Ingelheim, Novartis, Roche and Sanofi-Aventis. Over 40 speakers and more than 200 delegates attended the event. Companies represented included Abbott, Ablynx, Adnexus/ BMS, Astra-Zeneca/ CAT/ Medimmune, BiogenIdec, BioRad, Centocor (Johnson & Johnson), Crucell/ DSM, Domantis, Dyax, Genmab, Genzyme, Glycart/ Roche, Haptogen, Immunogen, Kyowa-Kirin, LFB, Medarex, Merck-Serono, Micromet, Novartis, Pierre Fabre Laboratories, Roche, Sanofi-Aventis, Seattle-Genetics, Transgene, UCB Celltech and Wyeth. Other attendees included those based in academe or government (University of Amsterdam, University of Zurich, Univeristy Hospital-Lyon, Ecole Polytechnique Federale de Lausanne, INSERM, Tufts University, US National Institutes of Health), consultants, and patent attorneys (Edwards, Angell, Palmer & Dodge). The meeting was very interactive and included exchanges during the many scheduled networking times (exhibitions, speed-networking, lunches and evening receptions). The first day of the three day conference was dedicated to advances in understanding antibody structure-function relationships. Challenges and opportunities in antibody development were the focus of the second day and the third day featured discussion of innovative antibodies and antibody alternatives.

## December 1, 2008

### Day 1, Therapeutic antibodies: Advances in dissecting structure-function relationships

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The chairman, Alain Beck (Centre d'Immunologie Pierre Fabre), opened the meeting with the following remarks: Monoclonal antibodies (mAbs) and related-products (immunoconjugates, radioimmunoconjugates, Fab fragments and Fc-fusion proteins) are the fastest growing class of pharmaceuticals, with nearly 30 products currently approved for a wide range of indications.<sup>3,14</sup> In just the last three years, six new antibodies and derivatives have reached the market. These included molecules that are novel formats, as well as first in class drugs in new therapeutic indications. In 2006, panitumumab (Vectibix) was the first fully human IgG2 mAb generated by immunization of humanized transgenic mice and the second anti-EGFR mAb to gain approval. Also in 2006, ranibizumab (Lucentis), the first *E. coli*-produced Fab fragment and the first affinity matured antibody, was approved as a treatment age-related macular degeneration. Later, tocilizumab (Actemra) a conventional IgG1, but directed against a new target (IL-6R), was registered in Japan; BLAs are pending both in the US and in Europe. In 2007, eculizumab (Soliris) was approved for paroxysmal nocturnal hemoglobinuria. Eculizumab was constituted by an original IgG2/4 hybrid format, and is unable to bind Fc receptors or activate the complement cascade. In 2008, rinolcept (Arcalyst), an IL-1R-Fc fusion protein also called IL-1 trap, was registered for cryopyrin-associated periodic syndromes. Also in 2008, certolizumab pegol (Cimzia) became the first PEGylated Fab fragment to gain approval. The product, indicated for Crohn disease, is produced in *E. coli* and conjugated to large PEG residues (40 kDa). Interestingly, from a structure-function standpoint, certolizumab was crystallized and the 3D model of this original PEG-Fab was recently reported.<sup>4</sup> In addition to these six new antibody or antibody-related product approvals, the first two biosimilar antibodies, Reditux (a copy of rituximab developed by Dr Reddy) and Clotinab (a biogeneric of abciximab developed by ISU ABXIS), were recently launched in India and in South Korea, respectively. Active discussions are ongoing regarding whether such generic biopharmaceuticals may also be approved in Europe, following approval of other glycoproteins such as erythropoietin.<sup>16</sup>

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**Choosing the right antibody isotype and the right format.**

All currently approved therapeutic antibodies are G-type immunoglobulins (IgGs) and derivatives of mouse, human or mixed origin. Human IgGs are divided into four subclasses or isotypes defined by different heavy chains ( $\gamma 1$ ,  $\gamma 2$ ,  $\gamma 3$  and  $\gamma 4$  in a 66/23/7/4 ratio in plasma) and different disulfide pairings. The 3D structures of IgGs are maintained by non-covalent interactions and by disulfide bridges, with specific numbers and characteristic connections for each isotype. These precise linkages can be established by liquid chromatography coupled to on-line mass spectrometry, as illustrated by peptide maps of IgG1, IgG2 and IgG4. IgG3s are characterized by a longer and more flexible hinge domain and the presence of 11 inter-heavy chain disulfide bridges (vs 2 for IgG1s and IgG4s, and 4 for IgG2s). Despite a high antibody-dependent cellular cytotoxicity (ADCC) potential, IgG3s are generally not selected for therapeutic antibody development mainly because the plasmatic half-life is shorter than that for the three other isotypes (7 vs 21 days, respectively). Interestingly, there is no direct functional and structural correlation between human IgG1, 2, 3 and 4 disulfide bridge connections and their mouse "homonyms" (IgG1, IgG2a, IgG2b and IgG3).

To date, most of the current therapeutic chimeric, humanized and human antibodies are based on an IgG1/ kappa backbone.<sup>17</sup> Nonetheless, IgG4 and IgG2 isotypes are being chosen more often when effector functions are unwanted. Conversely, IgG1 are frequently selected for killing pathogenic cells such as those with an over-expressed target antigen or viruses. IgG2 and IgG4 show specific structural and functional features such as in vitro and in vivo dynamic structural rearrangements that are not observed for IgG1. As the first marketed human IgG2, panitumumab is the prototype for this isotype; the product is followed by at least four other members targeting CTLA4, RANKL, IGF-1R and CD3 antigens that are in phase III studies.<sup>11</sup> Remarkably, in the last twelve months Chen et al.<sup>6</sup> at Amgen has published a number of extensive structure-function studies with IgG2 that have reported on new isomers not previously described.<sup>9,21</sup> These reports explored the molecule's functional consequences and disulfide rearrangement in vivo, and included data for recombinant and natural plasmatic IgG2. Unlike IgG1 and 4, human IgG2 have also been shown to form covalent dimers in vivo involving hinge Cys pairing that may increase the avidity effect. On the other hand, it is well-known that IgG4 form half-antibodies due to hinge CPSC instability, which can be stabilized by a single amino acid mutation, thereby mimicking an IgG1 hinge (CPPC). GenMab also showed recently that IgG4 can form bispecific antibodies in vitro and in vivo associated with the possibility to crosslink different antigens.<sup>18</sup> As a further consequence, IgG4s may be functionally monovalent in vivo if the molecule is not stabilized by an IgG1 S241P hinge mutation.

Following the establishment of markets for approved Fab fragments such as abciximab, ranibizumab, certolizumab, new monovalent formats like a one-armed antibody and IgG4 derived unibodies have recently emerged in specific structure-function cases where, for example, both cross-linking of the antigen and effector functions are unwanted. These points were presented and

extensively discussed by Andrew Goodearl (Abbott Bioresearch Center) and Rob Aalberse (Sanquin and University of Amsterdam).<sup>10</sup>

**Antibody/antigen complex stoichiometry and epitope and paratope mapping.** Epitope and paratope mapping and antigen/ antibody binding stoichiometry are essential features for therapeutic antibodies characterization. Both biophysical and physicochemical sets of data contribute to the elucidation of antibody mechanisms of action once the epitope is known. Alain Beck (Centre d'Immunologie Pierre Fabre) provided an overview of several successful epitope mapping case studies for first, second and third generation therapeutic mAbs. He also addressed the advantages and limitations of different mapping methods. Paul Parren (Genmab) delivered a presentation on the research and development of ofatumumab, an anti-CD20 human mAb currently in phase III studies.<sup>15</sup> Specifically, he demonstrated how epitope mapping and other structure-function studies helped to distinguish ofatumumab from rituximab and other anti-CD20 mAbs. Antonio Maschio (Edwards, Angell, Palmer & Dodge) discussed the use of mAb epitope mapping to define patent claims. Fabrizio Gianotta (ProGenosis) described a new and original epitope mapping method that is based on bifunctional hybrid proteins and capable of defining non-linear epitopes.

**Antibody engineering to reduce immunogenicity and improve pharmacology properties.** IgG are large tetrameric glycoproteins composed of 1,300 to 1,400 amino acids. A single amino-acid change or degradation in a mAb complementarity-determining region (CDR), such as the isomerisation of a single Asp residue, the deamidation of an Asn residue or the oxidation of a Trp residue, may be responsible for a dramatic loss of binding.<sup>19</sup> This is also true for modifications occurring in the Fc domain. For example, veltuzumab is a humanized anti-CD20 monoclonal antibody with CDRs identical to rituximab, except for one residue in CDR3 of the variable heavy chain (Asp101 instead of Asn). When compared to rituximab, veltuzumab has significantly reduced off-rates in the human lymphoma cell lines tested due to the single amino acid change in CDR3-VH. Juan Carlos Almagro (Centocor)<sup>1</sup> and Tristan Vaughan (Medimmune) discussed these points and showed several successful advances in improving antibody "drugability" and "developability." Scott Glaser (Biogen Idec) discussed antibody engineering to improve potency and stability. Specifically, he showed that modification of the highly soluble IgG structure and the design of new formats like single-chain variable fragments of bispecific mAbs are often detrimental to their stability.<sup>8</sup>

**Structural and functional implications of altered antibody glycosylation.** Glycosylation is highly dependent on the production system, the selected clonal cell population and the culture process.<sup>5</sup> Chinese Hamster Ovary cells (CHO) and mouse myeloma cells (NS0, SP2/0) have become the 'gold standard' of mammalian host cells used for the production of therapeutic antibodies and Fc-fusion proteins that have reached the market.<sup>20</sup> Of the approved mAb products, 48% are produced in CHO cells, 45% in mouse-derived cells (21% in NS0, 14% in SP2/0 and 10% in hybridoma) and 7% in *E. coli* (non-glycosylated Fab fragments).

Many alternative production systems and improved constructs are also being actively investigated. On average, IgGs glycans represent only about 3% of the total mass of the molecule. Despite this low percentage, particular glycoforms are involved in essential immune effector functions. However, glycoforms that are not commonly biosynthesized in human may be allergenic, immunogenic and accelerate the plasmatic clearance of the linked antibody. These glyco-variants must be identified, controlled and limited in products used as therapeutics. For example, cetuximab-induced anaphylaxis was recently correlated to the presence of 30% of galactose- $\alpha$ -1-3 galactose at a second N-glycosylation site in the variable domain. Conversely, antibody clearance of a CHO-produced human IgG did not appear to be significantly affected by Fc glycans, including high-mannose species.<sup>7</sup>

The growing pharmaceutical interest in glycan tailoring and profiling of mAbs is illustrated by the recent acquisition of GlycArt by Roche (2005) and GlycoFi by Merck (2006), as well by the creation and development of biotechnology companies specializing in recombinant glycoprotein production, glyco-engineering and glyco-analysis. These companies include AviGen (USA), Biolex (USA), BioWa (Japan), GlyCode (France), GlycoDiag (France), GlycoForm (UK), GlycoTope (Germany), Greenovation (Germany), Glynce (Japan), LFB (France), M-Scan (UK/Switzerland/USA), Neose (USA), ProBiogen (Germany), Procogonia (Israel), ProteoDynamics (France), Prozyme (USA) and Vivalis (France).<sup>2,3</sup>

During the last session of Day 1, the glycosylation patterns observed for the currently approved therapeutic antibodies produced in mammalian cell lines, the analytical methods used for their characterization, the expected benefits of manipulating the carbohydrate components of mAbs by engineering, as well as the possible advantages of alternative biotechnological production systems were discussed.<sup>13</sup> **Mitsuo Satoh (Kyowa Hakko Kirin)** gave an updated on the Biowa/Kyowa Potelligent technology licensed by several companies and applied to glyco-engineered mAbs directed against GD3, CCR4 (KM2760, Amgen), CD30 (MDX-1401, Medarex) and IL5R (BIW-8405, Medimmune), and currently investigated in early clinical trials in different indications (cancer, inflammation and asthma). The Potelligent<sup>®</sup> technology is based on fucose removal from Fc-linked oligosaccharides, which greatly enhances ADCC. The Potelligent<sup>®</sup> system was recently combined to engineer constant region of human IgG1/IgG3 chimeric isotypes to further enhance complement-dependent cytotoxicity (CDC) (Complement<sup>®</sup> technology, reviewed in ref. 12). **Qun Zhou (Genzyme)** presented the production of non-fucosylated oligomannose antibodies in CHO cells grown in the presence of kifunensine an alpha-mannosidase I inhibitor. These antibodies showed an increased affinity for Fc $\gamma$ RIIIA receptors, higher ADCC and reduced C1q binding. The serum half-life in mice was not altered, but remains to be investigated in primates.<sup>22</sup> **Christian Klein (Roche)** summarized the latest developments of GA101, a novel glyco-engineered type II CD20 mAb currently in Phase I/II clinical trials for the treatment of non-Hodgkin's lymphoma. Glycart GlycoMab<sup>®</sup> technology was used to produce batches of bisected afucosylated carbohydrates.

GA101 recognizes a type II epitope that is different from the type I recognized by rituximab. The combination of both type II epitope and enhanced ADCC is expected to translate into greater clinical efficacy in CD20-positive malignancies.

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December 2, 2008

**Day 2, Antibodies: Challenges and opportunities**Sherif Hanala<sup>1</sup> and Janice M. Reichert<sup>2</sup><sup>1</sup>Global BioFocus LLP; San Francisco, California USA; <sup>2</sup>Tufts Center for the Study of Drug Development; Boston, Massachusetts USA

The second day of the congress focused on a variety of topics addressing the challenges and opportunities faced by monoclonal antibody discovery, development and commercialization efforts. The session, chaired by **Dr. Clive Wood (Dyax)**, began with an overview of the development and approval trends of monoclonal antibodies. Discussion then focused on strategies for the development of successful pipelines, as well as formulation and delivery challenges. Other important topics included an overview of recent developments and trends in patenting and intellectual property law. Later presentations focused on enhanced production and control systems, and new approaches for the generation of antibody therapeutics.

**Dr. Janice M. Reichert (Tufts Center for the Study of Drug Development)** presented an overview of development and approval trends of monoclonal antibodies based on research done at Tufts CSDD since 2001.<sup>1-5</sup> She also presented recent results that clearly showed that the number of mAbs entering the clinic is on the increase globally. Her presentation covered all relevant therapeutic categories and mAb classes. The data presented, was classified in a manner to make possible the assessment of the productivity of the industry as a whole. Dr. Reichert tabulated all mAbs in development as well as key milestones along the development path. This research makes possible important calculations such as clinical development and approval times and the likelihood of approval. By stratifying the data, important insight can be generated for various cohorts (or subsets of the larger data set) to support important strategic decisions. Another important insight generated from Dr. Reichert's analysis is the increase of mAb activity in Asia. Clearly, mAbs as therapeutic agents have come of age. Details of the new data set include: over 500 antibody-based therapeutics that entered clinical studies sponsored by commercial firms, over 200 are currently in clinical studies, 22 are approved in the US, and an additional four are approved outside the US. In terms of therapeutic categories, oncology, immunological and anti-infective candidates comprise about 50%, 25% and 12%, respectively of the total number of mAbs that have been studied in humans. The number of mAbs entering clinical studies is now close to 40 per year, with human mAbs entering studies more frequently compared to humanized or chimeric candidates. For humanized mAbs, (n = 131), cumulative success rate is 17% based

on knowing the fate of 49% of these mAbs. The approval success rate for mAbs is generally higher than the rate for small molecule therapeutics. In terms of therapeutic categories, the cumulative success rates are 15% and 21% for humanized oncology and immunological candidates. Other interesting trends worth noting is the growing prevalence of antibody fragments (fAbs), pegylation and modified versions of mAbs (changes in glycosylation and Fc region engineering). No shift from the big three therapeutic areas is expected, but new therapeutic categories are being considered. Dr. Reichert concluded by wondering if the approval success rate for mAbs will remain as favorable as novel antibody candidates move through the clinical development process. If we keep the 20% success rate for mAbs, we will certainly see more monoclonal antibodies coming to the market to address the growing medical needs of in important therapeutic areas.

**Dr. Paul Parren (Genmab)** described the transgenic mouse platform and Genmab's discovery engine with a focus on functional screening of antibodies.<sup>6</sup> Genmab is focused on oncology therapeutics because there are many well understood and novel targets for which new mAbs can be developed; Genmab seeks to balance its portfolio with respect to targets (validated vs novel). High throughput and in vivo techniques are at the core of Genmab's selection process, generating large libraries that can be screened against the targets of interest. Confocal microscopy is used for binding studies, to further narrow the selection to the most promising candidates. Dr. Parren went on to describe various ongoing studies for HuMax CD-20 (ofatumumab) for refractory chronic lymphocytic leukemia (CLL). Ofatumumab is also being investigated for non-Hodgkin's lymphoma (NHL) and rheumatoid arthritis (RA), diffuse large B-cell lymphoma (DLBCL), as well as relapsing remitting multiple sclerosis (RRMS). Ofatumumab targets a unique binding site on the cell surface, recognizing a small-loop epitope of CD20, causing effective lysis of B cells which results in more efficient cell killing, when compared to rituximab (Beum et al 2008). CLL represents 25% of all leukemia; the age of onset is often over 70, and a well tolerated therapy is still required for refractory patients. Interestingly, Genmab developed ofatumumab in a relatively short period of time—the lead was identified in March 2002, in vivo proof of concept was shown in 2003 and the drug entered the clinic at the end of 2004. The biologics license application filing for ofatumumab is planned for this year. Lastly, Dr Parren touched upon collaborations for asthma and vascular disease with Roche (four ongoing studies) and other collaborations in oncology with GlaxoSmithKline, as well as efforts targeting HER-2, IGF and CD32b aimed at producing mAbs that are more effective than currently available therapeutic agents.

**Dr. Andreas Plückthun (University of Zurich)** started his talk with a historical perspective discussing the structural progression from antibodies to other scaffold molecules currently available and in development. He commented that a method was developed in his laboratory to make antibody fragments in *E. coli* twenty years ago; this method has allowed the production of variants and a set of selection technologies. However, many fragments of IgG had well known shortcomings, such as a tendency for aggregation. Dr. Plückthun's team took a radical approach for the time and

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decided to use the library concept and established screening and selection technologies to develop other scaffold structures that solved known biophysical problems associated with mAbs and fragments. The goal of the work was to develop molecules that have similar or improved targeting capabilities to antibodies and that are significantly more stable. The effort concentrated on the ankyrin repeat proteins, a class of human mammalian repeat proteins that are constructs with repeating units of structure. These designed structures known as designed ankyrin repeat proteins (DARPs) can be made into multivalent molecules or fusion proteins with multiple specificities when desired.<sup>8,9</sup> Also, to be good alternatives, these structures must also be as versatile as the structures now in use for various biomedical applications. DARPs are highly stable, fast-folding proteins that are composed of repeating units of 33 amino acids. The binding surfaces created by DARP structures can be thought of as a groove-like surface that can extend in length depending on the number repeats used. Interestingly, DARP binding surfaces structurally mimic antigen recognition sites on antibodies. By manipulating the amino acid sequence of the units (randomizing residues), a large library of up to 1012 molecules (for three randomized units) is generated. From there, suitable structures can be selected for various antigens by ribosome display or phage display. The DARPs have some very attractive benefits that make them viable alternatives, such as very high levels of expression—200 mg/ml in shake flasks, more than 10g/l in the fermenter (corresponding to 150 g/l of an IgG in molar equivalents). The molecules are very stable and display very good affinities; routinely at low nano-molar levels, with some at mid pico-molar level affinities observed (by ribosome and phage display). In terms of targets, versions of DARPs have been shown to effectively target HER2<sup>10</sup> and CD326 (EpCAM), with the added benefit of extended half-life. Work is ongoing to identify the most effective DARP structure to bind these and other targets in mouse models, with high tumor accumulation observed for some constructs. The team is also optimistic that DARPs will have low immunogenicity, because binders devoid of T-cell epitopes can always be found in selections.

**Dr. Tudor Arvinte (University of Geneva, Therapeomic)** discussed the challenges of formulating protein based drugs. A key point was that understanding the biophysics of a given drug is as important as understanding the biochemistry. With the large number of ongoing clinical trials, developers must consider that failure is not always due to the structure or mode of action of a given molecule, but can quite likely be due to the way the drug is formulated and administered during trials. Proteins are complex structures and as such, they aggregate, degrade, bind to non-target surface and make fibrils. Dr. Arvinte noted that commonly the less we know about the biophysics of a given molecule, the more stable we think the molecule is. Often, the analytical methods used are not well suited to understanding how a given molecule behaves as it enters the body. With the conversion of evidence approach however, there is a way to understand the behavior of these molecules. For example, in some cases the presence of loose aggregates can trigger a toxicity effect, so clearly the formulation can seriously disrupt the biology. These kinds of biophysical effects are at play in many drug formulations, but researchers may be

unaware of it. Dr. Arvinte went on to describe how special dyes can be used to stain aggregated protein or degraded molecules. He then went on to discuss the case of trastuzumab, a marketed antibody, and he showed how various buffer systems can impact the formation of aggregates.<sup>11</sup> Trastuzumab is a lyophilized powder, with a complex formulation that incorporates a novel chemical entity used to stabilize the antibody. He also highlighted how various parameters involved in handling of the solution, including speed of injection, can create aggregates. A warning by the manufacturer to not use dextrose in reconstitution solutions prompted Dr. Arvinte's team to conduct a study that concluded that if dextrose had been used in the formulation, trastuzumab would not have reached the market. Using a set of high throughput methods developed by Therapeomic, one can eliminate formulation options that can trigger aggregation or other deleterious effects. Another key point was that antibodies function only if they are flexible, therefore the loss of flexibility can eliminate effectiveness and often flexibility is not tested. To address the difficulty of developing an optimum formulation, careful biophysical study is required; no two molecules are the same biophysically, therefore each formulation must be carefully adapted. Clearly, care and experimentation are required to determine the best formulation, to eliminate any biophysical effects that can undermine the effectiveness of protein based drugs. Dr. Arvinte's final point: the biology of a molecule may be well understood, however understanding the biophysics of what is really happening in the vial and during the administration is equally important to success.

On a related topic, **Dr. Steffan Bassarab (Boehringer Ingelheim)** discussed the formulation and delivery challenges faced when developing a highly concentrated solution of a given protein based drug that also meets all the medical, physical and economic requirements. Dr. Bassarab, highlighted the importance of analytical services to guide the formulation and packaging process. Key questions to answer before establishing a formulation strategy include: Is the drug indicated for a chronic vs acute condition? What is the frequency of administration and dosage regimen? Will administration be done by the patient or a professional? Is it a single use or multiple use format? From a market perspective, what is the competitive situation (e.g. IP)? Dr. Bassarab highlighted the various parameters and techniques used to develop an accurate and complete view of the product from a biophysical perspective. Dr. Bassarab then discussed the pros and cons of various formulation strategies and their impact on product stability and manufacturability, such as induced aggregation or loss of protein caused by interface and surface interactions. Finally, Dr. Bassarab, commented on various well-known and novel drug delivery technologies. Dr. Bassarab's presentation served to impress upon attendees that the role played by the design of formulation and analytics groups is critical to the successful development of protein based drugs.

**Dr. Charles Dumontet (University Hospital, Lyon)** reviewed preclinical methods to evaluate and potential ways to circumvent resistance to rituximab. He first pointed out the difference between in vitro and clinical definitions of resistance. In the clinic, resistance is the absence of response to therapy. A comparison between sensitive and resistant cell lines, often obtained by selection or genetic

modification, is used to define 'resistance' in vitro. In general, in vitro cell lines used to evaluate antibodies have limitations because, as opposed to small molecule drugs, many lines are not sensitive to antibody alone. An additional effector mechanism is required to more closely reproduce the in vivo case (addition of non-activated human serum as a source of complement or use of accessory cells). 'Laboratory' cell lines such as Burkitt cells may not be clinically relevant, but fresh human samples can be difficult to obtain in sufficient quantities for assays. Dr. Dumontet then discussed potential mechanisms of resistance to rituximab, including alterations in the apoptotic, CDC and ADCC pathways, and the evidence suggesting resistance can be overcome. One method to overcome resistance involves sensitizing cells to rituximab through concomitant use of proteasome inhibitors, inhibitors of signaling pathways and enhancement of effector mechanisms. An alternate approach is to combine rituximab with antibodies having complementary functions, such as alemtuzumab, anti-CD22 mAbs or anti-CD23 mAbs. Finally, novel anti-CD20 mAbs may also overcome resistance to rituximab.

Immunogenicity assessment of antibodies is certainly one of the most challenging topics facing developers. **Dr. Patrick Liu (Genentech)** gave delegates some insight into several techniques to address the assessment of immunogenicity. He used the wording of product inserts to highlight the fact that immunogenicity depends on many factors. Such as chemical modifications (glycosylation), product degradation (fragments, aggregates, denaturation) and clinical factors (presence of other diseases or other medication), may not be directly related to the structure of the drug in question. He also noted that the sensitivities of assays used to assess immunogenicity can have an important impact on the manufacturer's capacity to predict immunogenic response. Dr. Liu then described the assay strategy adopted by Genentech to study these effects, which involves an approach using screening assays, confirmatory assays and functional assays in a sequential process. Among the assays presented, were ELISA and bridging ELISA, as well as other assays such as anti-therapeutic response and the more specific anti-therapeutic antibody (ATA); these assays can also be used for drug interference/tolerance studies. Dr. Liu also discussed ATA characterization using cell-based assays, immunochemical approaches like ELISA and the impact of ATA on drug pharmacokinetics. The case of panitumumab was presented to illustrate these points. Dr. Liu also described neutralizing antibody (Nab) assays used to identify the presence of entities that can directly block the binding of the target to the active site on the therapeutic or to indirectly inhibit the binding of the target to the therapeutic by inducing conformational changes.

Patenting and intellectual property have always been central to progress in the discovery of novel therapeutics. With the maturation of biotechnology and the appearance of biosimilars, understanding patentability over prior art is of utmost importance to the biopharmaceutical industry. **Mr. Jonathan Klein-Evans (MedImmune)** compared and contrasted the approaches to patenting in the United States (USPTO) and the European Union (EPO). He presented a brief history of developments and trends in patenting law in the last 20 years, most notably that the focus

has shifted from new targets to new molecules. The current focus is increasingly on improved molecules. As part of this perspective, Mr. Klein-Evans highlighted the increasing specificity of patent claims, going from simple targeting claims in the early days, to claims describing the effects on the target in the 1990's, to claims today that augment the targeting and effect aspects with detailed sequence information on the entities in question. He then went on to compare the US and EU approaches to patents, where he touched upon a key point of distinction. In the US, obviousness is the key obstacle to the granting of a patent; structural non-obviousness, irrespective of the method used to arrive at said structure, can be relied on. By contrast, in the EU, inventiveness (problem/solution approach) or the degree of difficulty of arriving at the invention seems to determine patentability. Questions to be answered in the EU include: Did the presented solution involve inventive skill? Did the solution provide unexpected benefit? Mr. Klein-Evans highlighted several cases to illustrate these key distinctions. As the field continues to mature and the gap between novel and prior art structures narrows "inventive skill" or "unexpected advantage" may become more important in supporting "non-obviousness" even in the US. Patent law is a field that is always shifting and Mr. Klein-Evans gave delegates a clear framework to understand the current mindset of patenting bodies and suggested future directions in patenting law.

Cell line optimization strategies were covered by **Dr. Hitto Kaufmann (Boehringer Ingelheim)**. Dr. Kaufmann rhetorically wondered why the topic of cell line optimization is worth discussing in light of the impressive productivity gains by the industry. The answer to his question lies in the fact that most new therapeutic proteins will be expressed in mammalian cells and, to supply a growing global patient population, new therapeutics may need to be produced in quantities from multi-hundred kilogram to a ton. Dr. Kaufmann described BI HEX<sup>®</sup>, an integrated, proprietary fast-track platform for the development of high-titer production cell lines (CHO cells), and associated high titer fed-batch processes. He noted that the expression system utilizes elements such as a proprietary media and feed platform and involved extensive work to develop an effective vector, as well as superior single cell cloning methods.

Dr. Kaufmann then reminded the delegates of the demands that will be placed on production cell lines of the future. These include the ability to produce increasingly higher titers of protein of specified quality, which is not a trivial task in light the many possible post translational modifications. Lastly, such processes must be scalable and robust and must utilize serum-free, chemically defined media. To achieve these ends, developers must use screening processes that take all requirements into account, and select the best host cell to accomplish the task. Dr. Kaufmann went on to describe the approach taken by Boehringer Ingelheim for host cell engineering. He noted that much work has been done over the years in the areas of transcription (to ensure high number of transcripts) and some good work has been done on translation. However, less research has gone into understanding the cellular machinery involved in protein secretion and post-translational modifications. Dr. Kauffman described some of the

work that contributes to the success of BI HEX<sup>®</sup>, such as the discovery that CERT, a lipid transport protein and an associated complex feedback loop process with PKD, works to facilitate the transport of ceramide from the endoplasmic reticulum to the Golgi complex and onward to the plasma membrane. Research done in collaboration with the University of Stuttgart revealed that over-expressing CERT, as well as an associated gain-of-function mutant (S132 A) does increase the specific productivity and final fed batch titer in CHO cell systems. In short, this process (involving CERT-S132 A) widens the bottle neck at the Golgi complex, thus allowing increased secretion of protein. Dr. Kauffman concluded with a description of another major component of the BI HEX<sup>®</sup> process that focuses on selecting the best possible producer clones. To accomplish this, the Boehringer Ingelheim process development team uses an automated high-throughput system capable of screening 4,000 clones in a period of 12 hours. The system enables “immediate and early” clone screening, where productivity is screened at the earliest possible time point. To achieve high productivity, ELISA was replaced by a simple HTRF assay. From there, titer curves and a specific productivity measurement using a clone select imager, are used to rank the clones. The combined use of titer curves and the imager, allows more accurate prediction of specific productivity compared to relying solely on titer curves, which may be misleading.

**Mr. Andreas Schneider (Innovatis, AG)** discussed real-time analysis and optimization of fermentation processes. He updated the delegates on the process analytical technology (PAT) initiative. A key point was that PAT rests upon an understanding and close monitoring of critical process parameters, with the goal of continuous process improvement. The US Food and Drug Administration is using PAT to instill a Quality by Design (QbD) mindset, and to encourage the industry to strive for improved, well understood processes that can be modified within accepted ranges to produce therapeutics of consistent quality. Mr. Schneider also gave an overview of the current data management efforts to support PAT, noting that the systems now used are non-standard, making process harmonization, automation and process portability very difficult, even within the same company. For example, there is no standard way to interface Historian, MES and LIMS systems, to ensure fluid and accurate data flow and information sharing. From an engineering perspective, Mr. Schneider gave an overview of the “closed loop approach” made possible by an integrated in-line and off-line analytical/data/analysis system that can make process modification possible in real time. He then spoke of a project done in collaboration with Bayer that aimed at automating sample extraction and delivery of the sample to analytical instrumentation located elsewhere in the processing suite (minimum 10 meters away), to determine important parameters such as Cell Density and Cell Viability. The system was also supported with a data processing and management system. Innovatis worked with Bayer to successfully design and install the sample acquisition, sample transport and analysis system. The system was designed to handle multiple reactors and used an open architecture to enable the use of various types of analyzers (HPLC, Cedex, etc.) with a sample transport capability of up to 30 meters away from the

acquisition site. Currently, the parameters that have been successfully monitored in quasi real-time using this approach are cell count, cell viability and titer. Work has started to expand the capabilities of the system to also analyze IgG, metabolite concentration and other parameters deemed critical to process control and also to scale-down the system to fit bioreactors of all sizes.

**Dr. Frank Detmers (Bio Affinity Company)** described the development of CaptureSelect<sup>®</sup> ligands, which are specialized affinity capture ligands designed to address the growing need for novel, and very specific, technologies for the purification protein based therapeutics. The use of an affinity capture step during product purification is a widely used technique by the industry. BAC's work may make possible the use of simpler two-step (capture and polish) processes that are less costly and more effective than the three-step purification processes now in use. The ligand discovery process at BAC starts with llama immunization to generate a VHH library, from which messenger RNA is isolated. From the RNA, cDNA is produced. Using PCR, a PPH library is then identified. The binding properties of individual proteins is assessed through colony picking. If insufficient binders are present in the PPH library, yeast display can be used to increase the amount. The chosen set of proteins are then screened against representative process operating conditions such as pH, buffers, cleaning agents and other process parameters to produce a short list of candidates. The best binders are then stabilized on polymer matrices and are tested further in a chromatography process. Once a suitable ligand is selected for a given application, it can be produced at large scale in baker's yeast, then purified by filtration and ion exchange chromatography. BAC licenses these affinity ligands to therapeutics and chromatography media producers. Ligands can be immobilized on any surface (membranes or beads) that can be activated with aldehyde, epoxide or NHS chemistry. The example of a human plasma library was used to illustrate the development and application concepts, demonstrating the capture of a broad range of IgG in flow-through mode. BAC's development pipeline includes ligands that bind a variety of proteins such as ApoA2, C1 inhibitor, Factor V, Factor X, Factor XII, Factor XIII, hVWF, EPO and IFN $\alpha$ -2b.

The discovery of anticancer monoclonal antibodies targeting the junction adhesion molecule A (JAM-A) by a functional approach was discussed by **Dr. Nathalie Corvaia (Centre d'Immunologie Pierre Fabre)**. JAM-A is located at the tight junctions of epithelium and endothelial cells, and is involved in the regulation of junctional integrity and permeability. A literature search has revealed no published information on the role of JAM-A in oncology. However, immunohistochemical analysis of a panel of human tissues from normal and tumor origin was performed at Pierre Fabre using a commercially available anti-JAMA-A mAb and results indicated that JAM-A was strongly overexpressed on tumor tissues, especially breast tumors. Mice immunized with MCF-7 breast cancer cells were used to generate mAbs able to block cellular proliferation. These candidates were further tested in vivo for their ability to induce MCF-7 tumor regression in engrafted nude mice. MAb 6F4 was able to completely inhibit tumor growth in mice after i.p. treatment with 1mg dose twice

a week. Proteomic analysis using 6F4-tagged beads, MALDI-MS and LC-MS/MS analysis, as well as database searching indicated that 6F4 specifically recognized human JAM-A. 6F4 activity at a dose of 1 mg twice a week was confirmed in A431-xenografted mice. Mechanism of action studies indicated that the anti-JAM-A mAb inhibits tumor growth in vivo through inhibition of cell proliferation, downregulation or shedding of the target, and downregulation of genes involved in translation machinery. The potential of the mAb as a therapeutic will be explored in future preclinical studies.

**Dr. Francis Bitsch (Novartis)** presented a comparison of methods for quantifying monoclonal antibodies in biological fluids. Knowledge of the time-dependent concentration and distribution of antibodies in biofluids and tissues is important in developing effective dosing strategies. Enzyme Immunoassays (EIA) or enzyme-linked immunosorbent assays (ELISAs) have traditionally been used for this purpose. However, the assays have limitations—assay development is time-consuming and expensive, linear dynamic range is narrow, precision is low and variability exists between vendors. To circumvent these problems and enable fast, efficient triage of candidates, Novartis utilizes a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system to quantify antibodies.<sup>12,13</sup> The approach is based on a combination of serum protein depletion methods and chromatography coupled with tandem MS of a unique antibody signature peptide produced by trypsin digestion of the protein. The method displays precision, accuracy and specificity across a wide dynamic range, is adaptable regardless of matrix, isotype or construct and is applicable to murine surrogate antibodies for target validation. The limitations of the method are inability to measure bound vs free or active vs inactive molecules, the time (5–10 min per sample) and cost for large scale studies and sensitivity.

The synergistic effects of the anti-epidermal growth factor receptor (EGFR) antibody combination Sym004 were described by **Dr. Michael Kragh (Symphogen)**. Sym004 comprises two chimeric IgG1 antibodies that target EGFR domain III at uniquely positioned, non-overlapping epitopes. The antibodies have been generated using Symphogen's mSymplex technology involving the following steps: splenocyte isolation from immunized animal, single cell sorting, Symplex PCR, repertoire cloning and arraying, preparation of DNA, repertoire expression in mammalian cells and high throughput screening to yield recombinant antigen-specific antibodies. The candidate was selected after 52 single anti-EGFR mAbs were tested for efficacy in a cell viability assay (HN5 and A431NS); greater than 80 combinations of two antibodies and greater than 450 combinations of three or more antibodies were tested similarly. The lead compositions were then tested in an A431NS xenograft model, and the Sym004 two mAb combination was selected for further development. In A431NS cells, 0.5–10 mg/mL concentrations of Sym004 decreased metabolic activity as a percentage of untreated control to a greater extent than other anti-EGFR mAbs tested and their respective combinations, including cetuximab, zalutumumab and panitumumab. Sym004 also showed superior efficacy in vivo as

compared to cetuximab and/or panitumumab in four animal tumor models (A431NS, A431, HCC827 and H1975). Sym004 induces EGFR internalization that is dependent both on binding by both antibodies and bivalency of full length Sym004 IgGs, and leads to EGFR removal by degradation. Furthermore, preliminary safety data in cynomolgus monkeys indicates tolerability at a 12.6 mg/kg dose followed by 8 mg/kg doses for six weeks. A full panel of toxicological studies has been initiated.

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## December 3, 2008

### Day 3, Innovation in antibody and antibody alternatives development

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The final day of the conference was dedicated to discussion of antibodies that have been designed with additional functionality compared to 'naked' molecules, such as conjugated and bispecific antibodies, alternative scaffolds, and domain antibodies. The characteristics of conjugated antibodies comprising an antibody, cell-killing agent, and linker were discussed by **Robert Lutz (ImmunoGen)**. The importance of tailoring the linkage between antibody and cytotoxin and adjusting the number of cytotoxic molecules per antibody to optimize the biological effect was emphasized. A series of new hydrophobic linkers (POL1, 2 and 3) with improved pharmacokinetic properties at high drug loads and improved potency against cancer cells, including multi-drug resistant (MDR) tumors, have been developed at ImmunoGen. When compared with SPDB and SMCC as linkers in mAb-DM1 candidates, a candidate with the POL1 linker had similar in vitro activity against COLO205, but had greater in vitro and in vivo activity against both COLO205-MDR and HCT15 colon carcinoma.

A clinical update of an advanced antibody-drug conjugate (ADC) based on trastuzumab was provided by **Mark Sliwkowski (Genentech)**. The cytotoxic component is maytansine derivative DM1 developed by ImmunoGen and the linker is MCC, a non-reducible succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate.<sup>1</sup> When compared to a reducible linker (SPP), the MCC linker had greater stability in rats, gave results closer to the control in a liver function test (serum enzyme AST), and showed improved efficacy in in vivo preclinical HER2+ models. Trastuzumab-DM1 had equivalent activity compared to trastuzumab in a variety of assays (HER-2 binding affinity, inhibition of HER-2 shedding, cytotoxicity mediated by antibody-dependent cell cytotoxicity, inhibition of AKT phosphorylation). The antibody-drug conjugate gave improved results compared to trastuzumab in in vitro assays, including inhibition of proliferation of MDA-MB-361.1 (resistant to trastuzumab and lapatinib) and SKBR-3 (resistant to lapatinib) cells, and growth of MDA-MB-361.1 xenografts. Trastuzumab-DM1 is being tested in three on-going Phase 2 single agent trials in HER2+ metastatic breast cancer patients. Interim results from one Phase 2 study showed a 43% (13/30) response rate in evaluable patients. Of 31 patients, thrombocytopenia was the most frequent Grade 3/4 drug-related adverse event (3/1, respectively). A randomized Phase 3 trial

in second and third line HER2+ metastatic breast cancer is planned.

Preclinical and clinical updates on auristatin-based ADCs were presented by **Hans-Peter Gerber (Seattle Genetics)**. The importance of optimizing the linkers and drug components of ADCs was again emphasized.<sup>2</sup> For example, a non-cleavable linker combined with a low cell permeability drug (mcMMAF combination) is expected to have reduced off-target toxicity and provide more selective drug release with minimal bystander activity. The combination of a cleavable linker and drug with high cell permeability (vcMMAE) is expected to release free drug to adjacent cells and therefore have bystander effects on stromal and antigen-negative tumor cells. Preclinical results for candidates utilizing each approach were presented. SGN-75, an anti-CD70-mcMMAF(4) ADC, showed dose dependent potency in models of renal cell carcinoma (786-O or patient tumor cells)<sup>3</sup>; an IND for SGN-75 may be filed in 2009. CD19 is an attractive target because the antigen is rapidly internalized. Both versions of anti-CD19 ADCs were tested in a model of rituximab resistant non-Hodgkin lymphoma. The anti-CD19-vcMMAE(4) was more efficacious than the mcMMAF version, with tumor volume reduced to near zero 21 days post tumor implant by the 3 mg/kg dose (q4dx4, IP). The anti-CD30-vcMMAE(4) candidate SGN-35<sup>4</sup> is currently in Phase 1 studies of CD30+ hematologic malignancies. Preliminary data from 22 evaluable patients treated at doses greater than 1.2 mg/kg indicated objective response in 45% (complete response and partial response = 10), with the majority of adverse events of Grade 1 and 2.

**Nils Lonberg (Medarex)** reviewed human antibody-minor groove binding alkylating (MGBA) conjugates for cancer therapy. These ADCs are prodrugs activated by intracellular carboxyesterases. In a xenograft (786-O kidney cancer) efficacy study in mice, MDX-1203, an anti-CD70 ADC, reduced tumor volume at doses of 0.03 and 0.1  $\mu\text{mol/kg}$  (approximately equivalent to 2.25 mg/kg and 7.5 mg/kg, respectively). The candidate also produced less percent body weight change than vehicle in a Balb/c mouse safety study. In a cynomolgus macaque model, minimal effects were observed in platelet count; some reduction in white blood cell counts were observed through day 20 after dosing, with rebound by approximately day 30. DNA-MGBA adduct accumulation was observed in tumor (6 pmol/mg of adduct per weight of organ), with limited accumulation observed in normal tissue (highest in lung, at approximately 1 pmol/mg of adduct per weight of organ). A Phase 1 study of MDX-1203 in renal cell carcinoma is planned in 2009. MDX-1204, an alpha mesothelin ADC was also discussed. Mesothelin is a useful biomarker; compared to healthy volunteers and random hospital patients, elevated serum levels have been reported in mesothelioma and ovarian cancer patients. Results of a lung cancer model indicated MDX-1204 dosed at 0.3  $\mu\text{mol/kg}$  maintained tumor volume at approximately the implantation level for over 170 days.

The focus of the day then turned to antibodies designed to bind two different targets. **Patrick Baeuerle (Micromet)** presented an update on bispecific T-cell engager (BiTE) technology, which

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provides stable recombinant monomers of approximately 55 kDa from CHO cells. BiTE antibodies are composed of two flexibly linked single-chain antibodies, with one half designed to bind to a surface antigen and the other designed to recruit T-cells by binding to CD3. T-cells are not activated by BiTE antibodies alone, but get activated by an appropriate BiTE only in the presence of tumor cells.<sup>5</sup> BiTE-activated T cells serially kill, which is why they are active even at ratios of 1 effector to 5 target cells. Blinatumomab, a bispecific anti-CD19/anti-CD3 BiTE, is in a Phase 1 study of non-Hodgkin lymphoma and a Phase 2 study of acute B-lymphocytic leukemia. Complete and partial tumor regressions were seen at doses as low as 0.015 mg/m<sup>2</sup> per day when given to non-Hodgkin lymphoma patients for 4–8 weeks (the dose range tested was from 0.0005–0.09 mg/m<sup>2</sup>/24 h).<sup>6</sup> At 0.06 mg/m<sup>2</sup> per day, 7 out of 7 patients responded. In total, 10 of 39 patients permanently discontinued treatment due to adverse events, most of which (n = 7) were due to CNS events. MT110, a BiTE antibody bispecific for EpCAM and CD3, is in Phase 1 study as a treatment for patients with late stage adenocarcinoma. Micromet's future plans include development of BiTEs from single-chain antibodies of human or humanized sequence (blinatumomab is murine; MT110 uses a deimmunized anti-CD3) and conversion of commercial antibodies, such as trastuzumab, panitumumab, cetuximab and omalizumab, into BiTE antibodies for engagement of T cells.

Development of a dual-specific antibody technology was described by **Chengbin Wu (Abbott Laboratories)**. Dual variable domain (DVD)-Ig molecules are tetravalent, target two different soluble or cell surface proteins, and can be expressed well in stable CHO cells. 1D4.1-ABT325, a DVD-Ig that binds hIL-12 and hIL-18, was produced using the technology. In a huPBMC-SCID mouse model, 1D4.1-ABT325 reduced serum huIFN gamma levels to levels comparable to those achieved by the combination of the parental antibodies 1D4.1 (anti-IL12) and ABT325 (anti-IL18). The pharmacokinetic profile in male SD rats dosed at 4 mg/kg IV and SC indicated a half-life of 11 days on average (range 9–13 days), with clearance of 0.26 mL/h/kg; these values were similar to those for 1D4.1. A C<sub>max</sub> of 33.4 mg/mL was reached at 6 days T<sub>max</sub>. In both rat and cynomolgus monkey PK studies, IL12 or IL18 capture ELISAs produced similar results.

The final major topic of the day was new binding scaffolds and emerging technologies. **Eric Furfine (Adnexus)** provided an overview of Adnectins, which are variants of the approximately 10 kDa 10th type III domain of human fibronectin. The binding characteristics of Adnectins are altered by making changes in up to three solvent-exposed loops on the fibronectin domain backbone. The first clinical candidate is CT-322, a pegylated antiangiogenesis agent that blocks the VEGFR-2 pathway. CT-322 was studied in patients with a variety of tumor types in Phase 1 and is in Phase 2 clinical development in recurrent glioblastoma multiforme.

The versatility of phase display technology was discussed by **Clive Wood (Dyax Corporation)**. Phage display libraries based on the first Kunitz domain of human tissue factor pathway inhibitor were developed at Dyax; diversity was created by varying regions of parental protein responsible for binding while regions responsible for maintaining the structure of the protein were

kept constant. DX-88, a candidate based on the human tissue factor pathway inhibitor scaffold, has successfully completed two Phase 3 studies for treatment of acute attacks of hereditary angioedema. A biologics license application (BLA) for the candidate was submitted to FDA in September 2008; the BLA received a priority review rating. Antibodies derived from phage display are also in development by Dyax, as well as collaborators such as ImClone Systems, Biogen Idec and Merrimack Pharmaceuticals. Preclinical data for DX-2400, an anti-matrix metalloproteinase (MMP)-14 IgG1 antibody, was presented. MMP-14 is a key regulator of pericellular proteolysis and tumor progression. The importance of selectivity was emphasized as there are multiple members of the MMP family. The K<sub>i</sub> of DX-2400 v. MMP-14 was 0.8nM compared to greater than 1,000 nM vs 13 other MMPs or tumor necrosis factor-alpha converting enzyme (TACE). In a MDA-MB-231 breast cancer xenograft, DX-2400 administered q2d at 10 mg/kg significantly reduced tumor burden compared to IgG control (p < 0.05); the candidate also slowed tumor growth compared to no treatment at doses as low as 1mg/kg (p < 0.05). Work by Dr. Shafaat Rabbani at McGill University suggests that DX-2400 also reduces metastasis incidence.

Stability engineering of human domain antibodies (dAb) was reviewed by **Ben Woolven (Domantis)**. Introduction of stresses (e.g., thermal, pH, protease) during phage selections can improve the attributes of library outputs such as improved resistance to proteases, thermal/shear stress or aggregation upon denaturation, as well as improved thermodynamic stability and affinity/potency.<sup>7,8</sup> Domain antibodies with improved biophysical properties may also have improved in vivo properties (e.g. serum half-life), and the ability to withstand shear and thermal stress induced by nebulisers. A case study of selection for improved resistance to proteases including trypsin was presented. Examples included one anti-IL-1RI dAb (DOM4-A), one anti-TNFR1 dAb (DOM1-A) and two anti-VEGF dAbs (DOM15-A and 15-C). Protease digest conditions varied (100–1,000 mg/mL for 2 hrs to overnight at 37°C); output was subcloned for dAb expression, purified, and characterized in a range of assays. In these experiments, higher concentrations of trypsin (1 mg/mL vs 0.1 mg/mL) resulted in selected leads that had greater resistance to digestion and higher T<sub>m</sub>. In particular, selected lead DOM1-B retained greater in vitro potency in a receptor-binding assay after being kept at 50°C for up to 14 days compared to the parent molecule DOM-1A. DOM1-B was also shown to be stable to nebulisation. To assess in vivo stability, the parent and stability engineered molecules were converted into dAb-Fc fusions. In a rat PK model, T<sub>1/2</sub> increased from 42 h for the parent DOM 15-A-Fc fusion to 75.3 hrs for the selected lead DOM15-B-Fc molecule, and from 19.5 h for the parent DOM 15-C-Fc fusion to 85.7 hrs for the selected lead DOM15-D-Fc molecule.

**Dimitar Dimitrov (National Institutes of Health)** discussed human antibody variable and constant domains as scaffolds, with a focus on domain antibodies and nanoantibodies.<sup>9</sup> Work in the Dimitrov laboratory has led to the identification of a novel domain antibody, m36, derived from a domain antibody library via sequential panning against two HIV envelope glycoproteins.

The approximately 15 kDa, monomeric molecule has a high level of expression, specific high-affinity binding to gp120 complexed with CD4, and neutralizes primary isolates from different clades.<sup>10</sup> The topic of nanoantibodies as potential therapeutics was also presented. Nanoantibodies make use of CH2 domains as scaffolds and can be engineered to contain antigen-binding sites, as well as binding sites mediating effector and stability functions. Isolated human CH2 domains are much smaller than IgG (approximately 15 kDa vs 150 kDa, respectively), and can be stabilized by constraining the N- and C-terminal strands with disulfide bonds. Several stabilized CH2 mutants, including m01 and m02, have been produced. These show increased stability to urea-induced unfolding and elevated melting temperatures (in 70–80°C range) compared to the parent CH2. In addition, large libraries were generated by mutagenesis and grafting of antibody CDRs to produce nanoantibodies targeting HIV. Two of these, m61 and m62, are HIV-specific binders and have been found to inhibit HIV-1 infection. Further work to develop novel nanoantibodies is in progress.

Finally, **Christian Heinis (Ecole Polytechnique Federale de Lausanne)** presented a novel strategy to generate small antibody-mimicking structures for therapeutic applications which he had recently developed with Sir Greg Winter at the Laboratory of Molecular Biology (LMB) in Cambridge, UK. The antibody-mimics are built of peptide loops that are anchored to a small molecule core and have a molecular mass of less than 2 kDa. The molecules are isolated in affinity selections from large combinatorial libraries that are generated by reacting cysteine-rich peptides on phage with tris-(bromomethyl) benzene. In iterative affinity selections, binders with high affinity ( $K_i$  as low as 1.5 nM) to the human disease targets plasma kallikrein, urokinase-type plasminogen activator and cathepsin G were isolated.

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