5th European Antibody Congress 2009
November 30–December 2, 2009, Geneva, Switzerland

Introduction

The 5th European Antibody Congress (EAC), organized by Terrapin Ltd., was held in Geneva, Switzerland, which was also the location of the 4th EAC.1 With more than 220 delegates, this event was the largest antibody congress held in Europe during 2009. Numerous pharmaceutical and biopharmaceutical companies active in the field of therapeutic antibody development were represented, as were start-up and academic organizations. The global trends in antibody research and development were discussed, including success stories of recent marketing authorizations (catumaxomab, certolizumab pegol, rilonacept, ustekinumab and ofatumumab developed by Fresenius, UCB-Celltech, Regeneron, Centocor and Genmab, respectively) and success and attrition rates for this fast expanding class of therapeutics. Case studies covering clinical progress in anti-CD20 (Genmab, LFB) and anti-IGF-1R mAbs (Biogen Idec, Imclone, Merck/Pierre Fabre), antibody-drug conjugates (ImmunoGen, Genentech, Seattle Genetics, Wyeth/Pfizer) and new scaffolds (Ablynx, Adnexus/Bristol-Myers Squibb, Damanthi/2x3x1Kline, Dyax, Molecular Partners, Scil Proteins) were presented. Major antibody structural improvements were showcased, including the latest global developments in 2-in-1 antibodies (Genentech), dual antibodies (Abbott), trifunctional antibodies (Trion Pharma, Fresenius), agonist antibodies (MedImmune, Kyowa Hakko Kirin), Fc-engineered (Centocor, MedImmune), glyco-engineered (Centocor, Kyowa Hakko Kirin, Lonza) aglycosylated IgGs (University of Cambridge) and non-activating formats (Genmab). Improvements of drugability (Pierre Fabre, Pfizer), alternative quantification methods based on mass spectrometry (Novartis, CEA), progress in manufacturing (Biogen Idec, Boehringer-Ingelheim, Merck KG) and patent strategies were also discussed. Last but not least, identification of mAbs against new therapeutic targets (Pierre Fabre, Roche, Crucell) and translations to clinical studies (Novartis) were presented, as well as progress in antibody humanization and engineering (Université de Montpellier, French Army Health Department, Merck-Serono, Pierre Fabre).

November 30, 2009: Day 1

Alain Beck
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The EAC chairman, Alain Beck (Centre d’Immunologie Pierre Fabre), opened the meeting with remarks on trends in antibody development in the last three years. Monoclonal antibodies (mAbs) and related-products (e.g., immunoconjugates, radioimmunoconjugates, trifunctional antibodies, Fab fragments and Fc-fusion proteins) are the fastest growing class of pharmaceuticals,2-4 with nearly 35 products currently approved worldwide for a wide range of indications.5

In just the last three years, ten new antibodies and derivatives have reached the market. These products include humanized and human IgGs, but also molecules based on novel formats, as well as first to fifth-in-class drugs in both traditional and new therapeutic indications. Specifically, eculizumab (Soliris) was approved for paroxysmal nocturnal hemoglobinuria (PNH) in 2007. Eculizumab comprises an original IgG2/4 hybrid format, and is unable to bind Fc receptors or activate the complement cascade. In 2008, three IgG-derived molecules, rilonacept, certolizumab pegol and romiplostim, reached the market. Rilonacept (Arcalyst) is an interleukin (IL)-1 receptor-Fc fusion protein also called IL-1 trap, which is indicated for cryopyrin-associated periodic syndromes (CAPS). Certolizumab pegol (Cimzia) became the first PEGylated Fab fragment (produced in E. coli) to gain approval in Crohn disease.6 Romiplostim is the first approved peptibody; the molecule binds and antagonizes the thrombopoietin (TPO) receptor via a TPO peptide mimetic fused to an Fc moiety (8 kDa peptide genetically fused to an aglycosylated Fc domain to increase the plasmatic half-life). Romiplostim was approved as a treatment for immune thrombocytopenic purpura (ITP).7

Remarkably, six antibody therapeutic products were approved by the European Medicines Agency or the US Food and Drug Administration in 2009. Ustekinumab (Stelara) was the first IL12/23 directed antibody indicated in moderate to severe plaque psoriasis. Golimumab (Simponi) is the fifth anti-tumor necrosis factor (TNF) biologic approved, but the first to be administered subcutaneously with an auto-injector on a monthly schedule.7 Tocilizumab (Actemra, RoActemra), a conventional IgG1 directed against IL-6R available in Japan since 2005, also reached the market in the US and in Europe as a treatment for patients with rheumatoid arthritis. Catumaxomab (Removab) is a unique tri-functional, bispecific antibody composed of a hybrid mouse IgG2a and rat IgG2b, and directed simultaneously against...
two different antigens (EpCAM and CD3) that was approved for treatment of malignant ascites. Finally, canakinumab (Ilaris), an anti-IL1 mAb for treatment of CAPS, and ofatumumab (Arzerra), an anti-CD20 treatment for chronic lymphocytic leukemia that has a different epitope and a different mechanism of action compared to rituxan, also reached the market.

Another important event in the last 3 years is the arrival on the market of biosimilar antibodies. The first two of these, Reditux (a biosimilar of rituximab developed by Dr. Reddy) and Clotinab (a biosimilar of abciximab developed by ISU ABXIS), were launched in India and in South Korea, respectively. Interestingly, active discussions are ongoing regarding whether such biopharmaceuticals may also be approved in Europe, where other glycoproteins such as erythropoietin are marketed, as illustrated by workshops on biosimilar mAbs organized by the European Medicine Agency in London on July 2, 2009, and in the US. The European Medicine Agency is very proactive concerning regulation of biosimilar products, as illustrated by the recent approvals of biosimilar somatropin, filgrastim, and epoietins alpha and zeta, the publication of concept papers, and guidelines and the organization of the workshop on the feasibility of biosimilar mAbs. The format of the workshop included open discussion on the pros and cons of biosimilar mAbs development, as well as the feasibility. The discussions might help to clarify the path ahead in the EU, and also in the United States, Asia and other countries. Patents on several first generation antibodies will expire soon, which will enhance interest in the development of biosimilar or follow-on mAbs in the near future.

William Strohl (Centocor) discussed modifications of IgG2 that result in antibodies reduced or even devoid of effector functionality. Several such antibodies are on the market or in clinical development. They include modified Fcs for either enhancing antibody-dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), opsonophagocytic killing (OPK) or reduction of those activities. Examples of reduced FcγR were discussed, including marketed products eculizumab (IgG2/4 hybrid), romiplostim (aglycosylated Fc-peptide), abatacept (Ocrenria, CTLA-4-Fc engineered fusion receptor), as well as teplizumab (IgG1-Fc Ala-mutated at position 236 and 237) and otelixizumab (mutated at Asp297Ala, aglycosylated IgG4), both of which are in Phase 3 studies.

More recently IgG2m4 (His268Gln, Val309Leu, Ala330Ser, Ala331Ser) was engineered as a silenced Fc and applied to both anti-amyloid and IL13R antibodies. IgG2m4 is characterized by a dominant ‘muting’ relative to aglycosylated Fc, no apparent binding to C1q, reduced FcγR binding as compared with IgG2, and no apparent immunogenicity directed toward Fc in rhesus studies. This new format also shows a long half-life by subcutaneous (s.c.) dosing compared to IgG1 isotypes (18–21 days for anti-ligand mAbs) and seems suitable for pharmaceutical development (high level expression, solubility up to 100 mg/mL; no isotype-specific aggregation). Nevertheless, the effector function is only reduced and not fully silenced. A new construction named IgG2Σ was designed and appeared to be entirely silenced. To address possible safety issues, surrogate versions for IgG2Σ have also been designed in mouse (IgG3/m) and rat (IgG2/m) with a mutation for limiting C1q binding (K322A). Additional tests are ongoing with both IgG2Σ and surrogate versions.

Janine Schuurman (Genmab) provided an update on wild type and mutated IgG4s, Unibody and other nonactivating antibody formats. Two humanized IgG4 antibodies, natalizumab (Tysabri) and gemtuzumab ozogamicin (Mylotarg), are approved for human use, and several others, e.g., TGN1412, are or have been investigated in clinical trials. IgG4 antibodies can dynamically exchange half-molecules. IgG4 can form half-antibodies due to hinge CPSC instability, which can be corrected by a single amino acid mutation, thereby mimicking an IgG1 hinge (CPPC). Gemtuzumab ozogamicin is hinge-stabilized whereas natalizumab is not. Considerations for the clinical development of non-activating formats were discussed.

Until recently, Fab-arm exchange with therapeutic antibodies had not been demonstrated in humans. Labrijn et al. showed in 2009 that natalizumab exchanges Fab arms with endogenous human IgG4 in natalizumab-treated individuals. In contrast, gemtuzumab ozogamicin contains the IgG4 core-hinge mutation that blocks Fab-arm exchange to undetectable levels both in vitro and in a mouse model. The ability of IgG4 therapeutics to recombine with endogenous IgG4 may affect their pharmacokinetic (PK) and pharmacodynamic (PD) properties. Mutations that completely prevent Fab-arm exchange in vivo should be considered when designing therapeutic IgG4 antibodies. Genmab is also developing half-antibody derivatives (75 kDa UniBodies) as a nonactivating format.

Antibody and Related-Products: Choosing the Right Format for the Right Indication

To date, most therapeutic chimeric, humanized and human antibodies have been based on an IgG1/kappa backbone. Nonetheless, IgG4 and IgG2 isotypes are being chosen more often when effector functions are unwanted (e.g., for receptor blocking without cell depletion). Conversely, IgG1 are frequently selected for killing pathogenic cells, such as those with an overexpressed 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; at least four other members targeting CTLA4, RANK-L, IGF-1R and CD3 antigens are in Phase 3 studies. IgG2 are characterized by isomers described recently. Unlike IgG1 and 4, human IgG2 have also been shown to form covalent dimers in vivo involving hinge cysteine 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). IgG4 can also form bispecific antibodies in vitro and in vivo that may crosslink different antigens. As a further consequence, IgG4s may be functionally monovalent in vivo if the molecule is not stabilized by an IgG1 S241P hinge mutation.

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Improving Efficacy and Drugability of Antibodies

Among all analytical methods used to characterize mAbs, mass spectrometry (MS) is playing an increasingly important role for both global and fine structural characterization of therapeutic candidates, and MS data is helping in the design of more homogeneous and drugable mAbs. Alain Beck (Centre d’Immunologie Pierre Fabre) presented different complementary spectrometers and methods used for primary structure assessment, glycosylation and production-system fingerprinting, structural isotyping by disulfide pairing determination and hot-spot mapping. All IgGs show common post-translational modifications and micro-variants that can be visualized by liquid chromatography, electrophoretic and MS-based methods. With the last generation of mass spectrometers (LC-ESI-TOF), the mass of intact antibodies (approximately 150 kDa) can be measured with a precision reaching 4 Da of total mass, which accelerates screening, routine identification and structure assessment of mAbs. The current resolution of mass spectra also allows investigation of the non-symmetry of N-linked biantennary oligosaccharides between the two heavy chains and may contribute to the better understanding of Fc gamma receptor binding structure relationships.

Glycans represent only an average of 2% of the total mass of IgGs and are located generally on Asn. Despite this low percentage, particular glycoforms are involved in important immune effector functions (e.g., ADCC, CDC). Glycosylation plays also an essential role in the long plasmatic half-life via binding to neo-effector functions (e.g., ADCC, CDC). Glycosylation also affects antibody conformation in the Fc. With the last generation of mass spectrometers (LC-ESI-TOF), the mass of intact antibodies (approximately 150 kDa) can be measured with a precision reaching 4 Da of total mass, which accelerates screening, routine identification and structure assessment of mAbs. The current resolution of mass spectra also allows investigation of the non-symmetry of N-linked biantennary oligosaccharides between the two heavy chains and may contribute to the better understanding of Fc gamma receptor binding structure relationships.

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Shantha Raju (Centocor/Johnson & Johnson) summarized the current understanding of IgG Fc glycosylation structure-function relationships. Crystal and solution structural studies show that minor variations due to the heterogeneity of terminal glycan sugars affect antibody conformation in the Fc. The presence or absence of various terminal sugars of Fc glycans increases microheterogeneity, which affects not only antibody effector functions, but also antibody stability and binding to certain cell surface antigens, and safety. Structural studies of intact antibodies and Fc fragments suggest that hydrophobic and hydrophilic interactions between sugar residues and amino acid residues in the CH2 domain of the Fc fragment affect the fine structural conformation of IgG molecules. Terminal-sugar residues mainly dictate these molecular interactions and therefore determine the impact of Fc glycosylation on antibody functions and stability. Terminal Gal, GlcNAc, and Man residues affect C1q binding and CDC activity, whereas terminal N-acetylmuramic acid (NANA), mannos, core fucose and bisecting GlcNAc residues affect FcyRIIIa binding and ADCC. Furthermore, terminal mannos residues may affect serum half-life of some IgGs and terminal NANA may affect antibody binding to some cell surface antigens.

Owing to the potential impact of Fc glycans microheterogeneity, it is necessary to be vigilant in quality control of antibody glycosylation and to limit the impact of Fc glycans on product quality and efficacy of antibody-based therapeutics. Glyco-engineered mAbs with improved cytotoxicity for malignancies are currently being investigated in clinical trials and glyco-engineered mAbs with improved anti-inflammatory properties for autoimmune and inflammatory diseases are in preclinical studies.

Fas receptor (CD95) is a tumor-associated antigen which, when ligated by Fas ligand, triggers cell apoptosis. CD95 belongs to a death receptor family that includes several members like Fas, TNF-R1, TRAIL-R1, TRAIL-R2, Dc-R1 and Dc-R2. All these receptors are characterized by a trimeric extra-cellular domain, a trans-membrane domain and an intra-cellular death domain that trigger apoptosis when activated. CD95 is expressed on many tumor cells, and is known to mediate apoptosis and play a key role to regulate lymphocytes. Using phage display, Ralph Minter (MedImmune/AstraZeneca) and colleagues have isolated E09, an agonistic antibody to Fas receptor that can initiate apoptosis in cancer cells (e.g., early apoptotic event such as Caspase 3/7 activation or late apoptotic event such as fragmentation). Through protein engineering and structural studies, they have explored the agonistic mechanism, which could also have implications for agonistic antibodies to other therapeutic targets.

Co-crystallisation studies of E09 Fab with extra-cellular domain (ECD) of Fas receptor were performed in collaboration with Markus Gruetter and colleagues at the University of Zurich resulting in a structure determination at 1.75 Å and the first crystal structure of Fas ECD. E09 Fab binds to two distinct epitopes, FAS ECD top and center domains; interestingly, one loop of Fasl interacts with the same epitope as E09. Affinity maturation was also used to generate more potent agonistic anti-Fas antibodies, resulting in the selection of clone EP6bB01, which exhibited approximately 50-fold improvement in affinity compared to parent E09. Both on-rate and off-rate were improved, but unfortunately, cell killing efficiency decreased as affinity improved. Further deciphering of agonism activity of anti-Fas antibodies is in progress. Interestingly, this case study shows that affinity maturation does not necessarily lead to biologic improvement. Agonist
activity is a complex multi-factorial process that requires fine-tuning of affinity, valency and epitope and binding interactions. Understanding the biology is essential to design more efficient lead optimization strategies.

Shiro Kataoka (Kyowa Hakko Kirin) gave an update on the Biowa/Kyowa Hakko Kirin Potelligent® technology licensed by several companies and applied to glyco-engineered mAbs directed against GD3, CCR4 (KM2760, Amgen), CD30 (MDX-1401, Medarex) and ILSR (BIW-8405, MedImmune), that are currently being investigated in early clinical trials in different indications (cancer, inflammation and asthma). The Potelligent® technology is based on fucose removal from Fc-linked oligosaccharides, which greatly enhances ADCC. The Potelligent® system was recently combined to engineer the constant region of human IgG1/IgG3 chimeric isotypes to further enhance complement-dependent cytotoxicity (CDC) Complegent® technology. Both technologies are used to complement the transgenic KM mouse (Kirin/Medarex) used to generate antagonist and agonistic human antibodies.

The generation of a CHO recombinant cell line (CHOK1SV) suitable for cGMP manufacture and expressing 100% non-fucosylated antibodies was discussed by Alison Porter (Lonza). ADCC activity is considered to be a critical effector function for many therapeutic antibodies. Non-fucosylated antibodies, harboring a tri-mannosyl core structure of complex-type N-glycans of Fc without fucose residue, may provide mAbs with greatly enhanced ADCC. Non-fucosylated antibodies can also eliminate human serum IgG's inhibitory effect on ADCC through its high binding activity to FcγRIII; the homogeneous glycoforms composed of fully non-fucosylated glycans are required to maximize in vivo efficacy of ADCC while avoiding the inhibitory effects of the fucosylated counterparts through the competition for antigen binding. Therefore, production of antibody therapeutics consisting of fully non-fucosylated forms may be a way to develop antibody therapies with higher efficacy and reduced costs.

With regard to clinical efficacy and regulatory requirements, a key element is the development of a robust production process to ensure the structural consistency of the product. So far, the manufacturing system using FUT8 knockout CHO/DG44 cells (Kyowa Hakko Kirin) is the only method that provides clinical samples approved by a regulatory authority, and it is considered to be the most feasible and reliable system for manufacturing fully non-fucosylated therapeutic antibodies that now exists. The Potelligent® technology was also successfully applied to Lonza's CHOK1SV cell line. The cell selection process is based on the fact that the parent CHO-K1 cells have a low glutamine synthetase (GS) expression level. Transforming the cells with a plasmid co-expressing GS and an IgG of interest and cultivating the transfected cells in the absence of glutamine and the presence of MSX allows only those cells that have stably incorporated the foreign genes to grow. Since it only relies on weak expression rather than on metabolic deficiency, this system requires the presence of MSX to be maintained during cell expansion in order to keep a sufficient genetic pressure to prevent deletion of the foreign DNA. These two genetic strategies rapidly allowed use of the CHO cells since it conferred a rapid method of selection for identification of high mAb producers. CHO cells can be cultivated in suspension in serum-free and chemically defined cultivation media in large-scale conventional bioreactors. They display a high resilience to cultivation conditions, do not require cholesterol and tend to remain viable for a longer period of time when compared to NS0 cells.

Fab/Antigen or Fc/Fc-Gamma Receptors
Co-Crystallization Studies to Gain Insights on Structure and mAb Mechanism of Action

Carl Webster (MedImmune) highlighted the importance of the human neonatal Fc receptor (FcRn) as a regulator of IgG transport and discussed the implications for use of antibodies as therapeutics. An IgG variant of motavizumab anti-RSV mAbs bearing the so called “YTE” triple mutation in the Fc domain (M252Y/S254T/T256E) and selected to increase the plasmatic half-life has reached the early clinical studies. The FcRn receptor plays a pivotal role in regulating the transport and distribution of IgG. This receptor is related to MHC class I molecules, it binds to the Fc region of IgGs, it interacts in a pH dependant way (binding at pH 6, not at neutral pH) and transports IgGs within and across cells. Recent studies have focused on analyzing how antibodies traffic within cells and the engineering of FcRn-IgG interactions to generate inhibitors of FcRn function. Data concerning the limitations of using mice as a preclinical model for FcRn function were presented.

The first three-dimensional structure of a human Fc fragment genetically engineered for improved PK properties was recently reported. When introduced into the CH2 domain of human immunoglobulin G (IgG) molecules, the triple YTE mutation causes an approximately 10-fold increase in binding to FcRn in vitro. Interestingly, this translates into an almost 4-fold increase in vivo in the serum half-life of YTE-containing human IgGs in cynomolgus monkeys. A recombinantly produced human Fc/YTE fragment was crystallized and its structure solved at a resolution of 2.5 Å using molecular replacement. This revealed that Fc/YTE three-dimensional structure is very similar to that of other human Fc fragments in the experimentally visible region spanning residues 236–444. The enhanced interaction between Fc/YTE and human FcRn is likely mediated by local effects at the substitutions sites. Molecular modeling suggested that potentially favorable hydrogen bonds along with an increase in the contact surface between the two partners may account in part for the corresponding increase in affinity.

The interface between antibody and antigen is often depicted as a lock and key, suggesting that an antibody surface can specifically bind to only one antigen. Jenny Bostrom (Genentech) and colleagues have recently reported in Science an antibody with an antigen-binding site that binds two distinct proteins with high affinity (nM range). They have isolated a variant of trastuzumab that binds the human epidermal growth factor receptor 2 (HER2) on the basis of its ability to simultaneously interact with vascular endothelial growth factor (VEGF-A), like bevacizumab and ranibizumab. Crystallographic and mutagenesis studies revealed that distinct amino acids of this antibody called...
bH1, engage HER2 and VEGF energetically, but there is extensive overlap between the antibody surface areas contacting the HER2 and VEGF-A antigens. An affinity-improved version of bH1 inhibits both HER2- and VEGF-mediated cell proliferation in vitro and tumor progression in mouse models.

Dual specific antibodies are derived from a monospecific antibody through mutations in the periphery of the antigen-binding site in the light chain (LC) complementarity-determining regions (CDRs). This strategy is a general one and can be applied to create dual specific antibodies against two distinct antigens. The mutational analysis of bH1 and another variant (bH1-44) suggested that the dual specificity could be switched to monospecific binding to either antigen. Indeed, bH1-44 lost binding to VEGF but retained HER2 binding when two LC residues were mutated. Similarly, two alanine mutations in the heavy chain drastically reduced the affinity for HER2 while preserving tight binding for VEGF. This finding highlights how a limited number of mutations in the antigen-binding site can alter specificity or add a distinct specificity. During development of the natural antibody repertoire, the antigen binding sites often undergo diversification by exchanging the VL that pairs with a VH. Somatic mutations also occur frequently, in particular among the residues in the periphery of the antigen-binding site. In contrast to previous class specific formats, the dual specific “two-in-one” antibody has the molecular structure of a regular IgG. It has all the favorable drugability properties of an IgG for therapeutic development, such as predictable PK properties, well-established manufacturing protocols, choice of Fc-mediated effector functions and bi- or mono-valencies.

**Antibody Pharmacokinetic and Metabolic Studies: Quantitative Analysis of mAbs in Biofluids by Mass Spectrometry**

Eric Ezan (Institute of Biology Techniques-Saclay, CEA) argued for the use of MS to quantify therapeutic antibodies in the serum of animals or patients; this approach would complement classical enzyme-linked immunosorbent assays (ELISAs). Analysis of therapeutic antibodies in biological fluids is necessary for preclinical studies of PK and toxicity. MS is emerging as an alternative method to immunoassay since it offers speed of development, appropriate sensitivity and high specificity. His presentation covered recent advances and the advantages of MS for the quantification of therapeutic antibodies in biofluids illustrated by several papers published in 2008. For proof-of-concept, CEA, in collaboration with Merck-Serono, developed a liquid chromatography method coupled with tandem MS for the accurate absolute quantification of cetuximab (Erbitux). Magnetized beads, functionalized with recombinant epidermal growth factor receptor (EGFR) extra-cellular domain, were used for specific immunocapture of the anti-EGFR antibody. Following digestion with trypsin, specific peptides from light and heavy chains were monitored by MS. Assay variability was found to be below 20% following optimization of the digestion step and using an internal standard. The lower limit of quantification (20 ng/mL) was similar to that of ELISA methods. Added value is derived from mass spectrometry quantification of peptides from the variable regions and involved in the binding to soluble EGFR, which is highly specific since non-specifically bound endogenous antibodies are not quantified. The simultaneous monitoring of an Fc region peptide (HT23) shows the importance of this interfering phenomenon in human serum. Moreover, the protocol can be fully automated by using an online immunocapture cartridge and trypsin chamber already developed for other analytical applications.

Olivier Heudi (Novartis Institute for Biomedical Research) presented case studies on similar quantitative MS-based methods for PK assessment of mAbs. Immunological techniques such as ELISA are the current analytical gold standard for therapeutic proteins and are considered to offer sufficient sensitivity and specificity for toxicokinetic or PK studies. Such assays preferentially use a non-competitive or sandwich format in which two mAbs bind different epitopes of the protein, or, in the case of antibodies, a capture protein (the biological target, or anti-idiotype or anti-species antibodies) traps the therapeutic antibody from the sample. These assays are simple ones and have high throughput capabilities, but their development is rather long and costly since it requires recombinant technologies or in vitro immunization to obtain the specific reagents. For peptide detection, MS combined with liquid chromatography usually employs triple quadrupoles operated in the multiple reaction-monitoring (MRM) modes, which is appropriate for quantification in complex matrices because of its high specificity and sensitivity. In the MRM mode, only parent/product ion pairs of interest are detected while all others are excluded. The target peptide mass is selected, based on its mass-to-charge ratio, in the first quadrupole for subsequent fragmentation in the collision cell. Then, the products of interest are selected in the third quadrupole and finally detected. Direct application of the MRM mode to therapeutic proteins depends on their size, as large proteins must be reduced in size by trypsin digestion.

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Robert Lutz (ImmunoGen) expertly moderated the second day of the EAC, which began with an overview of antibody development trends, and then focused on specific marketed products and antibodies in the pipeline. Finally, the ‘patent cliff’ and challenges in biopharmaceutical manufacturing were discussed.

The first speaker, Janice Reichert (Tufts Center for the Study of Drug Development), summarized data on development trends for human antibody and antibody fragments. The Tufts data set used to determine these development trends is composed of data for approximately 540 therapeutic antibodies, of which nearly 130 are human mAbs that entered clinical study during 1997 to 2008. Of therapeutic antibody candidates that entered clinical study sponsored by commercial firms during 2000 to 2008, 45% were fully human antibodies, nearly 40% were humanized and the remaining candidates were either chimeric or murine. The fully human mAb candidates were derived primarily from display or transgenic mouse platforms. A total of six have been approved in the US and other countries (adalimumab, panitumumab, golimumab, canakinumab, ustekinumab, ofatumumab) and another two (denosumab and raxibacumab) were in regulatory review as of December 2009. Based on a cohort analysis, i.e., each candidate in the cohort is followed over time, the current cumulative US approval success rate is 17%, and this will increase to 22% if the two candidates in regulatory review are approved. The current phase transition rates are 89% for Phase 1 to 2, 50% for Phase 2 to 3 and 80% for Phase 3 to regulatory review, and 100% for review to approval. It is important to note that many of the human mAb candidates entered clinical study recently and so the final fates for few of the candidates are known (27%). Rates may change as more candidates proceed through the process.

Antibody fragment therapeutics comprise a small percentage (11%) of the total number of antibody therapeutics in the Tufts data set of candidates that entered clinical study sponsored by commercial firms. Of the 54 fragments, 30 (56%) were antigen-binding fragments (Fab), 19 (35%) were single chain variable fragments (scFv), and 5 (9%) were third-generation fragments, which include domain antibodies. In addition, at least 38 antibody fragments were in preclinical development. As of December 2009, three Fab were approved in the US and other countries (abciximab, ranibizumab, certolizumab pegol), with another (metuximab) approved in China. Based on an analysis of the clinical entry of the three classes of fragments (Fab, scFv, third-generation) over time, industry development is focusing on scFv and third-generation molecules, which comprise nearly 80% of the antibody fragments now in clinical study. The variety of therapeutic categories studied has increased over time, with a de-emphasis on oncology and concomitant increase in the number of candidates studied for immunological, ophthalmic and cardiovascular/hemostasis indications. Compared to the candidates in the clinic, the antibody fragments in preclinical development were more likely to be multivalent or bispecific, and less likely to be conjugated, e.g., to a cytokine, toxin, drug or radioisotope.

Diane Seimetz (Fresenius Biotech) described the bispecific, trifunctional antibody catumaxomab, which was approved in the EU in April 2009. The anti–EpCAM, anti–CD3 product binds to EpCAM+ tumor cells, T cells and accessory cells. Comprising mouse IgG2a and rat IgG2b, catumaxomab exerts its anti-cancer effects through both antibody- and T cell-mediated cell killing, as well as anti-tumor immunization. The product was approved for the treatment of malignant ascites, which is characterized by an increased accumulation of protein-containing fluid within the peritoneal cavity caused by intraperitoneal (ip) spread of cancer, especially epithelial tumors that express EpCAM. The condition manifests in late-stage cancer patients and leads to abdominal pain and swelling, dyspnea, nausea and vomiting, as well as malnutrition and anorexia.

Dr. Seimetz discussed the pivotal study of catumaxomab in EpCAM+ carcinomas, which was a two-arm, randomized 2:1, open-label study of 129 patients with malignant ascites due to ovarian cancer and an equal number with malignant ascites due to non-ovarian cancer. Patients received four ip infusions at 10, 20, 50 and 150 mg catumaxomab on day 0, 3, 7 and 10 of the study. The primary objective was puncture-free survival, defined as the time to either next therapeutic puncture to drain fluid or time to death, whichever occurred first. Catumaxomab was found to significantly prolong puncture free survival, with a median time of 46 days for patients who received drug compared to 11 days for control patients (p < 0.0001), and significantly prolong time to first puncture, with a median time of 77 days for patients who received drug compared to 13 days for control patients (p < 0.0001). Overall survival showed a positive trend for the catumaxomab group and was significantly prolonged in patients with gastric cancer (71 versus 44 days; p = 0.0313). Most adverse events, e.g., pyrexia, nausea, vomiting, abdominal pain, appeared to be triggered by cytokine release as part of the mode of action or method of administration, were limited in time and intensity, and were manageable with medication.

The marketing application for catumaxomab was submitted to the European Medicines Agency in December 2007 and approved by the European Commission on April 20, 2009. Dr. Seimetz remarked that key factors for successful drug development and approval included early and continuous involvement of regulatory agencies, awareness of the complexity of a centralized procedure application, and implementation of appropriate infrastructure and dedicated teams as early as possible.

Andrew Nesbitt (UCB Celltech) provided an overview of the experimental differences between certolizumab pegol, a PEGylated, humanized Fab’, and other TNF-blocking biological therapeutics, which include full-size mAbs (infliximab, adalimumab, golimumab), and a fusion protein (etanercept). He emphasized the structural differences between certolizumab pegol (CZP) and the other agents, e.g., CZP is PEGylated,
univalent and does not have an Fc, whereas the other agents are bivalent and have an active isotype Fc.

Dr. Nesbitt explained that PEGylated molecules have different characteristics compared to non-PEGylated ones because PEG is heavily hydrated, leading to a 3–5x increase in hydrodynamic volume, which affects tissue distribution. PEGylated molecules tend not to diffuse into normal tissue, but will diffuse more readily into the more permeable inflamed tissues. This effect was observed in a study of the distribution of certolizumab pegol, adalimumab and infliximab in the inflamed paws of mice with collagen-induced arthritis, in which CZP penetrated tissue to a greater degree and for a prolonged duration compared to adalimumab and infliximab. Because CZP is monovalent, the molecule does not form large immune complexes with TNF. CZP does not have an Fc portion and so does not recruit secondary effector functions such as complement-dependent cytotoxicity (CDC) or antibody-dependent cell cytotoxicity (ADCC). In addition, CZP blocks LPS induced cytokine production in vitro, resulting in a unique profile of gene expression that is unlike other TNF-blockers.

CZP was approved for Crohn disease in Switzerland in September 2007 and in the US in April 2008. In clinical studies of patients with Crohn disease, one notable difference between CZP and adalimumab was the low incidence of injection site reactions in patients who received CZP. Dr. Nesbitt suggested several possible explanations for this phenomenon, e.g., formulation, Fc effects on mast cells, PEG effects on mast cells, and potential analgesic properties of PEG. Dr. Nesbitt also discussed the pivotal studies of CZP in rheumatoid arthritis (RA); in 2009, the product was approved in the EU, US, Canada for treatment of RA.

Brendan Classon (Regeneron Pharmaceuticals) discussed rilonacept, which is an approved biologic for blockade of excessive IL-1 cytokine activity in auto-inflammatory diseases. The product is a cytokine trap comprising the constant region of human IgG and the extracellular domains of two cytokine receptor components. Second generation versions are single-chain homodimers that demonstrate improved expression and purification at scales up to 10,000 liters. Rilonacept is a potent agonist of IL-1β activity and is approved in the EU and US for treatment of cryopyrin-associated periodic syndromes. Two other biologics (canakinumab and anakinra) are approved for CAPS.

Regeneron is currently expanding the potential therapeutic utility of rilonacept into other clinical indications, including gouty arthritis. In a rodent acute gout model, inflammatory response was attenuated by antagonizing IL-1 signaling. A Phase 2 study of the product as a treatment in gouty arthritis has been completed. Rilonacept was dosed at 160 mg subcutaneously weekly for 16 weeks and results were compared to those of placebo. At week 12, the mean number of flares per patient was 0.79 and 0.15 for those administered placebo or rilonacept, respectively (p = 0.0011); at week 16, the mean number of flares per patient was 0.93 and 0.22 for those administered placebo or rilonacept, respectively (p = 0.0036).

A number of observations suggest that the quantitation of IL-1β rilonacept complex levels may prove useful in identifying IL-1β driven diseases that are more responsive to IL-1 inhibition in the clinical setting. For example, following rilonacept dosing, normal healthy volunteers do not exhibit detectable levels of complex, RA patients have slightly elevated levels, gout patients have approximately 2-fold elevated levels compared to RA patients, and CAPS patients have an average of 2-fold higher complex levels compared to gout patients.

Dr. Classon also discussed Regeneron’s VelocImmune technology. This technology generates human therapeutic antibodies from proprietary genetically-modified mice. After immunization with an antigen, antibody candidates are selected by conventional hybridoma methods and B cell sorting technology. The output is GMP-grade CHO cell clones expressing full length human antibodies at 5–10 g/L. There are currently five VelocImmune-derived antibodies in clinical development.

Jacqueline Benson (Centocor) discussed the discovery and mechanism of action of the anti-IL12/23p40 antibody ustekinumab. For context, she first provided background on the four categories of CD4 positive T helper (Th) cells, which are Th1, Th2, Th17 and T regulatory (Treg) cells. Th1 and Th2 have been described in the immunology literature for many years, and are characterized by the cytokines they produce. Th1 produce interferon gamma and TNFα, while Th2 are characterized by production of IL4. Th17 cells have been recently described, and are characterized by production of IL17A, IL17F, IL22 and TNFα. Treg cells, previously referred to as T suppressor cells or Th3 cells, regulate immune responses and can be associated with the production of IL10 and TGFβ.

Ustekinumab binds to IL12 and IL23, which are important in the Th1 and Th17 lineages. IL12 and IL23 are produced by activated antigen presenting cells such as macrophage and dendritic cells. Th1 T cells differentiate in the presence of IL12, while a Th17 T cell response is elicited by a combination of cytokines such as IL23, IL1β, IL6 and TGFβ. In combination, Th1 and Th17 T cells and their cytokines are associated with a broad range of autoimmune disease pathologies.

Ustekinumab was discovered in the laboratories of Centocor in collaboration with GenPharm, which was later acquired by Medarex. The antibody was derived from an early version of human immunoglobulin transgenic mice, the minilocus human immunoglobulin transgenic mice, first described in 1996. Hybridoma cultures were generated from immunized GenPharm mice and IL-12 neutralizing monoclonal antibodies were selected. Heavy and light chains were cloned, and standard technology was used to generate the original cell line. However, cell line changes were later made to support large scale manufacturing. No changes were made in the antibody sequence during the process.

Ustekinumab is approved for psoriasis, but Dr. Benson mentioned that Phase 2 studies in other indications (multiple sclerosis, Crohn disease, psoriatic arthritis) have been published. Dr. Benson explained that psoriasis is an inflammatory disease of the skin, and studies suggest that there is a genetic predisposition and an environmental component, but that the trigger initiating the disease is unknown. Activated dendritic cells are a likely source of IL-12 and IL-23 and T cells are important in the pathogenesis of the skin. Various cytokines and effector functions associated
with activated Th1 and Th17 cells contribute to pathogenesis, ultimately resulting in plaque formation.

IL-12 is a heterodimer comprising a p35 and p40 (molecular weight 40 kiloDalton), while IL-23 is a heterodimer comprising p19 and p40. Dr. Benson explained that ustekinumab binds to the common p40 subunit of human IL-12 and IL-23, but does not bind to mouse, rat, rabbit or dog IL-12 or IL-23. Ustekinumab forms a simple immune complex with IL-12 or IL-23 at the expected ratio of 2:1 ligand:antibody. The contact residues have been identified, and results will be published soon. There is currently no evidence that Fc effector function contributes to the mechanism of action of ustekinumab. Dr. Benson stated that ustekinumab’s mechanism of action is mediated through binding to the p40 subunit of IL-12 and IL-23 and preventing their interaction with the cell surface IL-12Rβ1 receptor.

Paul Parren (Genmab) provided an overview of the development of ofatumumab, which is a novel anti-CD20 human therapeutic antibody. The product was approved as a treatment for fludarabine- and alemtuzumab-refractory B cell chronic lymphocytic leukemia (B-CLL) in the US in 2009, and is undergoing regulatory review in the EU. Studies of ofatumumab as a treatment for cancer, RA and multiple sclerosis are ongoing. Genmab has licensed world-wide rights to GlaxoSmithKline.

As Dr. Parren explained, ofatumumab recognizes a unique, membrane-proximal epitope on CD20. The product is more active in vitro against low CD20 expressing tumors, has a slower ‘off-rate’, and has enhanced CDC and ADCC compared to rituximab. The epitope of ofatumumab is contained in the small and large loop of CD20; the high avidity C1q binding site created by ofatumumab positions complement activation close to the cell surface and results in efficient CDC. Ofatumumab-opsonized cells bind C1q with higher avidity, and C1q co-localizes more strongly with ofatumumab, compared to rituximab, which binds to the large loop of CD20. Ofatumumab also induces ADCC more potently compared to rituximab.12

Dr. Parren then described the pivotal study of ofatumumab as a treatment for B-CLL. The multicenter, open-label, single-arm study was conducted at sites in Europe and the US. Advanced-stage patients were either refractory to both fludarabine and alemtuzumab (double-refractory, n = 59) or refractory to fludarabine and inappropriate for alemtuzumab treatment due to bulky nodes (bulky fludarabine-refractory, n = 79). The overall response rate was 58% in the double-refractory group and 47% in the bulky fludarabine-refractory group. Responses were observed in the 17p del and 11q del subgroups. Response correlated with significantly improved survival outcomes, and ofatumumab was well-tolerated, with no unexpected adverse events.

Dr. Parren remarked on the rapid development of ofatumumab. The HuMab was selected on March 21, 2002, in vivo proof of concept was achieved in mid-2003, a Phase 1/2 study in B-CLL was initiated in 2004. A Phase 3 study in B-CLL was initiated in 2006, with results presented at the American Society of Hematology annual meeting in December 2008. Marketing applications were filed in the US and EU on January 31, 2009 and February 5, 2009, respectively. FDA priority review was granted on March 30, 2009 and the product was approved for marketing in the US as a treatment for fludarabine and alemtuzumab refractory B-CLL on October 26, 2009. The product may be studied alone or in combination with other drugs as a treatment for alternate indications such as lymphoma, arthritis or multiple sclerosis in the future.

Christian Behrens (LFB Biotechnologies) discussed two recombinant mAbs with enhanced ADCC, LFB-R603 and LFB-R593, which are both in clinical trials. He first described the LFB group, which is a company owned by the French state. LFB has a public health mission to develop, manufacture and provide hospitals with products for the treatment of severe diseases, some of which can be very rare. The company’s portfolio includes marketed plasma-derived medicinal products and biotechnology-derived proteins in development, e.g., antibodies and other proteins, and it has biomanufacturing capacity in cell culture (MAbgène, France) and transgenic animals (GTC Biotherapeutics, US).

Dr. Behrens then discussed LFB’s EMABling® platform, which includes a host cell line derived from YB2/0 (rat myeloma) for the production of antibodies with low fucose content, high affinity for FcγRIIIa, and high ADCC activity.13

Dr. Behrens then presented preclinical data for the chimeric anti-CD20 antibody LFB-R603. At a 30 mg/kg dose, the antibody delayed growth of RL tumor cells in SCID CB17 mice at approximately the same rate as 30 mg/kg rituxan; however, at 100 mg/kg, LFB-R603 appeared to eliminate growth of the tumor cells for over 40 days, whereas at 40 days tumor growth was apparent in mice that received 100 mg/kg rituxan. LFB-R603 also induced B cell depletion in cynomolgus macaques more efficiently compared to rituximab; administration of 0.06 mg/kg LFB-R603 resulted in 50% depletion at day 4, whereas 0.3 mg/kg rituxan was required to achieve the same level of depletion. A Phase 1 study of LFB-R603 as a treatment for B-CLL was started in the fourth quarter of 2008, and a Phase 2 study is planned for 2010.

Dr. Behrens also discussed the anti-Rhesus (Rh) D antibodies LFB-R297 and LFB-R593, which are candidate treatments for hemolytic disease of the newborn (HDN). This disease develops when there is an Rh incompatibility between a sensitized mother (Rh negative) and the fetus (Rh positive). Antibodies from the mother cross the placenta and lyse red blood cells (RBCs) of the fetus, resulting in mild to severe anemia. Polyclonal anti-RhD antibody is available, however, limited supply is anticipated and production requires immunization of healthy volunteers. Initial experiments were performed with LFB-R297. This human anti-RhD IgG1 antibody cleared RhD+ RBCs as quickly as a polyclonal anti-RhD preparation (Rhophylac®) in an open label clinical proof of concept study in male volunteers.14

Dr. Behrens explained that LFB then prepared a new clone, LFB-R593, for further studies. LFB-R593 binds to nearly all genetic variants of the RhD antigen and has a high affinity for FcγRIIIa. The antibody induces high ADCC activity against RBCs and strong RBC phagocytosis by macrophages. LFB-R593 was well-tolerated in a Phase 1 study that evaluated the safety and pharmacokinetics (PK) of the antibody in RhD negative healthy volunteers. A Phase 2 study was initiated in August 2009 to evaluate pharmacodynamics (PD) and the effects of various doses of...
LFB-R593 in RhD negative healthy volunteers challenged with RhD positive RBCs.

Stephen Demarest (Biogen Idec) presented results of Biogen Idec’s research directed toward discovery of novel anti-insulin like growth factor 1 receptor (IGF-1R) antibodies, with an emphasis on the mechanism of ligand blocking for various anti-IGF-1R antibodies and selection of BIIB022 as a clinical candidate. Dr. Demarest first described aspects of IGF biology, which is ancestrally related to insulin biology, e.g., ligands and receptors are homologous. IGF-1 levels and signalling have an effect on glucose levels and glucose uptake, and the ligand is important for cell growth and transformation, as well as physiology. IGF-2 activity is primarily attributed to fetal development.

Dr. Demarest explained that the IGF-1 receptor has approximately 50% identity and 70% homology to the insulin receptor. IGF-1R comprises approximately 1,370 amino acids, with 903 amino acids comprising the extracellular domain. The receptor is a constitutive homodimer, with the ligand binding pocket located in the L1/CRR/FnIII-2 region. Binding of the ligand induces a conformational change that results in activation of a tyrosine kinase domain in the intracellular portion. The receptor is expressed on most natural somatic cells, and downstream signaling leads to cell growth, transformation and cell survival. Elevated levels of IGF-1R, IGF-1 or IGF-2 are associated with many tumor types. The activity of IGF-1R promotes tumor cell survival, growth, transformation and metastasis.

The ability of inhibitory IGF-1R antibodies to slow tumor growth was known as early as the mid-1980s, and commercial discovery efforts have been ongoing since the late 1990s. Starting in 2004, Biogen Idec created a panel of mAbs from mice and a human phage library that was screened for inhibition of IGF-1R and IGF-2 binding. Dr. Demarest presented data on biochemical aspects of ligand/IGF-1R interactions and blocking of the interactions, and remarked that their data showed IGF-1R inhibition mechanisms to have greater variation and complexity than they expected. He noted that they found allosteric blockers to IGF-1 (mAb BIIB1), IGF-2 (mAb BIIB2), as well as allosteric blockers of IGF-1 and -2 (BIIB3 and BIIB5). They also found competitive blocker BIIB4, which bound IGF-1R at an epitope that overlapped with the ligand binding site. Dr. Demarest then described various experiments designed to assess the nature of IGF-1 blocking by the various inhibitory mAbs. A library of 64 IGF-1R mutants was used to map the epitopes of the mAbs, and most were found to bind the cysteine-rich and L2 domains, although one allosteric IGF-1 and IGF-2 blocker, BIIB5, bound an epitope on the FnIII-1 domain’s outer surface.

Biogen Idec selected BIIB5 (renamed BIIB022), which was identified as an allosteric inhibitor, for clinical development. The mAb is an aglycosylated IgG4 with a mutation in the hinge to eliminate half-antibody formation and swapping. It has an effector-less backbone to eliminate potential Fc mediated toxicity to normal tissue. In vitro data indicated the mAb reduced IGF-1 and -2 mediated tumor cell growth and signalling, blocked IGF-1 and -2 binding to IGF-1R (Fab affinity = 1.3 nM). BIIB022 maximally reduced in vivo tumor xenograft growth in several tumor models. The mAb downregulates IGF-1R and has no cross-reactivity towards the insulin receptor. BIIB022 has shown acceptable tolerability and PK in non-human primates. Safety data from a Phase 1 study was favorable. Dr. Demarest indicated that nine companies now have mAbs in clinical trials, including Biogen Idec with BIIB022 beginning multiple Phase 2 trials, and that it will be interesting to see how the different mAbs perform considering that each likely has a somewhat different mechanism of action.

Dale Ludwig (ImClone Systems, a wholly-owned subsidiary of Eli Lilly and Company) discussed ImClone’s experience in developing an anti-IGF-1R therapeutic antibody. He first provided background on IGF-1R as an important pathway in cancer. He noted that expression is associated with resistance to cytotoxic therapy, which suggests that anti-IGF-1R therapies may be effective when used in combination with cytotoxic drugs, even in a refractory setting. Dr. Ludwig explained that IGF-1R consists of two subunits connected by disulfide bonds. The receptor is a preformed dimer and dimerization is not an obligate step in receptor activation, which is mediated by a conformational change that occurs upon ligand binding. The kinase domain is highly similar to insulin receptor (IR), and it has proven difficult to generate IGF-1R selective small molecules. There are no known activating mutations and no evidence of resistant forms of the receptor. Low levels of the receptor are sufficient for mitogenic or transforming activity. Interestingly, due to its close structural homology to IR, IGF-1R can form hybrid receptors with IR; these hybrids act like IGF receptors.

Dr. Ludwig briefly discussed eight anti-IGF-1R mAbs currently in clinical studies. These are CP-751,871 (human IgG2; Pfizer), IMC-A12 (human IgG1; ImClone), R1507 (human IgG1; Hoffmann LaRoche); AMG479 (human; Amgen), SCH717454 (human; Schering-Plough), AVE-1642 (humanized; Sanofi-Aventis), MK-0646 (humanized; Merck/Pierre Fabre), BIIB022 (human IgG4; Biogen Idec). These antibodies differ in their affinity, IgG subclass, binding epitope, circulating half-life, and receptor internalization activity, and so may show differential activity in clinical studies.

Dr. Ludwig then focused on development of IMC-A12, which was derived from a human Fab phage display library that was screened for selective binding to IGF-1R and its ability to block IGF-1 binding to receptor. The candidate is a human IgG1 that competitively inhibits binding of radiolabeled insulin from binding to IR, and it has proven difficult to generate IGF-1R selective small molecules. There are no known activating mutations and no evidence of resistant forms of the receptor. Low levels of the receptor are sufficient for mitogenic or transforming activity. Interestingly, due to its close structural homology to IR, IGF-1R can form hybrid receptors with IR; these hybrids act like IGF receptors.

Dr. Ludwig then focused on development of IMC-A12, which was derived from a human Fab phage display library that was screened for selective binding to IGF-1R and its ability to block IGF-1 binding to receptor. The candidate is a human IgG1 that is capable of eliciting ADCC and possesses a high affinity for human IGF-1R (Kd = 0.04 nM). IMC-A12 selectively binds to IGF-1R and not to IR, and competitively inhibits binding of radiolabeled IGF-1 to IGF-1R. The antibody does not competitively inhibit radiolabeled insulin from binding to IR.

Dr. Ludwig explained that, as determined in preclinical models, IMC-A12 acts by three mechanisms: (1) direct blocking of ligand binding to surface-associated receptor, (2) effecting surface receptor down-modulation through receptor internalization and degradation, (3) eliciting anti-tumor immune effector functions, i.e., ADCC and CDC. As a single agent, IMC-A12 was shown to reduce tumor volume in human xenograft models pancreatic (BxPC-3), colon (Colo205), renal (Caki1), prostate (LuCaP35), myeloma (RPMM-8226) and lung (NCI-H226) cancer. The candidate also demonstrated single agent activity in a
panel of pediatric tumor models. An additive effect was observed when IMC-A12 was tested in combination with CPT-11 in colon tumor xenografts (Colo205, HT-29). In combination with radioactivity, IMC-A12 produced a profound increase in radiation therapy-induced double-strand DNA damage and apoptosis. Also, addition of IMC-A12 to hormone ablation therapy delays conversion to androgen-independent tumor growth in a LuCaP35v xenograft model. Dr. Ludwig noted that downstream effector molecules such as K-Ras, B-Raf, PI3K and PTEN may represent markers of resistance. He showed data that indicated that a change in PTEN status did not alter the responsiveness of cells to anti-IGF-1R therapy.

In Phase 1 studies, IMC-A12 has been studied at doses ranging from 3 to 20 mg/kg on a once weekly or once every 2 weeks schedule in adult patients with a variety of tumors. A pediatric Phase 1 study was also done (6 and 9 mg/kg administered once weekly in 28 day cycles). The Phase 2 program for IMC-A12 includes 10 studies sponsored by ImClone that will assess the safety and efficacy of the candidate as a single agent or in combination with other cancer drugs in patients with prostate, colorectal, head and neck, breast, sarcoma, neuroendocrine, lung and hepatocellular cancer. In addition, the candidate is in numerous studies sponsored by the National Cancer Institute’s Cancer Therapy Evaluation Program.

Patrick Mueller (Novartis Institutes for BioMedical Research) discussed safety assessment and dose selection for first-in-human clinical trials with immunomodulatory mAbs. Dr. Mueller first provided an overview of the modes of action (MOA) of immunomodulatory mAbs used for the indications oncology and immunosuppression. For oncology indications, the MOA is via indirect mechanisms, i.e., immunostimulation against tumor cells, mediated by T cells or natural killer cells (NK), including blockage of inhibitory receptors such as CTLA-4 and activation of co-stimulatory receptors such as CD28. For indications of autoimmune disease and suppressing transplant rejection, mAb MOA include suppression of immune cells by prevention of homing to lymphoid organs and inflammatory sites, induction of anergy (peripheral lymphocyte tolerance) or deletion, and blockade of soluble targets such as cytokines by mAb-mediated sequestration.

Dr. Mueller then discussed qualification of preclinical safety models. In general, therapeutic mAbs need to show the same pharmacological activity (potency) in preclinical in vivo safety models as expected in humans. Use of only one species is generally acceptable in cases where only one relevant model can be identified. In the majority of cases the species will be the cynomolgus monkey. He emphasized that there should be no animal/human safety-relevant differences in target distribution or turnover, pharmacological potency of antibody, Fc effector function of antibody (including cross-linking potential), shape of the in vitro/in vivo dose response curve, or the expected duration of PD effect.

On the topic of safety relevant differences between non-human primates (NHP) and human immune systems, Dr. Mueller focused on key points regarding T cell activation and Fc receptor distribution. He mentioned that human T cells give much stronger proliferative responses upon activation compared to T cells from other species, including chimpanzee, because human ancestors lost T cell expression of sialic acid binding Ig-like lectins (SIGLECS) during late evolutionary stages. CD33-related SIGLECS are inhibitory signaling molecules expressed on most immune cells and downregulate cellular activation pathways. Human T cells express few to none of these molecules, although they are expressed in chimpanzees and other NHPs such as cynomolgus macaques. Regarding Fc receptor distribution, Dr. Mueller noted that the human FcyRIII (CD16) is specific for IgG1 and IgG3; cross-linking of Fc receptors by antibody-opsonized antigen complexes initiates cellular immune responses. Humans have CD16a, found on monocyte subpopulations, macrophages and NK cells and CD16b, found on neutrophils, eosinophils and mast cells when activated by IFNγ. In contrast, baboon, rhesus and cynomolgus monkeys have CD16a on monocytes and NK cells, but these animals have no gene encoding CD16b.

Dr. Mueller then discussed key parameters that should be conserved between humans and species selected for the preclinical safety model. The Fc receptor binding properties of therapeutic mAbs and the amino acid sequence of the target epitope should be as similar as possible. The target affinity of the antibody should have a comparable range and the target expression level should be comparable as well. Dr. Mueller stressed that the most important point was that the signal transduction upon target binding of the antibody should be the same. Finally, he noted that the potency of the mAb, i.e., magnitude of the PD effect should be as similar as possible.

On the topic of preclinical safety study design, Dr. Mueller discussed dose selection, PK/PD, immunogenicity and additional safety endpoints. He first mentioned that finding the maximum tolerated dose is sometimes not feasible because of the low toxicity of antibodies, and protein overload is observed if very high doses (g/kg) are administered. Doses should be high enough to determine the maximum pharmacological response, even if target affinity in NHP is lower than in humans, and ensure complete target saturation. Doses leading to multiples (greater than 10x) of the clinical exposure should be included in preclinical studies. Dr. Mueller noted that determination of the ‘no observed adverse effects level’ (NOAEL) is not required for human entry in populations with late stage cancer (as per ICH S9) and the low dose can be the same as the intended starting clinical dose in the case of an expected U- or bell shaped dose response curve.

Dr. Mueller then proceeded to discuss PK/PD in preclinical safety studies. He commented that mAbs sometimes have shorter half-lives in preclinical models compared to the expected half-lives in humans, and so higher dosing frequency or higher doses may be needed to compensate. A recovery period should be 4–5 terminal half-lives in length followed by a 1 month true exposure-free period. A PD marker always should be used to confirm the pharmacological activity of the drug. Determination of immunogenicity, i.e., the generation of anti-drug antibodies (ADA), is essential for interpretation of PK/PD and toxicity and should be determined on a regular basis. Neutralizing ADA diminish pharmacological/toxicological action of the drug. Regarding additional safety endpoints, Dr. Mueller discussed the importance of the measurement of potential cytokine release, e.g., 2–6 hours.
Dr. Mueller than compared two approaches for selection of doses for first-in-human (FIH) studies. The classical NOAEL approach is based on toxicology and focuses on determination of a high dose. The ‘minimum anticipated biological effect’ (MABEL) approach is based on pharmacology and focuses on determination of the lowest dose leading to any PD effect. A minimal PD effect can be defined as a biological effect or some meaningful surrogate such as either receptor occupancy, ligand suppression, PK inflexion or cell deletion. The maximum recommended starting dose (MRSD) is calculated based on data from both NOAEL and MABEL.22

Dr. Mueller further discussed the principle steps in the determination of MABEL. All available in vitro, in vivo and modeling data should be incorporated, including in vitro pharmacology studies with target cells from humans and relevant species, and concentration-effect data from in vitro and in vivo studies. The data should be integrated into the PK/PD model, if feasible, in order to predict pharmacological response in humans at multiple dose levels, which should be updated during FIH dose escalation. Dr. Mueller stressed that the animal-human differences in affinity/potency and exposure/distribution, as well as the anticipated duration of effects (acute vs. chronic PD effects) should be accounted for in the determination and interpretation of MABEL.

Dr. Mueller also discussed the receptor occupancy (RO) model, which he described as the simplest PK/PD model. RO can be calculated from $K_p$, target expression level, and total antibody concentration. He noted that he was focusing on a ‘worst case’ scenario immediately post single iv dosing, and that there are confounding factors such as bivalent binding, distribution/excretion from the vascular space, target turnover rate or duration of effect that could change the results. An initial dose for FIH should provide a maximum 10% initial RO.

A limitation of the MABEL and RO models is that they are not predictive of anaphylactic or anaphylactoid reactions. The MABEL model might provide a sufficiently low dose to avoid cytokine storms. Dr. Mueller compared data for cytokine induction by TGN1412 in cynomolgus monkeys and human. He noted that the most striking differences were the massive TNF$\alpha$ (1670x) and IFN$\gamma$ (704x) induction in humans administered 0.1 mg/kg TGN1412 compared to the virtual absence of release in cynomolgus monkeys administered a 500x higher dose (50 mg/kg). Borderline induction (18x) of IL5 and IL6 was observed in the monkeys, which now needs to be considered as a safety-relevant signal indicating cytokine release.

Initial clinical doses calculated by MABEL and RO models are low and these doses must be escalated to achieve a pharmacologically active systemic exposure to the therapeutic mAb. For dose escalation in FIH studies, Dr. Mueller suggested that a split dose approach, which is used for mAbs with very steep dose/response curves expected in humans, may be an option for higher risk mAbs. In this scenario, 10% of the intended dose is initially administered, followed by 40% a few hours later, then the remaining 50% after an additional few hours.

Dr. Nathalie Corvaïa (Centre d’Immunologie Pierre Fabre) presented her company’s strategy for the discovery and development of new anticancer monoclonal antibodies. Two complementary approaches are used. The first approach is targeted and target-driven, i.e., starting with knowledge of targets such as membrane receptors, circulating ligands including insulin-like growth factor-1 receptor (IGF-1R), cMet, CD151 and other undisclosed targets. This approach focuses on validated targets. The second approach, which provides access to innovative targets, is focused on function, and involves immunization with cells or tissues, functional screens, followed by selection of antibody and target identification. Targets found using this approach include JAM-A, TSN-1 and other undisclosed targets. She described the strategy as one that balanced innovation with risk, taking into account factors such as value, safety, science, competition, time to clinic and intellectual property.

CIPF’s targeted strategy was also used to develop candidates targeting the c-Met receptor, which is a tyrosine kinase receptor. The ligand of this receptor is HGF/SF, and cMet is overexpressed in tumor tissue, where it has anti-apoptotic activity and is involved in transformation/mitogenesis and invasion/metastasis. Dr. Corvaïa then explained the method used to generate anti-c-Met mAbs which involved immunization of mice to yield plasmocytes that were fused with Sp2/0-Ag14 myeloma cells. Cells producing mAbs were then accessed by ELISA and FACS; of 39,552 wells tested, 98 double positive hybridomas were selected. These were accessed for proliferation and phosphorylation, and 19 antagonist hybridomas were selected. These were further assessed for dimerization and in vivo activity; 12 were selected. Two of these (11E1 and 224G11) were humanized, and one, h224G11 was selected for development based on in vivo data, including assessment in the U87-MG glioblastoma model, a ligand dependent model, NCI-H441, a c-Met overexpressed tumor model and EBC-1 lung cancer models and H464T and MKN-45 gastric cancer models, three ligand-independent tumor models. Marked anti-tumor activities for 224G11 alone and in combination with cytotoxic drugs, e.g., navelbine, temozolomide, was observed.

Dr. Nathalie Corvaïa (Centre d’Immunologie Pierre Fabre)
CIPF’s functional strategy has been used to identify JAM-A as a potential anti-tumor target. The literature describes JAM-A as a constituent of the tight junctions of epithelium and endothelial cells that has interaction with LFA-1 and FGFβ and is a receptor for reovirus, but there is very little information on the role of JAM-A in oncology. Dr. Corvaïa explained that CIPF has produced an anti-JAM-A mAb, and studies have indicated that the mAb, 6F4, inhibits tumor growth in vivo through inhibition of cell proliferation and downregulation or shedding of the target. The mAb has been humanized and pretoxicological studies in monkeys are ongoing.

The function strategy has also been used to identify tetraspanins (TSN) as an emerging class of targets in oncology. TSN-1 is a 241 amino acid protein that is involved in cell-cell interactions, adhesion and migration. The target is overexpressed on a restricted panel of tumor cells, e.g., pancreas, colon, when compared to normal cells, e.g., leukocytes, fibroblasts and endothelial cells. CIPF has produced an anti-TSN-1 mAb, 2E11, that inhibits tumor growth in vivo. In summary, Dr. Corvaïa noted that she believes that a strategy of mixing targeted approaches and functional ones will bring new promising therapies in the near future.

Daniel Pereira (Pharma Research Toronto, Hoffmann LaRoche) discussed the discovery and development of antibodies against new targets. Dr. Pereira initially outlined the classical ‘target first’ approach to cancer antibody discovery, which includes purchase or expression of recombinant target, validation of the target, establishment of a screening assay, selection of hits from the assay, and finally testing of hits for anti-tumor efficacy. He then discussed the drawbacks, such as (1) the conformation and epitopes of the recombinant antigen may not accurately reproduce those of the tumor antigen; (2) target validation does not always ensure drugability; (3) bias in assay development may result in a screen for inappropriate antibody modes of action. Hits with mediocre efficacy may result from inappropriate choices made during the process.

Dr. Pereira then presented the ‘FunctionFIRST’ approach, which has been used at Pharma Research Toronto since 2000. The approach begins with immunization of mice with human tumor tissue, so that relevant target conformation and epitopes are used. A general screening assay that is inclusive and agnostic is then established and hits are selected. These hits are tested for anti-tumor efficacy, and potent efficacy is required for target identification and drugability confirmation. Finally, targets are identified and assessed; undesired targets are abandoned and novel targets are pursued. Using this approach, time to target identification is in the 2 week to 2 month range.

He then provided further details of the process. Frozen (not fixed) human tissues are used for the immunization. Hybridomas are prepared and the supernatants are screened in a cell-based primary functional assay. The resulting hits are the pipeline of functional antibodies; selected antibodies are purified and scaled up. These antibodies are then screened in a tumor cell-based secondary functional assay that includes various tumor types. In vivo testing of the selected antibodies then occurs, after which a number of activities occur in parallel, including tests of cross-reactivity, target identification, target expression and mechanism of action. Finally, antibody engineering and cell line development occurs, and the optimized candidate is ready for clinical development. In terms of productivity, approximately $1 \times 10^3$ hybridomas were screened to produce over 600 antibodies that are cytotoxic to cancer but not normal cells. These yielded approximately 100 antibodies that showed $>40\%$ tumor growth inhibition in xenografts, and more than 10 targets have been identified. Over 70 immunization campaigns have been done using a variety of tumor types, e.g., colon, lung, ovarian, breast, liver, pancreas, prostate, stomach, kidney, head and neck.

The target classes recognized by antibodies discovered using the FunctionFIRST platform include cell adhesion molecules, integrins, immunomodulators, signaling receptors, multi-span receptors and novel receptors.

Pharma Research Toronto is studying an anti-CD44 antibody discovered using the FunctionFIRST approach. The molecule is potentially a first-in-class antibody; it demonstrates anti-tumor efficacy at 2 mg/kg doses in a breast cancer xenograft model and is a potential cancer stem cell inhibitor. The MOA and biomarkers are under investigation; no dose-limiting toxicity has been observed in cynomolgus monkeys. Dr. Pereira then discussed a second program involving the development of anti-CD9 antibodies discovered using FunctionFIRST. CD9 is differentially expressed in AML compared to normal hematopoietic cancer stem cells. The anti-CD9 antibody inhibits outgrowth and secondary transplantation of cancer stem cells in a patient-derived AML/NOD-SCID mouse model. In addition, the antibody is effective in several solid tumor models. The MOA and biomarkers are under investigation.

Lili Huang (Dyax Corporation) presented data on highly selective matrix metalloprotease (MMP) inhibitors for oncology and inflammation. MMPs are zinc dependent enzymes involved in cleavage of extracellular matrices. They play roles in normal processes such as bone remodeling and organogenesis, but also in pathological conditions including cancer and inflammation. MMPs are well-known targets, but dose-limiting toxicities have blocked development of drugs that target MMPs in a non-specific manner. This creates an opportunity for a selective antibody-based MMP inhibitor. MMPs share conserved domain structures, including a catalytic domain containing the zinc binding site, and can be classified into secreted and membrane-type MMPs. MMP-9 (gelatinase B) and MMP-12 (macrophage elastase) are secreted MMPs. MMP-14 (MT1-MMP) is a membrane-type MMP.

Dr. Huang explained that Dyax is developing antibodies to three MMPs (MMP-9, -12 and -14). To identify antibody inhibitors to specific MMPs, Dyax used phage display technology utilizing Fab libraries. Screening assays were used to select molecules that bound the catalytic domain, and the Fabs were further screened by ELISA and enzyme inhibition assays. Testing for specificity and cross-reactivity was then performed. Based on the data, lead candidates were selected and reformatted into IgGs. Using this method, DX-2712, an MMP-12 inhibitor, was developed. MMP-12 is implicated in a range of inflammatory diseases, including asthma, chronic obstructive pulmonary disease/
emphysema, rheumatoid arthritis, atherosclerosis and inflammatory bowel disease. DX-2712 is a highly potent and selective antibody inhibitor of MMP-12. It is a competitive inhibitor with IC₅₀ in the low single digit nanomolar range. MMP-9 plays a role in inflammation, as well as tumor invasion and metastasis. Her final topic was DyaX’s most advanced MMP inhibitor, DX-2400, which is a human anti-MMP-14 IgG1 mAb. MMP-14 is implicated in tumor progression and plays a role in invasion, migration, vascularization, metastasis and radioresistance of tumors. DX-2400 is a competitive inhibitor of human, mouse, rat, cynomolgus and rabbit MMP-14 with IC₅₀ values in the sub-nanomolar to single digit nanomolar range. The candidate has been evaluated in a number of cell-based assays and in vivo tumor models. As monotherapy, DX-2400 inhibited tumor progression, metastasis and angiogenesis in xenografts of breast and other cancers; the effect was dose-dependent. The candidate was also tested in combination with bevacizumab; effects of the combination were greater than the effects of either single agent. A CHO cell line that produces DX-2400 at 3 g/L has been developed.

Ted Kwaks (Crucell) presented Crucell’s work on an antibody therapy against seasonal and pandemic flu, with an emphasis on discussion of antibody recognition of a highly conserved influenza virus envelope. He first discussed the need for new therapeutics for seasonal and pandemic influenza, and then presented the selection strategy Crucell used to find heterosubtypic neutralizing antibodies against H5N1 and H1N1. The general strategy was to find antibodies with broad activity, e.g., against both H1 and H5, that were targeted to a conserved epitope, which would limit the risk of escape mutants. Specifically, Crucell started with B cell subsets isolated from H1 vaccinated donors, then went on to select H5 positive scFv-phages that were screened for H1 and H5 binding. Starting in February 2006, a 2 x 10⁹ IgM memory library provided 105 H5-specific phage antibodies that were screened. From this group, 68 H5 specific IgG were then selected, and additional screening provided 21 H5N1 neutralizing IgG. The in vivo efficacy screening of these was achieved by December 2006.

Throsby et al. published data on mAbs CR6261 and CR6323, which demonstrated heterotypic cross-clade neutralization of influenza subtypes. CR6261 neutralizes multiple strains that evolved during the last century; the IC₅₀ for CR6261 ranged from 3.53 to 3.69 mg/mL for strains A/Brisbane/59/2007, A/Solomon Island/VR-145, A/New Caledonia/6/2005 and A/PR/8/34, and was 2.79 and 8.89 mg/mL for strains A/WSN/33 and A/Hong Kong/201345/07, respectively. Crystal structures of Fab CR6261 with influenza HA indicated that three antibodies bound per HA trimer in their pre-fusion state. The antibody binds a conserved region in the stem section of HA involved in fusion of the virus in the endosome, and which is necessary for virus replication. Hydrophobic residues at the tip of the VH1-69 CDR2 homes in on the pocket of the stem region of HA; H1 and H9 clade viruses have similar pockets that differ from the pockets of H3 and H7 viruses.

Dr. Kwak noted that there is universal conservation of helix A of HA2 across influenza virus subtypes and the exceptional conservation of the CR6261 epitope is explained by its function in the fusion process. CR6261 inhibits the pH-induced conformational change of influenza HA that are required for fusion with endosome. CR6261 was shown to be protective in a murine lethal challenge study. Female Balb/c mice (10 per group) were administered CR6261 ip at doses in the range of 0.6 to 15 mg/kg at Day -1, with intranasal viral challenge of 25x LD₅₀ H1N1 strain A/WSN/33 or H5N1 strain A/VN/1194/04 occurring at Day 0. All animals that received the two highest doses (5 and 15 mg/kg) survived for the 21 day observation period in both studies.

Data from studies of the antibody as a therapeutic agent were also presented. Female Balb/c mice (10 per group) were challenged with intranasal doses of 25x LD₅₀ H1N1 strain A/WSN/33 or H5N1 strain A/VN/1194/04 at Day 0, and then administered either CR6261 or control antibody at 15 mg/kg on Day 1, 2 or 3. All the animals that received drug on any day survived challenge with the H5N1 strain. For those challenged with H1N1, 100% survival was observed in the group that received drug on Day 1. Koustaal et al. have shown that CR6261 administered as either a prophylactic or therapeutic protects against disease in Balb/c mice challenged with H5N1 A/HK/156/1997 virus.

CR6261 also prevents death in ferrets, which are the most clinically relevant animal model for human influenza. Ferrets administered 30 or 10 mg/kg at Day -1, then challenged on Day 0 with a dose of 5 log TCID₅₀ of H5N1 A/Indonesia/05/2005 virus survived for the 5 day observation period. Animals that received the same dose of virus at Day 0, and then were administered 30 mg/kg at 4 hours and at 1 day also survived for the 5 day observation period. Finally, Dr. Kwak noted that preparations for the CTM batch for an upcoming Phase 1 study are ongoing.

Michael Rudolf (Kenta Biotech) began his presentation by emphasizing the need for therapeutic antibodies targeting infectious diseases. He then provided an overview of Kenta Biotech’s development of human antibodies against infections acquired in hospital intensive care units (ICU), such as Staphylococcus aureus, Pseudomonas aeruginosa and Acinetobacter baumannii. Such antibodies may be combined with antibiotics, thereby adding a different mode of action, to potentially provide more rapid resolution of infection and inflammation, reduced mortality and shorter stays in the ICU.

Kenta uses an improved hybridoma technology (MabIgX) for production of human antibodies. The process involves initial isolation of peripheral blood lymphocytes (PBLs) from donors. The PBLs are stimulated and antibody-producing cells are subjected to several screens. The selected cells are fused to a proprietary myeloma cell through electrofusion. The total process takes approximately 2 weeks.
Dr. Rudolf presented data for panobacumab (KBPA101), a vaccine-induced human IgM composed of a human m-heavy chain, κ light chain and J-chain in a pentameric structure. The antibody is reactive with LPS serotype O11 of *Pseudomonas aeruginosa* and mediates complement-dependent phagocytosis at low concentration. The candidate was evaluated in an open, non-comparative, multicenter Phase 2a study of patients with hospital acquired pneumonia caused by *P. aeruginosa*. The primary endpoints were safety, tolerability and PK. A total of 13 patients were administered three doses of 1.2 mg/kg at days 1, 4, and 7, and an additional 5 patients received one 1.2 mg/kg dose. The treatment was well-tolerated and there were no systemic or local infusion reactions. The PK data, derived from patients who received three doses, followed a one compartment model; t1/2 was 77.8 hours. The 30 day survival rate was 100% for these patients. Of the patients who received one dose, the 30 day survival was 40% (2 of 5 patients). No immunogenicity has been observed.

Antonio Maschio (Edwards, Angell, Palmer & Dodge) provided an overview of the intellectual property landscape, and suggested that change was forthcoming. He noted that numerous pharmaceutical companies are facing expiration of patents for products that currently have large global markets, e.g., Pfizer’s atorvastatin calcium (Lipitor), GlaxoSmithKline’s fluticasone propionate and fluticasone salmeterol (Advair), AstraZeneca’s quiptiapine fumarate (Seroquel) and esomeprazole magnesium (Nexium), and Sanofi-Bristol-Myers Squibb’s product clopidogrel bisulfate (Plavix). IMS Health has projected total loss of sales at $140 billion, which is approximately 20% of the global pharmaceutical market. Pharmaceutical firms are preparing for the next decade by focusing on biologic products at least in part because generic competition is viewed as difficult by investors. The antibody market is expected to grow at a 14% compound annual growth rate during 2006–2012 while small molecules are expected to grow 0.6% in the same period. There have already been numerous acquisitions of biopharmaceutical firms by big pharmaceutical companies, e.g., Domantis and Corixia were acquired by GlaxoSmithKline, AstraZeneca acquired MedImmune and Cambridge Antibody Technology, and Bristol-Myers Squibb recently acquired Medarex.

Although antibodies hold promise for the pharmaceutical industry, Dr. Maschio further remarked that expiration of antibody patents should also be scrutinized, as a number of patents covering antibody engineering technologies have expired or will soon expire. He was careful to note that there are differences in the year of expiry for patents granted in the US and EU. For example, the Celltech/Boss patent EP0120694 and the Genentech/Cabilly patent EP0125023 covering antibody production from host cells expired in 2004. The Kauffman ‘stochastic’ patent (EP0590689), which is arguably the first case that covers phage display, the Winter I patent covering CDR grafting and the Winter II patent (EP0368684), as well as the PDL/Queen patent (EP0451216), have also expired in Europe.

Dr. Maschio suggested several types of molecules that might be included in future patents and strategies to improve patentability. The molecules include antibodies that have been modified to include human serum albumin, multispecific antibodies and those with altered Fc regions, as well as alternative framework and synthetic binding molecules. He noted that patentability of antibodies might depend on description of structural features, although mentioning just one chain or less than six CDRs, will lead to a sufficiency objection. Structure might be described by epitope specificity, although supporting data such as crystallization data would be useful to include in the patent. Antibodies resulting from a specific process of production, e.g., antibody obtained by immunization with an antigen, might be patentable if the antigen is novel and not obvious. In addition, antibodies that target epitopes might be patentable if the epitope is unknown. Dr. Maschio provided further information on the case of non-linear epitopes, when the target molecule should be defined in the claim, the disparate parts of the epitope and their positions are carefully defined in the context of the rest of the molecule, data showing that existing antibodies do not bind the epitope are included, and a way of preparing antibodies reactive with the epitope is disclosed. In the case of hybridoma, these must be deposited, commercially available or publically available and the claim must be limited to the deposited antibody unless techniques for making antibodies with the same specificity are described.

Rolf Werner (Boehringer-Ingelheim) discussed biopharmaceutical manufacturing trends for maximizing antibody yields. He first emphasized that most antibodies are made in mammalian cells, with antibody fragments and scaffolds produced in microbial cells. He noted that, overall, production from transgenic animals and plants would not play a major role in the future. Dr. Werner then discussed creative protein engineering that may provide antibodies, protein scaffolds, e.g., anticalins, ubiquitins, crystallins, DARPin, affibodies, aptamers, fynomers, tetranection and fusion proteins with properties that are enhanced compared to those of antibodies now on the market. Selection of the cell line for production depends on the properties of the molecules; the choices are usually mammalian cell, filamentous fungi, yeast systems or bacterial systems. He explained that once a candidate has been selected, it must be produced in a validated expression system that has a robust fermentation process and yields high titers. For production from *E. coli*, the refolding process must be high yield. For all systems, the downstream processing must be high yielding and lead to a high concentration liquid formulation suitable for patient-friendly application forms. Dr. Werner noted that the titer is important, but that run time, and thus specific productivity (mg/L/day), must be considered because this will determine how many runs can be made per year and the annual supply of product.

Dr. Werner explained that the standard platform for mAb purification involves an essentially generic process, but that the process needs to be fine-tuned for each candidate. The purification steps include affinity chromatography, acid treatment/depth filtration, cation and anion exchange chromatography, 20 nm nanofiltration, ultra- and dia-filtration, formulation, followed by fill and finish. A high throughput screening program is required for optimization of the downstream process for any given candidate. Dr. Werner also presented data to support the point that sequential multicolour chromatography (SMCC) has
advantages over use of a batch method. SMCC is less costly, has more capacity, uses less buffer, and is faster and more productive, as assessed by g/L/day output, compared to a batch process. Finally, Dr. Werner noted Boehringer Ingelheim’s current fermentation capacity for cell culture is 12 bioreactors of 15,000 L and 10 bioreactors each of 2,000 L, 400 L and 800 L; their capacity for microorganism fermentation is 2 bioreactors of 6,000 L and 3 bioreactors each of 300 L and 30 L.

Aziz Cayli (Cellca) presented an upstream technology and a philosophy devoted to manufacturability of biopharmaceuticals. He first explained that Cellca solves problems with scalability, productivity and time to market by using process tools to assess the inter-relation of the vector, cell, media and process required to produce any given product. Dr. Cayli discussed optimization of viability of CHO K1 cells, which resulted in consistent cell viabilities (range of approximately 40–50%) over 100 passages. Cellca uses a proprietary expression system derived from optimization of numerous genetic elements and proprietary cell culture media designed for optimal growth of the CHO K1 cells. They also reduced metabolic waste products such as ammonia, lactate, alanine and glycine that are produced during the fermentation process through optimization of the media and clone. Dr. Cayli presented data showing that the components and process are sufficiently balanced that pH did not drift from the target range of 7.0 +/- 0.15 during a 12 day, 5 L bioreactor run.

Cellca has also successfully scaled up antibody production from 5 L to 2,500 L. Dr. Cayli presented data showing cell growth was comparable, cell viability was similar and the product concentration was similar (approximately 2 g/L after 12 day run) for the two scales. He noted that Cellca’s simple process was particularly suitable for use with disposables, would provide cost efficient and fast manufacturing, and the current fed-batch process was consistent for production of antibody and non-antibody proteins. The time from cell line generation through bioreactor process development is approximately 7 months.

Wolfgang Noe (Biogen Idec) provided a status report on paradigm changes and technology gaps in the biopharmaceutical industry. He started by noting trends in production of marketed products from mammalian cells during the 1980s to 2010. In the early years of the biotechnology industry, marketed products included replacement factors and enzymes that were used in small doses so that low expression levels, e.g., 10–100 mg/L, were acceptable even for chronic treatments such as epoetin. In the late 1990s and early 2000s, antibodies and soluble receptors such as rituximab, infliximab and etanercept, which may be dosed at rates of 2–10 gram per patient per year, were approved, and 100–1,000 fold increases in titer were needed for the products to be viable on the market. In recent years, more blockbuster protein products have been approved, the number of protein-based candidates in clinical development has increased to over 200, and biosimilar products are now entering the market. These changes require greater facility flexibility so that large and small scale runs can be done to support different market needs, but many small scale runs need to be done to produce the large number of candidates in clinical study. He noted that the need for 12,000 L bioreactors is probably limited because the manufacturing trend is toward smaller scale runs.

Dr. Noe then discussed Biogen Idec’s analysis of unit operations, including upstream, downstream and formulation processes. The key areas noted as having high unmet need for development improvement included fed batch bioreactors (e.g., industry would benefit from better understanding of process impact on quality attributes at molecular level), capture (e.g., Protein A is acceptable if commercial product is being produced, but is expensive for use in production of clinical candidates; less expensive alternatives are needed), drug substance storage (e.g., need for improved freeze/thaw processes at large scale and better solid storage forms), drug product delivery (e.g., need to achieve 150–200 mg/mL concentrations for high dose strategies), and device design. He noted that Biogen Idec had surveyed 8 leading biotechnology companies about their views on trends in process development and manufacturing in May 2009. He summarized the key findings as trends toward facilities flexibility, high throughput development, with needs for better methods for protein characterization, better drug delivery systems and a rational clinical material strategy. Also noted was the continuous challenge of producing larger numbers of ‘post-antibody’ products (e.g., engineered proteins of all kinds).

Dr. Noe suggested that process productivity has improved dramatically due to use of better cell lines and optimized media and feeds. The current trend is toward 10 g/L and above for antibody fed-batch processes. Biogen Idec’s experience is that a high yield process is scalable to 1,000 L using disposables. Overall time from vial to harvest can be reduced from 45–50 days that is typical for a conventional cell culture scale-up strategy to 30 days using methods such as high cell density, large vial cell banks and perfusion technology for cell bank preparation.

Regarding analytical methods, Dr. Noe noted the need to utilize technology to evaluate conformational changes in molecules that might affect biological activity. He discussed use of hydrogen-deuterium exchange heat maps to examine differences between IFN-β-1a and a PEG-modified version, and use of 2D LC/MS for analysis of a bioreactor sample of IFN-β-1a. Finally, Dr. Noe emphasized the challenge of ‘syringe-ability’. Patients want convenient application devices, so the availability of prefilled syringes, other injecting devices or customized applicators is important for success on the market. Concentrated formulations are needed for these devices; however, proteins are viscous at high concentrations and so preparing drug for delivery in these devices can be a technical challenge.

Lothar Jacob (Merck KGaA) discussed improved protein purification strategies for antibodies. He explained how proteins, ions and the base bead matrix interact during tentacle-type ion exchange, but noted that superior binding capacity could be achieved through optimization of the tentacle technology. Merck’s optimization strategy included determination of the best open dimeric tentacle structure by dopants. Using a robotic system, static binding capacity (SBC) measurements of lysozyme and IgG as a function of salt and pH could be made while the type of dopant, the amount of dopant compared to SO₃ and the total amount of SO₃ were varied. The final result of the experiments was Eshmuno™ S, which uses a combination of advanced
tentacle technology and new base beads, and has improved dynamic IgG binding capacity compared to other resins.

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December 2, 2009: Day 3

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Moderated by Clive Wood (Bayer Schering Pharma), the final day of the 2009 EAC was devoted to antibody humanization and engineering, antibody-drug conjugates (ADC) and development of novel non-antibody scaffold proteins as new players in the post-antibody landscape.¹

Novel Insights into Antibody Humanization and Antibody Engineering

Marie-Paule Lefranc (University of Montpellier), founder and Director of the international ImMunoGeneTics information system® (www.imgt.org), discussed this unique bioinformatic system that associates six comprehensive databases (e.g., nucleotide, proteins, primers, structures) with 15 calculation tools (e.g., V-Quest, JunctionAnalysis, DomainGapAlign), thereby allowing accurate immunogenetic analyses of not only antibody, but also T-cell receptor and major histocompatibility complex sequences.² The major bases of this unique system were presented, like the axioms describing IMGT ONTOLOGY, and their LABELS. Probably the most important breakthrough made by IMGT was its unique numbering scheme for both variable (V) and constant (C) genes, allowing a better description of framework (FR) and CDR, based on both sequence alignment and structural definitions. This universal numbering scheme is of major importance with regard to germline definition and humanization by CDR grafting. Several examples were shown, including how to evaluate in silico immunogenicity using DomainGapAlign and the IMGT amino acid (aa) classes, and how to construct a ‘Collier de perles’ and analyze it. Novel additions in the IMGT toolbox were presented: IMGT/2Dstructure-DB and IMGT/mAb-DB contain critical information on antibodies and Fc fusion proteins on the market or in late clinical development.

Philippe Thuillier (Health Service Research Center of the French Army) presented an elegant approach for the generation of mAbs from immunized primates, and applying IMGT analyses to perform germline humanization of these primate mAbs. A series of antigens used as ‘bioweapons’ (anthrax, ricin, plague, botulinum) were used to immunize cynomolgus primates and immune phage libraries were constructed. Dr. Thuillier’s first example corresponded to the protective antigen (PA) of anthrax lethal toxin. Upon panning, high PA binders were selected and sequenced. Using the IMGT/V-quest tool, the closest human germlines were identified.³ A ‘germinality index’ [GI, defined as the percentage of identity between the FRs of any V-gene (human or non-human) and the FRs of the closest human germline V-gene] was calculated and compared to the mean GI of human V-regions (mean = 94%).⁴ ‘Germinization’ (or germline humanization) was performed by mutating divergent residues between monkey and human FRs. The final engineered candidate 35PA83 kept its high nM affinity and potency and yielded a GI of 98%. Two other examples of mAbs directed against the anthrax lethal factor and against ricin were also presented. Super-humanized candidates were isolated and ‘germlinized’ with success.

Olivier Leger (Merck-Serono) described critical trends in antibody humanization. CDR-grafting, although described in the late 1980s, remains the gold standard procedure for humanization of non-human mAbs. Precise identification of FR and CDR portions to graft remains a major challenge. The next step in a humanization process involves identification of the acceptor frameworks. Decision points such as percentage of homology and similar length of CDRs between donor and acceptor V-genes remain crucial for the success of humanization. More recent humanization techniques, such as specificity determining region (SDR) grafting, resurfacing and super-humanization, tend to reduce the number of grafted residues and keep part of the CDRs human. Chain shuffling techniques using antibody phage display libraries allowed generation of fully human mAbs e.g., adalimumab, an anti-TNFα mAb.³ Finally, by taking into account physico-chemical and biophysical characteristics during the humanization process, better mAbs with improved stability, ease of formulation, solubility are likely to be produced.

Peter Lowe (Centre d’Immunologie Pierre Fabre) discussed a novel cloning technique that can be used to recover a complete immune repertoire. Indeed, the current technique to clone given V-genes from a hybridoma rely on PCR techniques, thus necessitating use of degenerated primers because the exact sequence of the antibody variable domain is essentially unknown. To circumvent these drawbacks, Dr. Lowe and colleagues described an elegant approach to clone full-length variable domains, including cognate signal peptide and 5’ untranslated region (UTR), without knowledge of their V-gene portion.

Four major steps were described: (1) cDNA synthesis using a constant domain-specific, phosphorylated primer; (2) cDNA circularization using CircLigase ssDNA ligase; (3) rolling circle amplification by Phi29 DNA polymerase (isothermal polymerase) using primers carrying an unique restriction site (MluI) → amplification of large sized DNA concatemers; (4) recovery of monomers by either restriction digest or PCR with sense and reverse primers located respectively at the 3’ and 5’ ends of the constant domain. The monomers can be either directly sequenced or cloned in expression vectors carrying constant domain portions for expression; there is no need for exogenous signal peptide or 5’UTR. Several examples of sequences and expression were shown. Improvements in both the speed of bench work and accuracy of results were obtained as compared to the conventional PCR-based cloning approach.

Mike Clark (University of Cambridge) discussed improvement of therapeutic mAb tolerance, e.g., by generating aglycosylated mAbs or introducing mutations in the Fc portion to modulate binding to FcγRs. This was exemplified through the case study of otelixizumab (TRX4), a non-depleting anti-CD3 mAb (aglycosylated IgG1), that is being co-developed by Tolerex and GlaxoSmithKline and is currently in Phase 3 studies as a treatment for type I diabetes. In order to fine-tune engagement (or not) of FcγRs, several mutations were generated in the Fc...
portion by incorporating human IgG2 and IgG4 residues into an human IgG1 backbone and IgG2 into IgG4 and reverse. Two domains were evaluated: ELLG motif at the N-term end of CH2 (1.1 to 1.4 IMGT numbering) and A-AP motif at the C-term end of the same domain (110-115-116 IMGT numbering). Mutant mAbs were assayed for their binding to FcγRIIa and FcγRIIb. Two such mutants, an IgG1Ab and an IgG2Aa are currently in the clinic; they yield no ADCC nor CDC and weak monocyte activation, and weak ADCC, but neither CDC nor monocyte activation, respectively.

Stefan Dübel (Technical University Braunschweig) presented novel results from a research project aiming to generate mAbs against the entire human proteome, by using phage display and HTS screening technologies (The Antibody Factory). Of particular interest was a comparison of pannings on 20 different SH2 domains provided by the Structural Genomics Consortium (SGC). Streamlined methods for high throughput antibody generation developed within the ‘Antibody Factory’ of the German National Genome Research Network (NGFN) were demonstrated to minimize effort and provide a reliable and robust source for antibodies. For the SH2 domains, about 2,700 clones were analyzed in two successive series of selections, resulting in 347 primary hits in ELISA. The validation of selected antibodies by cross-reactivity ELISA, western blot and protein microarrays demonstrated the versatility of the in vitro antibody selection pipeline to generate a renewable resource of highly specific monoclonal binders in proteome scale numbers with substantially reduced effort and time.

Antibody-Drug Conjugates

Four speakers discussed the various existing technological and industrially-applicable approaches to generate ADCs.

Hans-Peter Gerber (Pfizer) presented ADCs based on calicheamicin, e.g., the marketed compound Mylotarg (anti-CD33). Calicheamicin by itself is an extremely cytotoxic drug (IC50 around 50 pM) by making DNA breaks in the nucleus. A detailed overview of CMC-544 (inotuzumab ozogamicin), a novel ADC currently in clinical development, was presented. CMC-544 corresponds to a humanized anti-CD22 IgG4 mAb, linked to calicheamicin. It yielded potent anti-tumor activities in RAMOS and RL B cell lymphoma models and strong synergy in combination with rituximab. Its safety profile was consistent with toxicity associated with calicheamicin. A Phase 1 study indicated tolerability of the drug with manageable and reversible side effects, with MTD at 1.8 mg/m² Q4W (50 µg/kg). Clear signs of clinical efficacy were observed (30% complete response and 69% overall response rate in follicular lymphoma patients). A Phase 1/2 study in combination with rituximab is in progress in CD22+ non-Hodgkin lymphoma (NHL) patients.

Iqbal Grewal (Seattle Genetics) presented ADC technology based on Seattle Genetics’ proprietary cytotoxic drugs from the auristatin family (monomethyl auristatin E and F; MMAE, MMAF) and improved linker technology (both protease sensitive and non-cleavable linkers). Three products from their pipeline were highlighted: (a) SGN-35 (brentuximab vedotin) is an ADC comprising an anti-CD30 mAb linked to MMAE. The target is expressed on Hodgkin lymphoma, various T cell cancers and other hematological malignancies. Brentuximab vedotin may also have applications in autoimmune disease due to CD30 expression on activated, but not resting, T cells. SGN-35 is currently in a pivotal Phase 2/3 trial in patients with relapsed or refractory Hodgkin lymphoma and in two Phase 2 lymphoma trials. It received orphan drug designation in the United States and Europe for both Hodgkin lymphoma and ALCL and Fast Track designation from the FDA in Hodgkin lymphoma. The candidate is being co-developed with Millennium (now Takeda) (b) SGN-75 is a humanized anti-CD70 mAb attached to MMAF via a non-cleavable linker. CD70 is expressed on a variety of solid tumors, including renal cell carcinoma, pancreatic, ovarian and lung cancer, as well as multiple myelomas and several types of NHL. CD70 has limited expression on normal tissue, is not shed, and is rapidly internalized. SGN-75 is currently in Phase 1 studies for NHL and renal cell carcinoma. (c) SGN-19A is an ADC targeting the CD19 antigen, a pan-B cell marker expressed on many hematological malignancies, including NHL, chronic lymphocytic leukemia and acute lymphoblastic leukemia. Preclinical data demonstrate that SGN-19A effectively binds to target cells, internalizes and induces potent cell-killing activity, including complete tumor regressions in multiple cancer models.

Robert Lutz (ImmunoGen) presented data on tumor activated produgs (TAPs), which are based on ImmunoGen’s proprietary cytotoxic drugs maytansinoids (DM1, DM4) and act as non-immunogenic tubulin polymerization inhibitors. He also discussed their portfolio of improved linkers. Novel, non-cleavable linker technology (POL) allows novel TAPs to be resistant to multidrug resistant (MDR) efflux pumps, thereby yielding accumulation of DM1 or DM4 in the cytoplasm. Additional cytotoxic drugs affective in a TAP format were also presented e.g., DNA alkylating agents from the IGN family. Six TAPs are currently in the clinic. The most advanced candidate is trastuzumab-DM1 (T-DM1), which consists of DM1 attached to the HER2-binding antibody trastuzumab. T-DM1 is in development by Genentech and Roche, and the candidate is currently in Phase 3 studies as a third-line treatment of HER2 metastatic breast cancer. A second TAP, IMGN901 targets CD56, a tumor marker overexpressed in solid tumors, e.g., small-cell lung cancer, Merkel cell carcinoma, ovarian and neuroendocrine/carcinoid tumors, as well as liquid tumors such as multiple myelomas and NK lymphomas. It is currently undergoing Phase 1 clinical trials in both solid and liquid tumor types to determine its MTD.

Jagath Junutula (Genentech) presented a novel ADC technology called THIOMAB™. This platform originated from a phage display-based method to screen for reactive cysteines on the Fab surface of mAbs (PHEselector). Two such residues were identified at the FR4-CH1/Ck junctions. The conjugation scheme comprises mild reduction to uncap the cysteines, re-oxidation of the interchain disulfide bridges to reconstitute antibody integrity, and finally conjugation of biotin-PEO-maleimide on each chain. This strategy provides ADCs with uniform and homogenous stoichiometry (approximately two drugs/antibody). THIOMAB
drug conjugates are currently being developed to improve ADC therapeutic index, linker stability and mechanism of action.

**Novel Antibody Formats and Protein Scaffolds as Alternatives to Conventional mAbs**

Horst Lindhofer (Trion Pharma) presented the major advantages of the Triomab technology of tri-functional antibodies. The Fc portion comprises a mouse IgG2a associated with a rat IgG2b arm, which elicits strong ADCC. The variable domain is composed of a bi-specific domain, with one arm targeting CD3 and the other a specific tumor-associated antigen.9 Triomab molecules display a range of properties, e.g., the specific and simultaneous activation of multiple immune defense mechanisms against cancer cells, the efficient induction of tumor cell elimination by activation of accessory cells and T cells.9 These properties can easily be adapted to various cancer indications via exchange of the tumor-associated antigen. TRION currently runs three development programs in several cancer indications in collaboration with Fresenius Biotech: (1) catumaxomab (Removab) is a Triomab targeting a specific tumor-associated antigen. The compound is marketed in the European Union for the intraperitoneal treatment of malignant ascites in patients with EpCAM-positive carcinomas. Phase 2 studies for the treatment of ovarian and gastric cancers are ongoing; (2) ertumaxomab (Remomun), a Triomab antibody targeting CD3 and HER2 is being tested against metastatic breast cancer in a broad Phase 2 program; and (3) FBT0A05 (Lymphomun) is in Phase 1/2 for the treatment of B cell lymphoma. The compound targets CD3 and CD20.

Ray Camphausen (Adnexus/Bristol-Myers Squibb) provided an overview of Adnectins™, which are novel scaffolds based on the 10th fibronectin type III domain of human fibronectin, a ubiquitous human protein. This domain corresponds to a β-sandwich, with seven β-strands and three connecting loops showing structural homologies to Ig domains without disulfide bridges. Randomization of these loops, representing a unit of about 15 to 21 aa, was performed and displayed on both phage and yeast. Several high affinity binders have been isolated to numerous protein targets9 such as TNFα, ubiquitin and VEGFR2. The most advanced compound is Angiocept (or CT-322) currently undergoing multiple Phase 2 trials in oncology. A case study was presented on the design and development of tandem Adnectins™ that target IGF-1R and EGFR simultaneously. Mono-adnectin units targeting each of these receptors were isolated by applying PROfusion technology (based on mRNA display). Bispecific Adnectins™ were generated by genetic fusion and addition of various linkers; the effect of a variety of orientations was also examined. Selected bispecific Adnectins™ showed potent and efficacious activity on both IGF-1R and EGFR axis (phosphorylation, proliferation, AKT signaling, receptor degradation). Strong inhibition of H292 NSCLC cell growth was achieved in an in vivo mouse xenograft model.

Arnd Steuernagel (Scil Proteins) presented an overview on Affilin® technology, which are based on the human serum protein ubiquitin.13 The molecules are generated by introducing new aa residues at defined positions on the surface of ubiquitin. As up to 19 different amino acids are allowed in 8–14 positions, extremely diverse libraries composed of up to 10^9 molecules are generated. Several binding patches have been modeled and tested on ubiquitin by randomizing surface-exposed aa residues. This work led to the generation of two highly diverse libraries. The Affilin® monomer library contains variants with eight randomized aa positions. Nineteen natural aa are allowed on each randomized position, leading to a library complexity of 1.7 x 10^10 individual proteins. In Affilin® dimer libraries, 14 aa positions are randomized, generating a complexity of up to 10^19. These libraries are displayed using either ribosome display or a TAT expression system. Affilin® are druggable molecules: this scaffold is stable at high temperatures (90°C), pH stable, unfolds and refolds readily in solution, resists degradation in intestinal fluid, and is stable in rat serum for days. It is soluble and has no aggregation potential. Regarding PK/PD properties, Affilin® can be administered i.v. and s.c., show good biodistribution and elimination, and they show no adverse effects in a 14-day sub-chronic toxicity study. All Affilin® programs are currently at the preclinical stage.

Covagen is a relatively new player in the scaffold filed. The company was founded in 2007 as a spin-off of the Eidgenössische Technische Hochschule (ETH) in Zurich, and based on the covalent DNA display technology discovered in the laboratory of Dario Neri at ETH. Simon Brack (Covagen) presented the major properties of Fynomers, which use the Fyn SH3 domain as a scaffold for the generation of drug candidates that can bind to target molecules with the same affinity and specificity as antibodies, but do not have the intrinsic drawbacks of the antibody structure.13 The Fyn SH3 domain is a fully human protein composed of 63 aa residues. It can be produced in bacteria with high yields.13 Moreover, several Fynomers can be linked to yield a protein with multiple binding specificities. Their small size as well as their stability might allow Fynomers to be administered to humans by routes other than i.v. injection. The most advanced program corresponds to a Fynomer targeting IL17A. Several binders with high affinity were selected, and reformatted into an Fc fusion protein that was produced in E. coli. Serum half-life of the Fyn-Fc fusion protein reached about 50 hours; the molecule is currently undergoing in vivo testing.

Ruud de Wildt (Domantis/GlaxoSmithKline) presented insights on the pulmonary application of the domain antibody (dAb) scaffold that was discovered by Domantis. dAbs represent the smallest engineered binding domain of conventional antibodies and consist of the variable domain of either a light (VL) or a heavy (VH) chain restricted to their three CDRs and associated framework residues.14 dAbs range in size from 11 to 15 kDa and several mutations have been introduced to accommodate lack of the second chain and avoid aggregation.14 Matured dAbs express well in the periplasm of E. coli and in the yeast Pichia pastoris, and exhibit unusually high thermal stability and reversible unfolding properties, which is not the case for conventional antibody fragments. High-diversity phage display libraries of both VH and VL-based human and murine dAbs have been constructed and used to screen a range of antigens including haptens, enzyme active sites, cytokines and proteins. The program investigating chronic obstructive
pulmonary disease (COPD) is targeting tumor necrosis factor α receptor 1 (TNFαR1). Potent dAbs showing therapeutic efficacy in a chronic tobacco smoke mouse model have been isolated and characterized. Pulmonary administration was evaluated using a jet nebulizer for intra-nasal route.

Debbie Law (Abynex) gave an overview of the nanobody technology developed by Abynex. Nanobodies correspond to the VHH domain of a certain class of IgGs found in Cameldids, and which are naturally devoid of light chains. VHH framework regions are closely homologous to human heavy chains, but conventional humanization procedures can be applied to reduce potential immunogenicity of therapeutic nanobody candidates. Nanobodies combine the advantages of conventional antibodies with important features of small molecule drugs; nanobodies have high target specificity and affinity, low inherent toxicity, extreme stability and ease of manufacturing. Several nanobodies against difficult targets such as G protein-coupled receptors have been identified. Extended serum half-life can be achieved either by PEGylation, or by genetic fusion to an albumin-binding nanobody. Several clinical studies of nanobodies have been initiated. The most advanced candidate, ALX0081 targeting von Willebrand factor, is currently in Phase 2 studies as a treatment for patients with acute coronary syndrome and thrombotic thrombocytopenic purpura. Another nanobody candidate, ALX0061 targeting TNFα, is being developed by Pfizer.

Andrew Nixon (Dyax) discussed the discovery and development of ecallantide (DX-88), their lead program targeting human plasma kallikrein. Ecallantide was developed by Dyax 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).16,17 and produced in Pichia pastoris as a non-glycosylated recombinant peptide of approximately 7 kDa. DX-88 inhibits the proteolytic activity of human plasma kallikrein with a Ki of 25 pM and a high selectivity versus other proteases. DX-88 has completed four Phase 1, and three Phase 2 clinical trials that included 134 HAE patients.18 Two Phase 3 trials (EDEMA3 and 4) including a total of 143 patients were completed and provided the basis for FDA approval of ecallantide (Kalbitor) on November 30, 2009. DX-88 is also currently 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.

Michael Stumpp (Molecular Partners) presented the discovery platform for the generation of Designed Ankyrin-repeats (AR) proteins (DARPins).19 ARs comprise motifs of 33 aa residues containing a β-turn followed by two α-helices connected by a loop. Rational design strategies have been developed based on multiple sequence alignments and statistical analysis to calculate the probability of aa usage at each position of an AR. Combinatorial AR libraries have been constructed based on the 33 aa AR motif with seven randomized positions. DARPins contain typically 2 to 4 of these motifs flanked by N and C-terminal capping motifs to shield hydrophobic regions and allow increased solubility.19 DARPin libraries are preferentially screened using ribosome display. Library members are produced in E. coli with good yields, do not aggregate, and display high thermodynamic stability. The most advanced DARPin program targets HER2, and may have utility both for diagnostics and therapy.20

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