mAbs 3:2, 133-152; March/April 2011; © 2011 Landes Bioscience

IBC's 21st Annual Antibody Engineering and 8th Annual Antibody Therapeutics International Conferences and 2010 Annual Meeting of The Antibody Society

December 5–9, 2010, San Diego, CA USA

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Key words: antibody engineering, antibody therapeutics, phage display, biosimilar antibodies

The 21st Annual Antibody Engineering and 8th Annual Antibody Therapeutics international conferences, and the 2010 Annual Meeting of The Antibody Society, organized by IBC Life Sciences with contributions from The Antibody Society and two Scientific Advisory Boards, was held December 5–9, 2010 in San Diego, CA. The conferences featured over 100 presentations and 100 posters, and included a pre-conference workshop on deep-sequencing of antibody genes. The total number of delegates exceeded 800, which set a new attendance record for the conference.

The conferences were organized with a focus on antibody engineering only on the first day and a joint engineering/therapeutics session on the last day. Delegates could select from presentations that occurred in two simultaneous sessions on days 2 and 3. Day 1 included presentations on neutralizing antibodies and the identification of vaccine targets, as well as a historical overview of 20 years of phage display utilization. Topics presented in the Antibody Engineering sessions on day 2 and 3 included antibody biosynthesis, structure and stability; antibodies in a complex environment; antibody half-life; and targeted nanoparticle therapeutics. In the Antibody Therapeutics sessions on days 2 and 3, preclinical and early stage development and clinical updates of antibody therapeutics, including TRX518, SYM004, MM111, PRO140, CVX-241, ASG-5ME, U3-1287 (AMG888), R1507 and trastuzumab emtansine, were discussed and perspectives were provided on the development of biosimilar and biobetter antibodies, including coverage of regulatory and intellectual property issues. The joint engineering/therapeutics session on the last day focused on bispecific and next-generation antibodies. Summaries of most of the presentations are included here, but, due to the large number of speakers, it was not possible to include summaries for every presentation.

Delegates enjoyed the splendid views of the San Diego Bay and proximity to the Gaslamp Quarter provided by the venue. The 22nd Annual Antibody Engineering and 9th Annual Antibody Therapeutics conferences, and the 2011 Annual Meeting of The Antibody Society, are planned for December 5–8, 2011 at the same location in San Diego, and will include two two-day short courses on Introduction to Antibody Engineering and Protein Characterization for Biotechnology Product Development.

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Submitted: 01/23/11; Accepted: 01/23/11
DOI: 10.4161/mabs.3.2.14939

Day 1: December 6, 2010
Antibody Engineering

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Neutralizing Antibodies and Identification of Vaccine Targets

The first session of the meeting focused on exploiting antibodies for the development of new vaccines. The session chairman, **Richard Begent** (University College London), welcomed delegates with the reminder that antibodies are the best way to diagnosis infectious disease. He then pointed out that while it was analytical techniques that first focused attention on antibodies that targeted infectious agents, the field has broadened to include a wide range of disciplines, such as genetics. Beginning with hybridoma technology (i.e., the formation of hybrid cell lines by fusing an antibody-producing lymphocyte and a non-antibody producing cancer cell), recent technological achievements, such as in vitro isolation of antibodies from combinatorial libraries and their functional expression in bacteria, have infused the field with great power. However, this capacity may be underutilized unless the targeted biological system, as well as the antibody, is fully understood.

The keynote presentation was delivered by **Dennis Burton** (The Scripps Research Institute). Professor Burton began with an overview of classical vaccine design and the fact that it does not require an understanding of the interplay between pathogen and immune system. Unfortunately, chemical inactivation or live attenuation are either too cumbersome or ineffective for highly variable vaccine targets, such as influenza or the human immunodeficiency virus (HIV). Professor Burton described a rational structure-based immunogen design effort that requires full understanding of the molecular interactions of a broadly neutralizing antibody (bNAb) and antigen. These efforts focus on overcoming obstacles created by the immune-evasion capabilities of host immune-selective pressures. Reverse engineering a vaccine involves the isolation of bNAbs from infected individuals, the molecular characterization of bNAb-pathogen antigen interaction, subsequent immunogen design and testing, and the eventual combination of several immunogens for a vaccine.

The major targets for HIV-1 neutralizing antibodies are the envelope glycoproteins (Envs): the exterior Env gp120 mediates receptor binding, and the transmembrane Env gp41 mediates viral entry. To generate an effective neutralizing antibody response against HIV-1, it is necessary to target functionally conserved, exposed regions of the Envs. With the laborious isolation of b12,[1] which was the first HIV-1 bNAb isolated from HIV positive donors, the primary receptor CD4-binding site (CD4bs) on gp120 became an attractive surface to target for focused immunogen design. Professor Burton discussed in vivo protection studies demonstrating that b12 protects against low-dose repeated mucosal simian HIV challenge in macaques[2] and that Fc-receptor, but not complement binding, is important in antibody protection against HIV.[3] Attempts to reverse engineer the CD4bs epitope have included immunofocusing on gp120 by hyperglycosylation and mutation, fragmentation of the outer domain constructs, peptide reconstruction of CD4bs and epitope cloaking.

The other gp120 directed bNAb, 2G12, binds to a cluster of conserved glycans on the outer domain of the exterior glycoprotein.[4] 2G12 recognizes the sugars on the gp120 glycan shield by its domain-exchanged structure that produces a rigid array of binding sites. While attempts to exploit the 2G12 epitope as a vaccine target using agents such as oligodendrons, protein-glycodendron conjugates and oligomannose on virus scaffolds have not elicited a neutralizing response, molecular studies on the mechanism of domain exchange have revealed a potential immunization strategy.

Professor Burton then discussed use of gp41-directed bNAbs 2F5 and 4E10 as templates for immunogen design. While both bNAbs bind to continuous regions close to the viral membrane, 2F5 recognizes an extended loop structure and 4E10 binds a helical epitope conformation.[5,6] Efforts to re-elicit 2F5 and 4E10-like antibodies using scaffold immunogens in guinea pigs resulted in good mimicry, but did not produce neutralizing antibodies. Therefore, research is now focused on membrane incorporation and the elicitation of hydrophobic character in gp41 immunogen design.

Attempts to reverse engineer an HIV-1 vaccine with the original four bNAbs had been ongoing for nearly a decade when Professor Burton began collaborating with the International AIDS Vaccine Initiative (IAVI) to screen donors for new bNAbs (i.e., the Protocol G project). Using high throughput technology, the scientists at IAVI were able to generate human monoclonal antibodies (mAbs) from broadly neutralizing serum donors and isolated two elite neutralizers (PG9 and PG16) that have outstanding breadth and potency. Both PG9 and PG16 neutralize approximately 85–90% of viruses in a 160-virus panel and display a potency of 1–2 logs better than the previous four bNAbs.[7] Most importantly, PG9 and PG16 recognize the V2/V3 variable loop epitope and are largely trimer specific. A high-resolution crystal structure has been solved and shows an inflexible hammerhead structure with a sulfated tyrosine that recognizes the virus. Both antibodies display uniquely long CDRH3 arms with a variable loop that determines their fine specificity. In addition to these new and impressively potent bNAbs, several more novel bNAbs are being isolated and tested. Professor Burton optimistically concluded his presentation by stating that the rate-limiting step is no longer the acquisition of bNAbs. The generation of a functional immunogen is now the biggest hurdle and the new bNAbs give great insight into surmounting this issue.

Kate Williams (University of California, Berkeley) reported on the role antibodies play in protection and enhancement of dengue virus infection in a mouse model of dengue disease. Ms. Williams began with an overview of antibody-dependent enhancement (ADE) and an explanation of why a tetravalent dengue virus (DENV) vaccine must elicit robust protection against all serotypes. There are four serotypes of DENV and infection with one serotype is typically asymptomatic and protects against re-infection with the same serotype; but secondary infection with another DENV serotype carries an increased risk of severe disease, including a life-threatening vascular leakage syndrome referred to as dengue hemorrhagic fever/dengue shock syndrome.

It has been reported that anti-DENV antibodies can mediate pathogenesis of a second DENV infection in vitro, but in vivo corroboration has not been possible because no animal model of antibody-enhanced dengue disease is currently available.

Ms. Williams then described a mouse-adapted DENV2 strain, DS10, that produces a tumor necrosis factor (TNF)-dependent fatal vascular permeability syndrome in interferon-α/β and γ-receptor-deficient (AG129) mice 4–5 days after intravenous infection.[8] The AG129 mouse model is permissive to infection with all four DENV serotypes, displays relevant tissue and cellular tropism, produces long-lasting serotype-specific and serotype-cross-reactive anti-DENV antibodies of a balanced isotope ratio, and generates a vascular leakage syndrome that is similar to human dengue disease. Ms. Williams confirmed the critical role of FcγR interaction in ADE in vivo. Using the F(ab)'2 fragment of 4G2 and the N297Q variant of hE60-IgG1, it was verified that interaction of the anti-DENV mAb with the FcγR, and not binding of C1q, is essential for ADE in vivo. Ms. Williams also remarked that mAbs engineered to prevent FcγR interaction also exhibit prophylactic and therapeutic efficacy against DENV, and thus have potential as an antiviral strategy.

The molecular interaction of protective mAbs and the DENV envelope protein was the final topic of the presentation. Binding sites were mapped to epitopes in regions in domain I (lateral ridge), domain II (dimer interface, lateral ridge and fusion loop) and domain III (lateral ridge, C-C' loop and A strand). The anti-C-C' loop and anti-domain I/II dimer interface mAbs are prophylactic against an ADE infection, but neither are therapeutic as wild-type or as a non-FcγR binding variant.[9] Ms. Williams concluded with an outline of future work that involved further characterization of mouse and human mAb determinants of protection and enhancement at the serotype domain and epitope specificity level.

Ronald Vogels (Crucell) first emphasized the importance of his company's motto: "Combating infectious diseases by bringing innovation to global health." He commented on the difficulty of treating influenza A and how bNAbs against influenza can help. An important option for the prevention and treatment of influenza is the administration of purified or recombinant antibodies for groups 1 and 2 influenza A viruses. Dr. Vogels noted that influenza hemagglutinin (HA) is an antigenic glycoprotein found on the surface of the influenza viruses and is the main antigenic determinant; neutralizing antibodies can affect HA binding to the cell receptor and subsequent fusion.

In 2008, Crucell discovered CR6261,[10] a mAb that is able to prevent and cure influenza by binding to a highly conserved epitope in the HA stem with heavy chain only.[11] Dr. Vogel highlighted that CR6261 is able to neutralize a broad range of group 1 influenza viruses, including the currently circulating H1N1 seasonal influenza strains and the highly pathogenic H5N1 ('bird flu') virus. However, group 2 HAs such as H3, H7 and H10 strains are not recognized by CR6261 because they have a glycan structure in the hydrophobic pocket that prevents recognition by the VH1-69 germline gene of CR6261.

Dr. Vogel then described the isolation of two new mAbs, CR8020 and CR8043, that neutralize multiple phylogenetically distinct H3 strains and H7 and H10 subtypes. Prophylactic administration of CR8020 and CR8043 protects mice against lethality from H3N2 challenge and there is mutually exclusive binding by CR6261 and the new mAbs. Using competition binding experiments and computational modeling, a second overlapping conserved epitope has been identified in group 2 influenza viruses. These H3 mAbs prevent infection through two different mechanisms. They either block pH-induced conformational change or prevent proteolytic cleavage. Dr. Vogel concluded by reiterating that group 2 mAbs have defined a second conserved epitope on HA that is key to virus replication and that these H1/H3 mAbs are an important addition to the arsenal against influenza.

Twenty Years of Phage Display

The second session of the day, which celebrated the 20 year anniversary of the development of antibody phage display, began with opening remarks by chairman **James Marks** (University of California, San Francisco). He began with a summary of the current state of antibody therapy by stating that all pharmaceutical companies now have antibody programs; there are currently 24 US Food and Drug Administration (FDA)-approved antibody drugs with four more undergoing review and hundreds of mAbs in clinical studies;[12] and the estimated global sales will exceed $50 billion by the year 2015. Professor Marks then discussed highlights of antibody-based technology development, including hybridoma technology, antibody phage display, and transgenic mice. He concluded with the reminder that phage display has been an invaluable tool in the development of human antibodies as research tools and therapeutics.

The first presentation was delivered by the grandfather of phage display, **George Smith** (University of Missouri), and detailed the successes and failures of the technology's development and use over the last twenty-two years. Professor Smith began with a comprehensive review of filamentous phage and the construction of phage display libraries. This technique uses recombinant DNA technology to create bacteriophages with a peptide or single chain antibody or Fab fused to multiple copies of a coat protein, thereby displaying it on the phage surface, while DNA encoding the displayed entity is packaged within the virion. Phage that bind a target molecule can then be identified through affinity selection, which has enabled the engineering of peptides, antibodies and other protein domains. While the technological advantage of phage display over use of animals or mammalian cell culture to generate human antibodies appeared to be clear at the outset, its biggest failure is that in the intervening 22 years antibody-library technology has not been widely disseminated. Professor Smith then described factors inhibiting its wide spread use, including the use of the phagemid system rather than phage for antibody display. He suggested that, were phage displayed antibody systems further upgraded and streamlined, this technology could be accessed by "non-specialist" researchers, just as phage-displayed peptide libraries are. Professor Smith concluded by stating that even though there are drawbacks to phage-displayed antibodies, it is still an ingenious innovation that has produced many important discoveries.

Richard Lerner (The Scripps Research Institute) discussed the capabilities and power of combinatorial antibody libraries, which lies within the chemistry of large numbers. To illustrate his point, Dr. Lerner discussed the insights gained from an antibody library constructed from cells taken from Turkish children infected with avian influenza using phage display.[13] The isolated antibodies, which use the VH1-69 family and have a low number of somatic mutations while displaying no similarity between CDR3 loops, bind to the conserved stem of HA. These libraries revealed unprecedented antibodies and offer hope for universal neutralization. Dr. Lerner then discussed using large combinatorial antibody libraries as a representation of the immunological repertoire in health and during disease. Researchers developed combinatorial libraries from patients suffering from chronic rejection of transplanted human kidneys in order to understand how B-cell infiltrates (lymphoid clusters) are important to the survival of the kidney. By comparing clones from donors of successful and rejected transplants, it was learned that the lymphocytes did not originate in the host, may be lymphoma-like, and that they may be escape mutants from T-cell regulation.

Sir Gregory Winter (Medical Research Council) discussed the use of chemistry to tailor phage repertoires for drug development. He recapitulated the importance of phage display by discussing the advances made with human therapeutic antibodies. He compared and contrasted antibodies with chemical drugs with regard to properties such as target affinity and specificity, and targeting of intracellular antigens. He then discussed single domain antibodies (dAbs). Elimination of one of the partner domains from an Fv allows dAbs to recognize its cognate antigen with lower affinity than parental mAb, but the absence of immunoglobulin constant domains removes the natural effector functions of dAbs, thereby making it necessary to equip them with an engineered Fc. Sir Gregory Winter's final topic was phage-encoded combinatorial chemical libraries based on bicyclic peptides.[14] Basically, novel accessory effector moieties are made using synthetic repertoires, encoding Cys-NX-Cys-NX-Cys (where x is a random amino acid and n = 0–8 amino acids), to mimic the rapid chemical reaction of trimethylbenzene. He concluded by describing two successful examples, PK15 and uPA in tumor invasion.

The session ended with a "fireside" chat (made possible by a televised fire) with the pioneers of phage display. The panel comprised **Richard Lerner** (The Scripps Research Institute), **John McCafferty** (Cambridge University), **George Smith** (University of Missouri), **Sir Gregory Winter** (Medical Research Council), **Andrew Bradbury** (Los Alamos National Laboratories), and **James Marks** (University of California, San Francisco). The open format elicited questions and comments on topics ranging from the quality of reagents to opinions about the utility of the technology.

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Day 2: December 7, 2010

Jean-Luc Teillaud

The second day of the conferences was divided into two parallel tracks, Antibody Engineering and Antibody Therapeutics, with morning and afternoon sessions for each track. Topics in the Antibody Engineering tracks included antibody biosynthesis, structure and stability and antibodies in a complex environment, while topics in the Antibody Therapeutics track included preclinical and early stage development of antibody therapeutics and clinical updates of antibody therapeutics.

Antibody Engineering: Antibody Biosynthesis, Structure and Stability

The morning session of the Antibody Engineering track, chaired by **Andreas Plückthun** (University of Zürich), was dedicated to discussion of the glyco-engineering of IgG and the structural changes and characteristics that may facilitate the folding and the production of IgG in yeast and bacterial cells.

The selection of aglycosylated full-length IgG from repertoires displayed on *E. coli* was presented by **George Georgiou** (University of Texas at Austin). Produced in the periplasm, these IgG are captured by an Fc binding protein expressed at the inner membrane of the bacteria. Permeabilization of the outer membrane makes it possible to screen for fluorochrome-labeled ligands. Multiple rounds of fluorescence-activated cell sorting (FACS) allow the selection of antibodies with affinity in the nanomolar range.[1] Antibodies against the protective antigen of

Bacillus anthracis have been isolated. Aglycosylated IgGs binding to activating Fcγ receptors (FcγRI, FcγRIIa and FcγRIII) but not to inhibitory FcγRIIb were also generated. These IgG have potent anti-tumor killing activities in vitro and in vivo due to their lack of engagement of the inhibitory receptors, thus favoring the activities of activating FcγRs.

Marc d'Anjou (GlycoFi/Merck & Co.,) discussed the control of post-translational modifications of recombinant proteins in humanized *Pichia pastoris* yeast. The capacity to engineer and to produce (with an excellent fidelity during the scaling-up of the process and from batch to batch) large amounts (>2 g/L) of proteins, notably antibodies, with pre-determined human N-glycan structures that impact the solubility, serum half-life and effector functions of antibodies paves the way to a new paradigm for the development of therapeutic proteins.[2,3] Therapeutic antibodies produced in humanized *P. pastoris* exhibit pharmacokinetic (PK) properties similar to the same antibodies produced in mammalian cells (e.g., CHO). Moreover, the in vivo activity of a neutralizing anti-virus antibody produced in humanized *P. pastoris* appeared to have a similar potency in viral infection prophylaxis as the same antibody produced in mouse NS0 cells. Also, an anti-CD20 antibody produced in humanized *P. pastoris*, devoid of fucose, was capable of depleting B cells in the presence of cells from an IgG1 low-binder F/F FcγRIII (CD16) donor, whereas there was no response to rituximab in the same assay.[4] This technology allows the generation of 100% afucosylated highly cytotoxic antibodies. Another interesting use of the humanized *P. pastoris* is the alteration of the N-glycosylation often present in the CDRs and framework regions of antibodies. It can lead to severe adverse events such as hypersensitivity, as observed with cetuximab which exhibits an N-glycan in its CDR-H2 when produced in SP2/0 cells. The humanized *P. pastoris* offers an alternative by fine-tuning the glycosylation profile of such antibodies.

Antibody Engineering: Antibodies in a Complex Environment

The first part of the afternoon session of the Antibody Engineering track, chaired by **Richard Begent** (University College London) focused on the long-term effects of antibody treatment on the immune system of the host, on the T-and B-cell immunogenicity of polyvalent vaccines made of mosaic proteins and on the role of affinity in the anti-tumor efficacy of antibodies.

Jean-Luc Teillaud (INSERM) showed that the treatment of tumor-bearing immuno-competent mice with an anti-CD20 mAb induces a long-lasting adaptive cellular anti-tumor response through the early recruitment of $CD4^+$ cells.[5] When the $CD4^+$ compartment is depleted before tumor engraftment and antibody treatment, mice are no longer protected and die rapidly. This long lasting effect, which requires the presence of an intact Fc portion, is a "vaccine-type" effect, as evidenced when mice are challenged with tumor cells several months later. The presence of T cells protects these mice from the tumor challenge, whereas the depletion of $CD4^+$ cells just before tumor challenge or the lack of $CD8^+$ cells (using CD8 knockout mice) abolish the protection. Thus, this in vivo model demonstrates that, besides inducing early events such as killing or phagocytosis of tumor cells, antibody treatment can elicit a cellular adaptive memory immune response that ensures a long-term anti-tumor protection. Injection of IL-2 after tumor challenge reinforces the anti-tumor protection whereas it has no potentiating effect at the initiation of the antibody treatment. Moreover, the mouse anti-CD20 mAb used in this study has been chimerized and a low-fucose antibody selected by LFB S.A.[6] This low-fucose chimeric antibody (LFB-R603) is currently undergoing evaluation in a Phase 2 study in patients with B chronic lymphocytic leukemia (B-CLL).

The design of mosaic proteins to achieve adequate T- and B-cell responses was then discussed by **Bette Korber** (Los Alamos National Laboratory). The initial model system is the HIV Env protein that exhibits extraordinary variability since up to 35% of amino acids can differ between two Env proteins and up to ~10% amino acid variation can be observed in a single patient over time. Thus, any anti-HIV vaccination strategy has to deal with two problems related to patient-to-patient variability: (1) the variability of the T-cell receptor (TCR) and (2) the polymorphism of the HLA molecules. Both molecules should adapt the enormous variability of the HIV-derived peptides in order to be able to present them to specific T cells. It is a major challenge as T-cell responses have limited cross reactivity.

Dr. Korber explained that, using computational modeling, mosaic proteins have been designed to optimize the coverage of T-cell epitopes, while keeping common epitope variants in a natural protein context.[7] Notably, a database map (termed LANL database) has made it possible to determine the $CD8^+$ and $CD4^+$ T-cell responses to p24 (positions 100–200). HIV evolution has been mimicked by in silico recombination using a genetic algorithm to optimize vaccine epitope coverage. Mosaic HIV-1 vaccines containing mosaic HIV-1 Gag, Pol and Env antigens have been shown to expand the breadth and the depth of cellular immune responses in rhesus macaques without compromising its magnitude.[8] They elicit $CD8^+$ T lymphocyte responses that confer enhanced immune coverage of diverse HIV strains in monkeys.[9] Of note, the setting of "B-cell" mosaics is even more complex as antibodies "see" discontinuous regions in folded proteins. However, some specific rules to build B-cell mosaics can be envisioned: (1) B-cell mosaics may present the most common mutations that impact antibody binding; (2) Affinity maturation in the presence of the most common variants might produce antibodies that could neutralize more broadly. However, whether B-cell mosaics represent useful vaccine molecules remains to be evaluated.

The relationship between the affinity and the efficacy of antibodies directed against solid tumors was then examined by **Gregory Adams** (Fox Chase Cancer Center). Using a set of single chain Fv (scFv) binding to the same epitope of HER2/Neu but with different affinities, it was demonstrated in the early 2000s that an increase of affinity above 10^{-9} M was not beneficial to tumor uptake. The lowest affinity molecules exhibited diffuse tumor staining whereas scFvs with a Kd around 10^{-11} M showed only a perivascular staining.[10]

Dr. Adams noted that recent experiments have demonstrated that high affinity anti-HER2/Neu scFv rapidly internalize into tumor cells and are degraded within the cells.[11] Thus, the balance between the IgG off-rate (making the antibody available again for further binding to tumor cells) and IgG internalization (followed by the degradation of the antibody) is in favor of the internalization/degradation pathway when high affinity antibodies are used. This leads to the rapid catabolism of high affinity antibodies in vivo, resulting in limited tumor penetration. In fact, use of high or intermediate affinity antibodies could also depend on the stage of tumor development at the time of the treatment. Experiments using a set of anti-human epidermal growth factor receptor (EGFR) IgG antibodies that cross-react with mouse EGFR are ongoing in a mouse model to better delineate the optimal time points for use of either high- or intermediate affinity antibodies.

A new gene-silencing technology mediated by antibodies was presented by **Yong-Sung Kim** (Ajou University). Professor Kim described "interfering transbodies" as cell-penetrating antibodies equipped with sequence-specific nucleic-acid-hydrolyzing activity that penetrate into the cytosol of living cells and preferentially recognize and hydrolyze a target mRNA, which leads to target gene silencing. His group is conducting studies with 3D8, a DNA/RNA hydrolyzing antibody isolated from an autoimmune MRL-*Ipr/Ipr* mouse using hybridoma techniques.[12] ScFv, VL single domain and VH single domain derivatives of 3D8 have been generated and humanized.[13,14] When incubated with HeLa cells, 3D8 scFv was shown to penetrate into cells via caveolae-mediated endocytosis and localize in the cytosol in a concentration (range of 1–20 μM) and time-dependent (range of 0.5–48 h) manner.[15] When incubated with HeLa cells for 2 h at a concentration of 10 μM, the 3D8 VL penetrated into the cells and localized to the cytosol without further trafficking into the nucleus.

Professor Kim described experiments to generate variants of 3D8 VL with improved properties. Variants were selected from a synthetic library of 3D8 VL randomized residues located in one of two beta-sheets by screening for target-specific binding using immunomagnetic cell sorting (MACS) and FACS. 3D8 VL variants that had 100–1,000-fold higher affinity and 2–5-fold greater selective hydrolyzing activity for target substrates than for off targets were isolated.[16] In one example, sorting was against the 18-bp ssDNA $HER2_{18}$ substrate corresponding to gene nucleotides 2,391–2,408 of the Her2/neu gene. Selected mutants showed preferential DNA/RNA hydrolyzing activity for the target substrate. Professor Kim noted that the $HER2_{18}$-selective 4MH2 variant penetrated into cells and showed comparable downregulation of HER2 expression to that of a small-interfering RNA targeting the same HER2 sequence, resulting in apoptotic cell death of HER2-overexpressing breast cancer cells.

Results from Professor Kim's laboratory thus suggest that interfering transbodies with cell-penetrating, sequence-selective and nucleic acid-hydrolyzing activities can induce target gene-specific gene silencing. Professor Kim concluded by noting that the interfering transbody approach has challenges, including achieving appropriate target specificity, cell/tissue specificity and PK, but has numerous potential prospects in the areas of RNA therapy, viral genome therapy, intrabody delivery and vaccine delivery.

Antibody Therapeutics: Preclinical and Early Stage Development

The morning session of the Antibody Therapeutics track was chaired by **Rathin Das** (Synergys Biotherapeutics). As an introduction, Dr. Das briefly reviewed relevant facts pertaining to the development of mAb therapeutics, e.g., global revenue was $40 billion in 2009, at least five $250 million–$2.1 billion deals involving antibody therapeutics have been done in the past year, human antibodies comprised 45% of the antibody pipeline in the 2000s and their approval success rate to date is 17.5%[17] and discussed the promise shown by new product formats such as antibody-drug conjugates, bispecifics and biobetters.

Daniel Lightwood (UCB-Celltech) described the development and characterization of a potent inhaled anti-IL-13 Fab for treatment of severe asthma. IL-13 is implicated in the pathogenesis of asthma through a variety of means, e.g., recruitment and activation of eosinophils, activation of chemokine and cytokine expression in the vascular endothelium. UCB-Celltech's strategy is to deliver a high-affinity neutralizing Fab using a nebulizer in the preclinical and early clinical studies, and follow with a dry powder formulation. The inhaled delivery has advantages because the drug can be delivered directly to the site of action with very limited systemic exposure, which allows the drug also to be quickly withdrawn.

IL-13 neutralizing antibodies were initially selected from candidates generated from the antibody discovery platform UCB selected lymphocyte antibody method (SLAM), which is a way to obtain heavy and light chain variable region gene sequences directly from single specific B cells. SLAM involves four steps: (1) culture of immune B cells; (2) primary screening; (3) consolidation of positives and secondary screening; and (4) V-region gene isolation. The IL-13 screening cascade included a homogenous bead-based assay that was used to identify hIL-13 binders, a STAT-6 secreted alkaline phosphatase reporter assay to identify IL-13 functional antagonists and a Biacore A100 analysis to estimate affinity and determine mechanism of neutralization. Starting with ~10^9 B cells, 7,500 positives from the primary binding assay were reduced to 100 VH-VL gene pairs that yielded 27 unique families of recombinant antibodies. A total of five unique antibodies met the selection criteria for continued development. Six CA652γ1 Fab grafts were evaluated for affinity, Tm, stress-induced aggregation and pH-induced unfolding. Significant variability in stability profiles were observed between humanized grafts despite only minor differences in amino acid sequence. The candidate selected for preclinical evaluation, CDP7766, demonstrated activity in a cynomolgus macaque model of allergic asthma when delivered via inhalation.

David Valacer (Tolerx, Inc.) discussed the preclinical development of TRX518, an aglycosylated, humanized IgG1κ mAb that is currently in a Phase 1 study (NCT01239134) in adults with biopsy-proven unresectable Stage III or Stage IV melanoma.

TRX518 targets the glucocorticoid-induced tumor necrosis factor receptor (GITR), which is a 228 amino acid residue transmembrane protein that has no death domain but which can induce apoptosis by binding with the death domain-containing protein Siva. GITR is found on multiple cell types involved in the regulation of tumor immunity, e.g., regulatory T cells, effector T cells, B cells, NK cells, activated dendritic cells. Modulation of GITR or its ligand thus may have many potential clinical applications.

Dr. Valacer then discussed the preclinical evaluation of three antibodies: an IgG2a rat anti-mouse GITR mAb (2F8), an IgG1 chimeric anti-human GITR mAb (Ch-6C8) and TRX518. 2F8 combination therapy was shown to reduce tumor growth and extend survival, e.g., in combination with either gemcitabine (80 mg/kg) or cyclophosphamide (150 mg/kg), 2F8 dosed at 4 mg/kg was shown to reduce tumor burden by 50–80% in a CT26 colon carcinoma model. Dr. Valacer postulated that an anti-GITR mAb may affect many aspects of a specific anti-tumor immune response to tumor-associated antigens. Ch-6C8 administration to cynomolgus monkeys resulted in increased humoral and cellular immune responses. In proliferation assays, TRX518 was shown to enhance proliferation of sub-optimally stimulated T cells, but it did not act as a superagonist. In a GLP toxicology study in non-human primates, the half-life of TRX518 dosed at 5 or 50 mg/kg was 3.8 and 2.6 days, respectively. No cell subset depletion was observed and there was no increase in cytokine induction or auto-antibodies. The first-in-humans ascending dose study of TRX518 was initiated in November 2010 and the estimated primary completion date for the study is June 2012.

The therapeutic use of cytokine/anti-cytokine complexes to intensify the in vivo potency of cytokines was discussed by **Charles Surh** (The Scripps Research Institute). The combination of IL-2 with certain anti-IL-2 antibodies stimulates either memory effector T cells or regulatory cells (Treg) depending on the antibody used. Thus, the choice of the antibody present in the complex should be adapted to the pathology to be addressed. A prostate tumor mouse model showed that a particular complex could efficiently stimulate the low $CD8^+$ anti-tumor response developed by the animals.[18] Conversely, the use of another anti-IL-2 antibody in the anti-IL-2/IL-2 complex induced the expansion of Treg and not that of effector cells, leading to resistance to experimental autoimmune encephalomyelitis, as well as a long-term acceptance of islet allografts.[19] These differences are related to the ability of IL-2 present in the complexes to bind over longer periods of time either to the high affinity IL-2 receptor (comprising the α, β and γ chains), provoking the expansion of Treg, or to the low affinity IL-2R (comprising only β and γ chains), inducing $CD8^+$ memory T cells. In addition, the use of $FcRn^{-/-}$ knockout mice abrogated the induction of these effects by anti-IL-2/IL-2 complexes, as well as the effect of IL-7/anti-IL-7 complexes on T-cell development. Thus, the prolongation of the IL-2 half-life and the capacity of IL-2 to interact with CD25 (the α chain of IL-2R) are critical parameters for achieving these effects.[20]

Mikkel Pedersen (Symphogen) detailed the synergistic anti-tumor effect obtained with SYM004, a mixture of anti-EGFR antibodies, as compared to the use of a single anti-EGFR antibody. The example illustrated use of Symphogen's integrated screening platform, which allows the functional ranking of mixtures of mAbs for therapeutic use. A mixture of two anti-EGFR antibodies was selected among twenty mixtures, based on IC_{50}, affinity, epitope-bin evaluation, and an in vivo tumor xenograft model. A 1:1 mixture of these two chimeric IgG1 antibodies, binding to non-overlapping epitopes, located within the domain III of EGFR, induced strong EGFR internalization and degradation (as opposed to cetuximab), whether the EGFR exhibited mutations or not. Sym004, therefore, may be appropriate as a treatment for a broader patient population than the currently available anti-EGFR antibodies because it leads to the inhibition of all EGFR-related signaling events. The mixture showed superior efficacy in vivo in animal models as compared with cetuximab and panitumumab.[21] This result is likely due to the combination of different inhibitory mechanisms, including receptor blockade, receptor internalization and degradation, as well as activation of the effector mechanisms ADCC and CDC, induced by the mixture of antibodies. A discussion with the US Food and Drug Administration about the way toxicity should be tested (mixture vs. individual antibody) is ongoing. Dr. Pedersen pointed out that whether the concept of targeting multiple epitopes on a single molecule can be extended to other tumor targets such as HER2/Neu is still a matter of debate. A Phase 1/2 clinical study in cancer patients with refractory or recurrent solid tumors is ongoing and should bring interesting results regarding the safety and efficacy of the approach.

The renaissance of bispecific antibodies (BsAbs) in oncology was then discussed by **Matthew Robinson** (Fox Chase Cancer Center). Based on the important role of ErbB3 in the mechanisms of ErbB2/EGFR resistance to targeting agents (due to the direct link of the ErbB3-dependent signaling with the PI3K/Akt pathway), a BsAb single chain Fv directed against ErbB2 (also known as HER2) and ErbB3 (MM111; Merrimack Pharmaceuticals) has been developed and evaluated in vitro and in vivo.[22] It was hypothesized that the use of this BsAb would increase the selectivity of the targeting and would induce a strong anti-tumor effect. By fine-tuning the affinity/avidity of the BsAb through an anti-ErbB3 arm with a Kd of 1.6×10^{-7} M and an anti-ErbB2 arm with a Kd in the nanomolar range, only tumors expressing both ErbB2 and ErbB3 are targeted as shown by ^{125}I-BsAB biodistribution studies in tumor bearing SCID mice. This BsAb also mediates inhibition of tumor cell growth in vitro. It is currently undergoing evaluation in two Phase 1/2 studies (NCT00911898, NCT01097460) of $HER2^+$ breast cancer patients.

Dr. Robinson then discussed the optimization of the use of BsAb (e.g., in combination with standard-of-care chemotherapeutics and biologics) and the construction of next generation BsAb formats (e.g., antibody drug conjugates, new BsAbs). Notably, he stressed that certain epitopes targeted by anti-ErbB3 antibodies are important because the in vitro efficacy of these antibodies segregates with epitope. Thus, panels of scFv have been generated that target defined epitopes on Erb family members; they have been formatted as scFv BsAb to test the most promising pairs in terms of in vitro and in vivo anti-tumor effects.

Christian Klein (Roche GlycArt) presented a novel strategy to generate new bispecific IgG formats, i.e., the Heavy (H) chain/Light (L) chain "cross-over." BsAbs are of particular interest when one wants to simultaneously block two soluble factors that exhibit complementary and overlapping activity, e.g., such as the two pro-angiogenic factors, vascular endothelial growth factor (VEGF) and angiopoietin (Ang)-2. The latter molecule is produced during inflammatory processes or when tumors are developing.

To investigate the possibilities, an anti-VEGF x anti-Ang-2 BsAb was generated using a "cross-over" strategy. Dr. Klein explained that the approach is based on the formation of HL dimers in which the VH-CH1 of the H chain is exchanged with the full L chain of each of the two antibodies (or only the VH and the VL are exchanged between the H and L chains). The VH-CH1 is produced as a light chain and the VL-CL is fused to the hinge-CH2-CH3 domains of the heavy chain. Alternatively, the VH is fused to the CL and produced as a light chain, while the VL is fused to the heavy chain constant domains. The hinge region is modified to form an elbow allowing each of the VH-VL pairs to bind their specific targets without any steric hindrance by the other pair. The HL monomers derived from each antibody are then associated through their Fc region by the method of the "knobs-into-holes" engineering of CH3 domains proposed by Paul Carter in 1996.[23]

The "cross-over" strategy leads to the generation of different BsAbs formats. These formats exhibit the same affinity for VEGF and Ang-2 compared to their parental counterparts as shown by surface plasmon resonance (SPR) and bind simultaneously to both antigens. In vivo assays in a tumor graft model and in a VEGF-induced cornea micro-pocket assay showed that the best format is the one comprising a VL domain fused to the heavy chain constant domains and the VH domain fused to the CL and expressed as a light chain. Thus the "cross-over" strategy is a promising engineering approach whereby antibodies are not heavily engineered, contain "knobs-into-holes" mutations and novel fusion points, but do not require artificial linkers or connectors.

Antibody Therapeutics: Clinical Updates of Antibody Therapeutics 1

The afternoon session of the Antibody Therapeutics track, chaired by **Trudi Veldman** (Abbott Laboratories), focused on updates for therapeutic antibodies that are undergoing evaluation in clinical studies.

The Phase 2a studies of intravenous (iv) and subcutaneous (sc) dosage forms of PRO140 in HIV-infected subjects were discussed by **William Olson** (Progenics Pharmaceuticals, Inc.). Dr. Olson reminded delegates that only 25 drugs are approved as treatments for HIV infection and none of these are cures. Challenges associated with HIV treatment include drug resistance, drug interactions, long-term morbidities, incomplete recovery of the immune system and the requirement for daily, lifelong adherence to treatment. PRO140 is a humanized IgG4 mAb that blocks HIV from entering and infecting healthy immune cells. It targets CCR5, which is a chemokine receptor that mediates normal trafficking and activation of immune cells and acts as the portal of entry for the most abundant strains of HIV. The mAb received FDA Fast Track designation.

Three studies of PRO140 have been completed. Two studies were designed as single dose, dose ranging (0.5–10 mg/kg) investigations of PRO140 in 30–40 patients,[24,25] while the third study (NCT00642707) evaluated sc administration of PRO140 at 162 mg or 324 mg dosed on days 1, 8 and 15 of the study.[26] PRO140 demonstrated potent, dose-dependent antiviral effects that were statistically significant, and the activity was observed for both iv and sc dosage forms. The virologic suppression was rapid and prolonged, and the increases in rates of antiviral response were dose-dependent, for both iv and sc dosage forms. Outgrowth of pre-existing CXCR4-using HIV-1 variants was observed in a small percentage (~7%) of subjects enrolled using a first-generation tropism assay, consistent with studies of other CCR5 co-receptor antagonists. No pre-existing R5 resistance to PRO140, i.e., resistance of the R5 HIV-1 strains to PRO140, has been observed, and no emergent R5 resistance was observed despite prolonged virologic suppression (up to six weeks) with monotherapy and slow elimination of drug.

Regarding tolerability, PRO140 was generally well-tolerated when administered via either i.v. or s.c. routes. There were no drug-related serious adverse events and no pattern of toxicity. The administration-site reactions were infrequent, mild, transient and self-resolving. Dr. Olson also mentioned that, to date, no dose-limiting toxicity has been seen in humans or in preclinical studies. The peak and overall exposure increased approximately in proportion with dose and the serum half-life was 3–4 days for both the iv and sc dosage forms. Regarding immunogenicity, anti-PRO140 antibodies were detected infrequently, at low titer and without impact on PK or antiviral outcomes. Dr. Olson noted that the sc dosage route was selected for further development based on its potent efficacy, favorable tolerability and potential for convenient self-administration by patients.

Lioudmila Tchistiakova (Pfizer) presented the development of two anti-IL-13 neutralizing mAbs. Both antibodies inhibit the binding of IL-13 to the (IL-4Rα/IL-13Rα1) heterodimeric receptor. However, one of the mAbs blocks only the binding of IL-13 to the IL-4Rα chain of the receptor, while the other blocks the binding to both the IL-13Rα1 chain of the receptor and to another IL-13 membrane receptor, IL-13Rα, that lacks canonical Jak/STAT signaling functions.[27] Both antibodies demonstrate comparable reduction of lung inflammation, serum titers of Ascaris-specific IgE and Ascaris-triggered basophil histamine release in cynomolgus monkeys.[28] Interestingly, although these two humanized antibodies exhibit the same affinity for IL-13R, only the antibody blocking the binding of IL-13 to IL-4Rα attenuated early and late stages of asthmatic responses following allergen challenge, to a similar degree as leukotriene antagonists, in two clinical Phase 2 studies where mild, atopic asthmatic patients were tested with either one of the two antibodies. Both antibodies had no effect on the level of total IgE in blood and had identical PKs. However, analysis of serum IL-13 levels showed an increase only after infusion of the antibody blocking the IL-13/IL-13Rα2 interaction, suggesting a role of this receptor in the clearance of IL-13 in vivo.

Tony Shock (UCB-Celltech) discussed the use of an anti-human CD22 IgG1 humanized antibody (epratuzumab, licensed from Immunomedics) in patients with moderate-to-severe systemic lupus erythematosus (SLE). CD22 is a member of the SIGLEC family of proteins and is an inhibitory receptor present on B cells that decreases B-cell activation without depleting the B-cell compartment. Epratuzumab stimulates CD22 internalization and decreases the expression of some adhesion molecules on B cells from SLE patients (CD62L and β7 integrin), while the expression of β1 integrin is increased; it cross-reacts with CD22 of non-human primates. Also, it enhances the spontaneous migration of B cells and the migration of $CD27^-$ B cells towards the chemokine CXCL12.[29] Moreover, it induces a modest ADCC in vitro when macrophages are cultured with primary B cells. In vitro, it does not inhibit the IgM and IgG production by human B cells stimulated with pokeweed mitogen (PKW), pansorbin, sCD40L or anti-IgM. Interestingly, it also does not affect the primary and secondary immune responses to specific antigens (keyhole limpet hemocyanin, KLH; tetanus toxoid, TT) in cynomolgus monkeys, although a modest decrease in peripheral B-cell number has been observed. This suggests that the use of epratuzumab in humans will not reduce the antibody response to specific pathogens. Epratuzumab has been tested in a Phase 2b study (EMBLEM™) (n = 227 patients stratified by disease severity). It was well-tolerated, the incidence of serious adverse events and infusion reactions was similar to that of placebo, and it induced clinically meaningful improvements in SLE in patients, e.g., enhanced improvements at week 12.

Finally, **Olaf Stüve** (University of Texas Southwestern Medical Center) presented data on the immunologic and radiologic status of a cohort of patients fourteen months after cessation of natalizumab (Tysabri®) therapy. Natalizumab is a humanized antibody against VLA-4 marketed for treatment of patients with multiple sclerosis (MS). A Phase 2 study failed to demonstrate a difference between natalizumab treatment groups and the placebo group with regard to gadolinium-enhanced lesions on magnetic resonance imaging (MRI) 3 months after discontinuation of therapy. Clinical MS disease activity was thus evaluated in 23 patients with MS after discontinuation of natalizumab therapy over a 14 month period.[30] Surrogate disease markers on MRI, immunologic parameters in peripheral blood and cerebro-spinal fluid (CSF), and safety were also evaluated. Adverse events were monitored. The majority of the patients were found stable for clinical disease activity, neuro-imaging and immune responses. The initial change in the CD4/CD8 ratio and the decrease in lymphocyte cell numbers returned to normal when patients were assessed 14 months after cessation of natalizumab therapy. Furthermore, no infectious complication (notably no progressive multifocal leukoencephalopathy),[31] was observed. An important question to be addressed now is how to define the patients who should be treated continuously vs. those who should receive natalizumab only as an induction therapy.[32]

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Day 3: December 8, 2010

Thierry Wurch and Janice M. Reichert

As was the case on Day 2, the third day of the conferences was divided into two parallel tracks, Antibody Engineering and Antibody Therapeutics, with morning and afternoon sessions for each track. Topics in the Antibody Engineering track included antibody half-life and targeted nanoparticle therapeutics, while topics in the Antibody Therapeutics track included clinical updates of antibody therapeutics and development of biosimilar and biobetter antibodies.

Antibody Engineering: Antibody Half-Life: The Long and the Short of It

The session was chaired by **Dario Neri** (ETH Zurich) and the keynote lecture of the session was delivered by **Sally Ward** (University of Texas Southwestern Medical Center). She first provided a comprehensive overview of the functions and mode of action of FcRn and mAb recognition.[1] This MHC class I type receptor transports immunoglobulin (Ig) G molecules within and across a diverse array of different cell types, allowing the maintenance of IgG levels and the delivery of antigen in the form of immune complexes to degradative compartments within cells.[1] FcRn-antibody interactions are pH-dependent (high affinity at pH 6.0 but low affinity at pH 7.4) and have been mapped essentially to histidine residues on IgGs;[1] this interaction is not dependent on Ig N-glycosylation. IgG with Fc regions engineered to bind with higher affinity and reduced pH dependence to FcRn potently inhibit FcRn-IgG interactions and induce a rapid decrease of IgG levels in mice. Such FcRn blockers, called 'Abdegs' (antibodies that enhance IgG degradation), may have uses in reducing IgG levels in antibody-mediated diseases, and in inducing the rapid clearance of IgG-toxin or IgG-drug complexes.[1] Professor Ward also presented a novel transgenic mouse model, in which this Fc receptor can be conditionally deleted.[2] In combination with mice that express Cre recombinase under the control of the Tie2 promoter (Tie2-Cre), the effect of site-specific deletion of floxed FcRn in endothelial and hematopoietic cells on IgG persistence could be analyzed.[2] The pharmacokinetics and steady-state levels of IgG in Tie2-Cre mice that are homozygous for the floxed FcRn allele reveal a complete loss of FcRn function in regulating the half-lives of wild-type IgG.[2] Novel microscopic imaging techniques have been developed by Professor Ward and colleagues to monitor intracellular events on the recycling pathway that leads from sorting endosomes to exocytosis at the plasma membrane.[3] In this system multiple planes can be simultaneously imaged within the cell in conjunction with visualization of the plasma membrane plane by using total internal reflection fluorescence (TIRP) microscopy. Using this imaging technique, Professor Ward showed that FcRn is transferred from the limiting membrane of such endosomes to lysosomes, and is rapidly internalized into the lysosomal lumen.[3] By contrast, lysosomal associated membrane protein-1 (LAMP-1) persists on the limiting membrane. Receptor transfer is mediated by tubular extensions from late endosomes to lysosomes, whereas full fusion is rarely observed. The persistence of FcRn on the late endosomal limiting membrane, together with selective transfer to lysosomes, allows this receptor to undergo recycling or degradation. Consequently, late endosomes have functional plasticity, consistent with the presence of the Rab5 GTPase in discrete domains on these compartments.[3]

David Szymkowski (Xencor) summarized the improvements observed by the Xtend antibodies resulting from point mutations in the Fc portion of antibodies to improve half-life. This is based on the Xencor proprietary XmAb® Fc domains with a wide range of immunological properties. Nearly 2,000 different Fc variants have been designed and tested on various Fc binding partners (FcγR, C1q, FcRn). Thus an appropriate XmAb Fc can be selected to improve nearly any antibody's half-life. Xtend-Fc relates to mutations that enhanced binding to the receptor FcRn, which results in a 2- to 4-fold increase in in vivo half-life in primates; no loss of antibody stability or disruption of binding to FcγR or protein A has been observed. Validated half-life improvements for several antibodies in primates and huFcRn transgenic mice were presented. For bevacizumab-Xtend, dosage of once every two months achieved the same therapeutic efficacy as dosage of bevacizumab every 1–3 weeks. An improved version of cetuximab was generated by humanizing the variable domain, removal of an N-glycosylation site present in the CDR-H3 of cetuximab, and by applying the Xtend technology, resulting in a large improvement of efficacy. Similar improvements were shown for Xtend-Fc derivatives (or biosuperiors) for adalimumab, abatacept, omalizumab, trastuzumab, tanezumab and rituximab.

Volker Schellenberger (Amunix) discussed the XTEN technology to prolong protein half-life. PEGylation of proteins and peptides is a classical way to extent their half-life. It has been validated clinically since several marketed products are based on this modification. Amunix developed a novel technology based on a polypeptide chain that mimics the properties of PEG. XTEN is 864 AA long, and composed only of the 6 AA Gly, Glu, Ser, Thr, Ala, Pro. Similarly to PEG, XTEN confers long serum half-life in humans, it is non-immunogenic and homogeneous. XTEN is produced recombinantly by genetic fusion to the protein drug of interest without requiring an independent conjugation step during manufacture. Due to XTEN's extended structure, it provides a bulking effect similar to PEG, with associated improvements in payload immunogenicity and stabilization. As an example,

the addition of XTEN to exenatide (Byetta®), a 39 amino acid peptide marketed by Amylin Inc., for diabetes treatment, is expected to lead to improvements: (1) because of much flatter PK profile, the dosing could be changed from twice a day to as little as once a month, (2) exenatide causes a high frequency of nausea and vomiting attributed to the rapid, sharp concentration changes, which should be decreased due to the much flatter PK profile of the XTEN product, (3) exenatide is made by a complex synthetic chemical process, but the XTEN fusion product is produced as a recombinant protein and can be concentrated to more than 60 mg/ml, (4) exenatide causes an immune response in 60% of patients, whereas the XTEN fusion is expected to be less immunogenic.

A novel antibody format developed by UCB CellTech was presented by **David Humphreys** (UCB CellTech). They reasoned that antibody therapeutics should start from Fab molecules, as they are robust and more stable than their IgG parent. IgG unfolding transitions are lower than that of the Fab, with the antibody CH2 domain being the "soft spot" of IgG1 and IgG2. However, the major drawback for therapeutic use of Fab fragments is their short half-life. This can be modulated by PEGylation or by adding a domain for binding to human albumin. Dr. Humphries presented a Fab-Fv fragment that carries an albumin-binding domain in addition to the classical antigen binding site, created by adding the VL of an albumin-binding antibody to the C-terminus of the C-κ domain, and its VH domain to the C-terminus of the CH1 domain of a recombinant Fab fragment. An additional cysteine bridge within this additional Fv domain was included in some constructs. Anti-albumin antibodies were generated in rabbits, two candidates (CA645 and CA648) were selected, which yielded nanomolar binding affinities to human, rat and mouse serum albumin, and their VH/VL domains were cloned into a Fab-Fv scaffold. These fusions showed a 20-fold lower clearance rate in mice as compared to the Fab; Fab-645 yielded a half-life of 62 h in mice, corresponding to about 1 week in human. In vivo efficacy of Fab-Fv was similar to Fab-PEG in a model of inhibition of human peripheral B lymphocytes engraftment in SCID mice. Improvement of the actual Fab-Fv format is ongoing with engineering of cysteines for interdomain disulfide bonds and design of alternative linkers (5–25 AA) between the Fab and Fv domains.

By applying the domain antibody (dAb) technology developed by Domantis (acquired by GlaxoSmithKline in 2006), **Oliver Schon** (GlaxoSmithKline) demonstrated how an human albumin-binding dAb could be applied to improve the half-life of peptides and proteins. AlbudAbs are albumin-binding dAbs: by binding to serum albumin, AlbudAbs decrease clearance of therapeutic drugs covalently linked to them.[4] This allows for tighter control of drug concentration in patients over longer dosing intervals. The coding sequence of an antagonistic dAb was genetically fused to that of an AlbudAb to generate a fusion protein with monovalent engagement with target and with tunable affinity of both partners of the fusion. No Fc-mediated effects are expected for such a fusion molecule, e.g., ADCC, CDC. Several families of AlbudAbs against rat serum albumin were generated, with nanomolar binding affinities. They were fused to interferon α-2B for functional efficacy evaluation in an A543 SCID mouse xenograft model (survival model);[4] significant survival was achieved with the DMS7322 AlbudAb derivative. This candidate also showed good binding to human and cynomolgus serum albumins, and was used to design a long-acting version of the 40 AA long peptide, exendin-4, a GLP-1 receptor agonist used in the treatment of obesity and diabetes. Several fusion proteins between exentin-4 and various AlbudAbs were constructed, produced and evaluated for their binding to albumin and potency to activate GLP-1R, with the best candidates being evaluated in relevant mice models. Overall, exendin-4 AlbudAbs were effective in pre-clinical models: they inhibited feeding for three days following a single dose and gave significant weight loss in diet-induced obese (DIO) mice, and they showed activity in a db/db mouse model of type II diabetes.

Dario Neri (ETH Zurich) presented data on antibody engineering to modulate PK by chemical modifications or gene fusions. The prototypical mAb used in these different studies is a scFv fragment, directed against the ED-B domain of oncofetal fibronectin, an isoform specifically associated with tumor vasculature and termed L19.[5] This antibody fragment has been conjugated to many different payloads, and Professor Neri discussed some of these.

Example 1. This case-study dealt with an iodinated L19 derivative currently developed by Bayer Schering Pharma. Three ED-B-derivatives of L19 were investigated:[6] dimeric single-chain Fv, "small immunoprotein" (SIP), and whole IgG1. These L19 derivatives were labeled with I-125 or with In-111 and evaluated in F9 (mouse embryonal teratocarcinoma cells) tumor bearing mice. The most favorable therapeutic index was found for I-131-L19-SIP followed by I-131-L19-IgG1.[6] The therapeutic index of all In-111-labeled derivatives was inferior. The best therapeutic efficacy was observed using I-131-L19-SIP, resulting in significant tumor growth delay and prolonged survival after a single injection.[6]

Example 2. L19 scFv was fused to interleukin-2 (IL2) and showed potent and efficacious activity in several orthotopic mouse models. Professor Neri presented data from a completed Phase 1/2 clinical study that evaluated the safety, tolerability, recommended dose and early signs of activity of L19-IL2 in a total of 21 patients with progressive solid tumors; the study was expanded to specifically include patients with metastatic renal cell carcinoma (mRCC).[7] The recommended dose was defined to be 22.5 Mio IU IL2 equivalents.[7] The pharmacokinetic parameters of L19-IL2 were dose proportional over the tested range, with a terminal half-life of 2–3 h. Toxicities were manageable and reversible with no treatment-related deaths.[7] Stable disease was observed in 51% of patients overall and 83% of mRCC patients after two cycles.[7]

Example 3. This example involved the portable albumin binder (AlbuFluor). The site-specific chemical modification {2-(3-maleimidopropanamido)-6-[4-(4-iodophenyl)butanamido]hexanoate albumin-binding moiety} of a C-terminal cysteine residue in scFv antibody fragments was presented:[8,9] a small organic molecule capable of high-affinity binding to serum albumin could substantially extend serum half-life in rodents.[9] The strategy was implemented using the scFv fragment F8, specific to the alternatively spliced ED-A domain of fibronectin, another tumor-associated antigen. The chemically modified

scFv-F8 antibody fragment exhibited a dramatic increase in tumor uptake.[8,9]

Antibody Engineering: Targeted Nanoparticle Therapeutics

The session was chaired by **James Huston** (Boston Biomedical Research Institute), and the first lecture was given by **John Park** (University of California San Francisco Comprehensive Cancer Center), who discussed immunoliposomes (IL) in cancer treatment. He first provided an overview of the current knowledge on nanoparticles/lipoparticles loaded with cytotoxic payloads, but without site-selective targeting. The most advanced compound, nanoliposomal irinotecan (CPT-11) showed markedly superior efficacy when compared with free CPT-11 in human breast (BT474) and colon (HT29) cancer xenograft models.[10] Additional improvements in nanoparticle treatments should be achieved with the addition of molecular targeting, e.g., using liposomes linked to ligands such as monoclonal antibody fragments directed against cancer-associated antigens. IL combine antibody-mediated tumor recognition with liposomal delivery and, when designed for target cell internalization, provide intracellular drug release.[11] Recent advances in IL design include rapid selection of phage antibody-derived scFv for targeting, and methods for conjugation of ligands to existing approved liposomal drugs such as PEGylated liposomal doxorubicin (Doxil®). A first example of an IL was presented as a novel anti-HER2 scFv F5 conjugated to Doxil®[12] that is currently in development. It selectively binds to and is internalized by HER2-overexpressing tumor cells. This IL yielded increased efficacy in a variety of preclinical mouse models including breast cancer (BT474, MCF7/HER2, MDA453, B585) and other HER2-overexpressing cancers (CALU-3, N87).[12] Liposome-antibody-targeting of long circulating liposomes does not increase tumor localization, but does alter microdistribution and enables tumor cell internalization in vivo.[12] The second example focused on an EGFR-targeted IL. Superior in vivo efficacy was established for glioma (U87, U87/EGFRvIII), breast carcinoma (MDA468), lung (NSCLC, A549), colorectal and drug-resistant cancers (MDA231R overexpressing MDR1). The last topic dealt with the identification/selection of novel scFv candidates with the primary goal of achieving internalization.[13] Primary panning was performed on MDA-MB231 cells (basal type) and counter-selection was applied versus luminal breast cancer cells (i.e., MDA-MB 453).

The next presentation by **Kerry Chester** (University College London) introduced a novel and original application of iron oxide nanoparticles for imaging and cancer therapy. Superparamagnetic iron oxide nanoparticles (SPIONs) can substantially improve the sensitivity of magnetic resonance imaging (MRI), therefore SPIONs could be used to target and image cancer cells if functionalized with recombinant single chain Fv antibody fragments (scFv). This hypothesis was tested by generating antibody-functionalized SPIONs using a scFv specific for carcinoembryonic antigen (CEA), an oncofetal cell surface protein. SPIONs of different hydrodynamic diameter and surface chemistry were investigated and targeting was confirmed by ELISA, cellular iron uptake, confocal laser scanning microscopy (CLSM) and MRI. The presented data demonstrated that antibody-functionalized-SPIONs bound specifically to CEA-expressing human tumor cells, generating selective image contrast on MRI.[14] In addition, the cellular interaction of the antibody-functionalized-SPIONs was influenced by hydrodynamic size and surface coating.[14]

Theresa Allen (Center for Drug Research and Development) presented an overview on the use of nanoliposomes targeted via scFv and their use in cancer therapy. Targeted liposomal drugs represent the next step in the evolution of liposomal drug delivery in cancer treatment. In various preclinical cancer models, antibody-targeted PEGylated liposomal drugs have demonstrated superior therapeutic effects over their non-targeted counterparts.[15] ScFv has gained popularity as a targeting agent of choice over other fragments or whole Ab. For clinical development, scFv are potentially preferred targeting agents for PEGylated liposomes over mAb and Fab', owing to factors such as decreased immunogenicity and pharmacokinetics/biodistribution profiles that are similar to non-targeted PEGylated (Stealth) liposomes.[15] In a first example, long-circulating (Stealth) immunoliposomes (SIL) that were targeted against the B-cell antigen CD19, via a whole HD37 monoclonal antibody (HD37 mAb) were described.[16] Compared to untargeted liposomes, SIL showed increased binding in vitro to CD19-expressing Raji cells and, when loaded with doxorubicin (SIL-DXR), increased cytotoxicity against Raji [CD19(+)], but not Molt4 [CD19(-)] cells. Pharmacokinetics and biodistribution studies showed that SIL-DXR targeted via HD37 Fab' exhibited the same long circulation half-life as SL-DXR.[16] All SIL-DXR extended the mean survival time of Raji-bearing mice compared to SL-DXR or free DXR. SIL-DXR targeted via HD37 Fab' had the longest circulation half-life, and appeared to be slightly more effective in prolonging survival times than SIL-DXR targeted via either HD37-c-myc-Cys-His5 single chain Fv fragment or HD37 mAb.[16] In a second example, Her2-targeted SIL were described. In a murine breast cancer model, the rate and the extent of bioavailability of doxorubicin entrapped in liposomes targeted by a single-chain antibody fragment against the HER2/neu antigen was compared to free DXR and non-targeted liposomal doxorubicin (Doxil®). Breast cancer tumors contained the highest total levels of doxorubicin and the highest levels of bioavailable doxorubicin when anti-HER2/neu-targeted liposomes were used, and the targeted liposomes also resulted in the greatest level of tumor control.[17]

James Paulson (The Scripps Research Institute) presented a novel way of targeting specifically B lymphoma cells using glycan ligands of CD22. CD22 is a member of the sialic acid-binding Ig-like lectin (SIGLEC) family that is known to be a regulator of B-cell signaling. Its B-cell-specific expression makes it an attractive target for immunotoxin-mediated B-cell depletion therapy for the treatment of B-cell lymphomas and autoimmune diseases. CD22 is well described as an endocytic receptor and that, after internalization, it is targeted for degradation. In contrast, Professor Paulson and colleagues have demonstrated CD22 is instead constitutively recycled to the cell surface.[18] The glycan ligand-based cargo attached to CD22 is released and accumulates intracellularly as CD22 recycles between the cell surface

and endosomal compartments.[18] Antibodies to CD22 do not accumulate but remain bound to CD22 and recycle to the cell surface. Thus, antibodies targeting CD22 glycan were developed in an IL approach using Doxil®. The targeted liposomes were actively bound and endocytosed by CD22 on B cells, and significantly extended life in a xenograft model of human B-cell lymphoma.[19] Moreover, they bound and killed malignant B cells from peripheral blood samples obtained from patients with hairy cell leukemia, marginal zone lymphoma and chronic lymphocytic leukemia.[19] Siglec-1 or sialoadhesin may also be an interesting target for IL therapy. It exhibits highly restricted expression on tissue and inflammatory macrophages and on activated monocytes. This target might have an impact in the treatment of inflammatory responses that promote rheumatoid arthritis and tumor metastasis.

The last presentation of this session was given by **Subhash Chauhan** (University of South Dakota), who presented novel strategies for sensitizing cancer cells, with applications in ovarian cancers. Polymer micelle nanotechnology aims to improve the therapeutic efficacy of anti-cancer drugs while minimizing their side effects, and it can be applied with a range of chemotherapeutics. Different types of polymer micelle technology based nanotherapies were presented, e.g., poly(lactic-co-glycolide) (PLGA); polymerosomes; acid cleavable, thermosensitive, pH sensitive and cross-linked micelles.[20] An important feature of polymer micelle nanotechnology is the small size (10–100 nm) of particles, which improves circulation and enables superior accumulation of the therapeutic drugs at the tumor sites.[20] One example developed is based on curcumin, a natural polyphenolic compound that has shown promising chemopreventive and chemotherapeutic activities in cancer.[21] But curcumin showed poor bioavailability and suboptimal pharmacokinetics that largely moderated its anti-cancer activity in pre-clinical and clinical models. Thus curcumin was encapsulated in PLGA nanoparticles, in the presence of poly(vinyl alcohol) and poly(L-lysine) stabilizers, using a nano-precipitation technique.[21] These curcumin nano-formulations were characterized for particle size, zeta potential, drug encapsulation, drug compatibility and drug release. Encapsulated curcumin existed in a highly dispersed state in the PLGA core of the nanoparticles and exhibited good solid-solid compatibility. An optimized curcumin nano-formulation (nano-CUR6) has demonstrated two- and six-fold increases in cellular uptake performed on A2780CP ovarian and metastatic MDA-MB-231 breast cancer cells compared to free curcumin.[21] In these cells, nano-CUR6 has shown an improved anti-cancer potential in cell proliferation and clonogenic assays compared to free curcumin. This effect was correlated with enhanced apoptosis induced by nano-CUR6.[21] Next, conjugation of the nano-CUR6 formulation to two antibodies targeting either transferrin or anti-TAG-72 (CC49) was presented. Western-blot confirmed antibody conjugation and imaging data demonstrated improved tumor specific targeted delivery of nano-CUR6 anti-cancer drug.[21]

To relate nanoparticles with the more classical antibody therapeutics, **Thierry Wurch** (Institut de Recherche Pierre Fabre, Centre d'Immunologie Pierre Fabre) presented novel data on the influence of the antibody hinge region and the control of its intrinsic activity. The particular hinge region provides structural flexibility to both variable and constant domains via Fab arm rotation and waving and Fc rotation and controls Fab-Fc planar folding. Numerous Cys residues are involved in interchain (H-H or H-L) bonds. These major structural features are directly associated with the primary amino-acid sequence of the hinge region and thus to the antibody isotype. Numerous mutants or chimeric hinge regions (by swapping portions from different Ig isotypes and especially human IgG1 and IgG3) have been constructed and evaluated for their effector functions. A clear implication of the lower hinge portion (part of the CH2 domain based on genetic criteria) in complement activation was demonstrated. Some mutants located in the middle hinge portion affected FcγR binding and ADCC. On the other hand, much less is publicly known on the role of the hinge region in antigen binding.

Dr. Wurch and colleagues constructed a large series of mutants in this hinge region that modulate the flexibility and rigidity of the Fab portion; mutations included insertion of additional cysteine residues and amino acid deletions (1 to 4). These modifications were associated with a strong impact on the intrinsic activity of the resulting antibody mutants towards the target antigen: both agonistic and antagonistic activities were strongly modulated, ranging from weak to strong partial agonist and weak to efficacious antagonist. Introduction of additional cysteine residues was in some cases associated with mispairing of heavy and light chains, as was already noted for wild-type human IgG2 mAbs.

Antibody Therapeutics: Clinical Updates of Antibody Therapeutics 2

The keynote address for the Wednesday morning session was given by **Barbara Rellahan** (Division of Monoclonal Antibodies, US Food and Drug Administration), who discussed new and emerging mAb and mAb-related products such as bispecific antibodies, mAb fragments and alternative scaffold proteins. She noted that the objective of targeting two antigens can be achieved by either one bispecific mAb or a cocktail of two mAbs, and that FDA does not have a preference for development of one type of product over the other. FDA's guidance for industry that discusses nonclinical safety evaluation of drug or biologic combinations does generally recommend testing of each new entity individually, but indicates that if mAb are to be marketed together only, it may be possible to conduct studies only on the combination. Dr. Rellahan explained that there are advantages to both types, e.g., bispecific mAbs may have simpler manufacturing processes, but cocktail products can be evaluated individually for activity and safety, and their ratio can be adjusted accordingly.

Dr. Rellahan noted that antibody fragments and scaffold proteins may have advantages, but the possible disadvantages of engineered binding proteins should not be overlooked, e.g., there may be less knowledge on the structure/function relationship, there may be greater off-target effects because the level of specificity may not be the same as a mAb, and immunogenicity may increase due to novel amino acid sequences in the protein. She expanded on the topic of immunogenicity by first noting

that, while in general the immunogenicity of chimeric, humanized and human mAbs is less than that of murine versions, there is still substantial variation seen between individual candidates. For example, immunogenicity rates of 55.6, 100 and 80% have been observed for specific chimeric, humanized and human (phage display-derived) mAbs. Dr. Rellahan noted that the lack of a sensitive assay may have led to an under-estimation of mAb immunogenicity for many products, and of particular concern are assays that are sensitive to product interference. She suggested that sponsors should not underestimate the importance of understanding product immunogenicity and the consequences of it on safety and efficacy, and that, if feasible, measures should be taken to reduce product immunogenicity, e.g., "de-immunizing" products, minimizing protein aggregates/particulates. She also noted that sponsors should develop a sensitive anti-drug antibody (ADA) assay, i.e., one able to detect ADA at levels of product present in patient samples, as early in development as possible, and that the immune response should be characterized, e.g., neutralizing or not, region of mAb the ADA is directed against should be identified.

In her concluding remarks, Dr. Rellahan provided suggestions for how sponsors can facilitate the review of novel proteins, e.g., request interaction with FDA early in development; for pre-IND candidates, specifically request product quality reviewer or ask CMC questions if input is needed; clearly describe the product in the submission cover letter; take advantage of all opportunities to interact with the FDA to gain feedback on your proposed development pathway.

David Stover (Agensys/Astellas) provided an update on ASG-5ME, which is an antibody-drug conjugate (ADC) that targets SLC44A4. The antigen is a novel cell surface transporter that is a putative 10 transmembrane protein (710 amino acids) with an anion exchanger motif. SLC44A4 is homologous to human choline transporter and is overexpressed in multiple solid tumors, e.g., prostate and pancreatic cancer. ASG-5ME comprises a human IgG2kappa mAb derived from the XenoMouse® technology that is conjugated to monomethyl auristatin E (MME; 3.7 drugs/mAb) through a protease cleavable dipeptide (valine-citrulline) linker. Its high affinity to SLC44A4 (0.4 nM) is similar to that of the naked AGS-5M2; the ADC cross reacts with cynomolgus monkey ortholog. Dr. Stover explained that, after ASG-5ME binds to cells, the ADC is internalized via endocytosis and the MME is released into the cell via enzymatic cleavage of the linker. The MME is then available to bind to tubulin, which causes cell cycle arrest at G_2/M and leads to apoptosis.

Dr. Stover presented data showing ASG-5ME mediates cytotoxicity in vitro and potent anti-tumor activity in xenografts that express the antigen. The terminal half-life estimates for ASG-5ME were 13.3 days in mouse (estimated from 3 mg/kg single dose), 15.2 days in rat (estimated post multiple 3 mg/kg doses) and 8.4 (±0.79) days in monkey (estimated from 3 mg/kg single dose). Dr. Stover noted that the ADC had excellent PK properties that were not significantly different from the naked mAb. GLP toxicity studies in cynomolgus monkeys suggest that the limiting toxicity is non-specific. ASG-5ME is currently in a dose escalation, Phase 1 study (NCT01228760) of the safety and pharmacokinetics of ASG-5ME monotherapy in patients with advanced prostate cancer, and a dose escalation, Phase 1 study [NCT01166490] to evaluate the safety and tolerability of ASG-5ME and to identify the maximum tolerated dose in patients with pathologically confirmed metastatic pancreatic adenocarcinoma.

The clinical proof-of-concept (POC) strategy applied to date for development of U3-1287 (AMG 888) was presented by **Thore Hettmann** (U3 Pharma GmbH/Daiichi-Sankyo). U3-1287 (AMG 888) targets HER3, which is a key dimerization partner for HER receptors. HER3 directly binds PI3K and induces PI3K/AKT signaling, and is implicated in resistance mechanisms to anti-HER therapeutic agents.[22] U3-1287 (AMG 888) is a human IgG1 derived from XenoMouse® technology with Kd in the range of 1–3 nM, cross-reactivity in cynomolgus, rat and mice, and a clean safety profile (no observed adverse effect level at 200 mg/kg in monkey and rat). The mAb inhibits proximal and distal HER signaling and induces rapid internalization of HER3.

Dr. Hettmann explained that the strategy for development included identification of appropriate indications, identification of therapeutic combinations to overcome acquired resistance and identification of predictive and prognostic biomarkers. He then described key features of the first clinical study. The IND was filed in May 2008 and a dose-finding Phase 1 study [NCT00730470] to assess the safety and tolerability of U3-1287 (AMG 888) in patients with advanced solid tumors was initiated in September 2008. The study was composed of two parts: (1) dose escalation phase (15–21 patients) and (2) dose expansion phase with an adaptive design (30 patients, 15 with non-small cell lung cancer and 15 with other solid tumors as in Part 1). In the study, U3-1287 (AMG 888) appeared to be safe and well-tolerated with an adverse event profile as expected in patients with advanced solid tumors. In part 1, 50% of patients had a best overall response of stable disease. The drug exhibited non-linear PK, presumably mediated by dual linear and nonlinear clearance pathways. After the first dose, at doses above 3 mg/kg, the Cmax and AUC increased in an approximately dose-proportional manner. The maximum tolerated dose was 20 mg/kg in Part 1 of the study. No neutralizing anti-drug antibodies have been detected to date. Dr. Hettmann concluded by remarking that the combination of U3-1287 (AMG 888) and erlotinib is currently undergoing evaluation in a Phase 1b/2 study (NCT01211483) of EGFR treatment naïve subjects with advanced non-small cell lung cancer who have progressed on at least one prior chemotherapy.

Lessons learned and questions remaining about development of human IgG1 antibodies targeting insulin-like growth factor 1 receptor (IGF1-R) for treatment of sarcomas were discussed by **Lee Helman** (National Cancer Institute, National Institutes of Health). He first noted that IGF signaling provides a survival signal that contributes to tumor cell resistance to DNA-damage induced cell death. This resistance is associated with mammalian target of rapamycin (mTOR) signaling and can be reversed with agents that block mTOR; conversely, mTOR activation of Akt can be reversed by IGF1-R blockade. Dr. Helman then reviewed preclinical studies with anti-IGF1-R antibodies that provided early evidence to suggest a beneficial combination of mTOR inhibition with IGF1-R inhibition. The effects

of IGF1-R inhibition were found to correlate with IGF1-R, i.e., low levels were a negative predictor. In addition, the effect of IGF1-R blockade on the decrease in pAkt was lost in long-term xenografts,[23] and this tachyphylaxis was abrogated with mTOR inhibition, leading to the question: What is the mechanism of tumor regrowth?

Top-level results of a Phase 2 study (NCT00642941) of the anti-IGF1-R human R1507 mAb administered to patients with recurrent or refractory Ewing's sarcoma (ES), osteosarcoma, synovial sarcoma, rhabdomyosarcoma (RMS) and other sarcomas were presented by Dr. Helman. The study is ongoing but not recruiting patients as of December 2010. The overall objective response rate for 115 eligible patients with refractory ES who were administered R1507 was 16.5% (11 durable, 8 short-lived). Toxicities observed included thrombocytopenia (7%), anemia (7%), pain (7%) and hyperglycemia (3%). Dr. Helman noted that response did not correlate with IGF1-R, IGF1, IGF2, IGF2-R, IGF binding proteins-2, 3, 4, insulin receptor A or insulin receptor B as assessed by quantitative real time polymerase chain reaction. The analysis of responders and non-responders for circulating nucleic acids, methylation patterns, RNA expression profiles and targeted sequencing is ongoing. Dr. Helman discussed the need to identify mechanisms of early response and then tumor re-growth and find ways to combine R1507 with other targeted therapies or chemotherapy. He concluded with remarks on a recent study by Huang et al. that defined and compared acquired resistance mechanisms for IGF-IR-targeted therapies.[24]

Antibody Therapeutics: Development of Biosimilar and Biobetter Antibodies

Mark Cragg (Southampton University) discussed CD20 as a target and why Type II anti-CD20 mAbs might make better therapeutics than Type I versions. Professor Cragg started by posing several critical questions: Are all anti-CD20 mAb equal? What are the critical effector mechanisms? What regulates the sensitivity of different diseases to anti-CD20? He then explained the differences between Type I mAbs, which include the marketed products rituximab, ofatumumab, as well as mAbs LT20, 1F5 and AT80, and Type II mAbs such as tositumomab, FGM1 and GA101. Type I mAbs induce TX-100 insolubility, i.e., CD20 translocation into TX-100 rafts and activate complement extremely efficiently, while Type II mAbs induce homotypic adhesion and cell death.[25-27]

Professor Cragg noted that Type I mAbs display good CDC, limited PCD and good ADCC, while Type II mAbs display poor CDC, good PCD and good ADCC. However, in vivo comparisons to determine which type is best are difficult due to differences in the human vs. mouse mAbs isotypes, e.g., in effector functions, half-life and potential generation of mouse anti-human antibodies, and due to problems with xenografts, e.g., small numbers of cells, human-mouse reaction and protection of human cells from mouse complement.[27]

Professor Cragg discussed a preclinical huCD20 transgenic mouse model developed by Professor Mark Shlomchik at Yale University that faithfully recapitulates the normal human B-cell counterpart. He presented data that indicated that Type II mAbs are superior to Type I mAbs in depleting B cells,[27] and that complement was not important, but FcγR are critical for both Type I and II mAbs.[28] He then presented experimental results for Type I anti-CD20 mAbs that indicated these mAbs internalize into cells and become degraded along with CD20 in an energy and temperature dependent process. Specifically, after internalization, the Type I mAbs traffic to the early endosome and lysosomal degradation follows. Modulation of CD20 by the Type I mAbs ultimately leads to reduced phagocytosis of tumor cells as well as consumption from the sera.[28]

He then presented data indicating that the modulation rate of CD20 from the surface of different NHL sub-types might be linked to the efficacy of rituximab in the clinic, with those sub-types modulating rapidly doing less well clinically. Finally, Professor Cragg, presented data indicating that the inhibitory FcγRIIB is a key component of this process, precipitating the internalisation of rituximab in a cis-fashion from the surface of the same cell. He showed that Type II mAbs do not engage FcγRIIB and so are not regulated by this process. Professor Cragg concluded by stating that Type II mAbs have better therapeutic potential compared with Type I versions because they do not modulate and are therefore not consumed or removed from the cell surface.

A comparison of the EGFR antibodies zalutumumab (HuMax-EGFR, Genmab), cetuximab (Erbitux®, Merck KGaA), panitumumab (Vectibix®, Amgen) and nimotuzumab (Biomab-EGFR/TheraCIM/Theraloc, Biocon/YM Biosciences/Oncosciences) was presented by **Paul Parren** (Genmab).

He first described the characteristics of the EGF receptor (EGFR), which is a 170 kDa Type I glycoprotein. EGFR is a tyrosine kinase growth factor receptor that binds EGF, as well several other EGF-like ligands. Signaling by EGFR plays a role in cell proliferation, differentiation and migration of normal cells and the receptor is frequently overexpressed in many tumors and associated with poor prognosis. Dr. Parren briefly reviewed format and the most advanced phase for the four EGFR antibodies: (1) zalutumumab is a human IgG1 in Phase 3 studies of patients with head and neck cancer; (2) cetuximab is a chimeric IgG1 marketed as a treatment for head and neck cancer, and colorectal cancer; (3) panitumumab is a human IgG2 marketed as a treatment for colorectal cancer; and (4) nimotuzumab is a humanized IgG1 marketed for head and neck cancer, and glioma, although it was not marketed in the US, Europe or Japan as of December 2010. All four antibodies bind a closely related, overlapping epitope on EGFR domain III. Only zalutumumab and nimotuzumab bind non-overlapping epitopes and are able to bind simultaneously to EGFR.

Dr. Parren provided specific details for zalutumumab, which was generated in a transgenic mouse (HuMAb-Mouse™), and selected for high affinity binding to human EGFR and effective inhibition of ligand binding (EC_{50} = 0.25 nM by ELISA). In terms of its in vitro concentration-effect relationships, inhibition of signaling by zalutumumab is most effective at saturating concentrations, induction of ADCC already at low concentrations, and overall, target saturation ensures maximum efficacy.

The dose-response relationship of zalutumumab treatment using different dosing schedules (10, 30 or 100 mg/kg on days 20 and 34 or repeated dosing at 100 mg/kg) was studied in a subcutaneous A431 tumor xenograft model.[29] Dr. Parren noted that a high dose level was required for full EGFR saturation. Effective eradication of established xenografts was observed with administration of zalutumumab on the repeated high dose schedule, and the in vivo effect was greater than expected from the in vitro results.

In comparing zalutumumab with cetuximab, panitumumab and nimotuzumab, Dr. Parren presented data that showed that these mAb differ in their efficacy to inhibit EGFR signaling and recruitment of effector cells for ADCC. In vitro, zalutumumab was significantly more potent in downmodulation of EGFR than the other mAb. Zalutumumab and cetuximab demonstrated comparable inhibition of A431 tumor cell growth and induction of ADCC and were in both assays significantly more potent than nimotuzumab and panitumumab. Specifically, panitumumab was as effective in recruiting ADCC by neutrophils and monocytes, but less effective in NK cell-mediated ADCC, compared with zalutumumab.[30] He noted that panitumumab-induced ADCC is mediated via FcγRIIa and affected by the functional FcγRIIa-R131H polymorphism, but IgG1 mAb are not affected. In established A431 xenograft models, tumor growth was inhibited by 50 mg/kg i.p. doses of zalutumumab or cetuximab, but not by panitumumab and nimotuzumab administered at the same dose.

Finally, Dr. Parren presented data that combination treatment with a mixture of zalutumumab and nimotuzumab resulted in enhanced anti-tumor efficacy, being significantly more effective than the single agents or other EGFR mAb combinations. Solely the combination of the non-cross-blocking mAbs zalutumumab and nimotuzumab resulted in enhanced anti-tumor efficacy, being additive for EGFR downmodulation and tumor cell growth inhibition, and synergistic for induction of CDC. In vivo evaluation of zalutumumab/nimotuzumab combination therapy revealed that combining these therapeutics significantly improved tumor growth reduction over single agent treatment, eradicating 8 of 10 tumors in a therapeutic A431 xenograft model. Interestingly, due to the selective binding characteristics of nimotuzumab the enhanced anti-tumor effects of the zalutumumab/nimotuzumab combination seems preferentially targeted to EGFR-overexpressing cells.

Antibody Therapeutics: Regulatory and Intellectual Property Issues for Biosimilar Antibodies

Antoinette Konski (Foley & Lardner LLP) reviewed the new US legislation on biosimilar products, which is officially titled the Biologics Price Competition & Innovation Act of 2009 (BPCI Act). The Act amended the Public Health Service Act (PHSA) to create a new pathway for the approval of biological products that are biosimilar to an approved reference product or biosimilar and interchangeable with an approved product; the Act became effective as of March 23, 2010 with full retroactivity. It applies to biologics already approved under the PHSA, i.e., any products for which a biologics license application (BLA) was filed, as well as biologics now under review. According to PHSA section 351(i), BLAs are required for "a virus, therapeutic serum, toxin, antitoxin, blood or blood component or derivative, allergenic product or analogous product…applicable to the prevention, treatment or cure of a disease or condition of human beings." The new legislation amends this definition to include "protein (except any chemically synthesized polypeptide)."

The definitions of "biosimilar" and "interchangeable" biosimilar products were provided by Ms. Konski. A biosimilar is highly similar to the reference product notwithstanding minor differences in clinically inactive components, with no clinically meaningful differences in terms of safety, purity and potency. An interchangeable biosimilar is a biological product that "may be substituted for the reference product without the intervention of the health care provider who prescribed the reference product."

Ms. Konski then reviewed three basic elements for a US biosimilar application: (1) analytical data showing the product is "highly similar" to a reference product despite "differences in clinically inactive components;" (2) animal studies, including the assessment of toxicity; (3) clinical study(ies) sufficient to demonstrate "safety, purity and potency in one or more appropriate conditions for use" that parallel an approved use of the reference product, although Ms. Konski noted that the FDA may determine that any of these three elements is not required in a given case. Other basic requirements are that the biosimilar have the same mechanism of action as the reference product (if known) for the approved indication, the label for the biosimilar must match the approved indication of the reference product, the route of administration, dosage form and strength must match the reference product, and the product must be produced in an approved manufacturing facility.

Under the standard for approval, the FDA will approve the biosimilar application if the product is found to be "biosimilar" and it can be expected to produce the "same clinical result as the reference product in any given patient." Ms. Konski noted that, under the standard for approval of an interchangeable biosimilar product, FDA will label a biosimilar as interchangeable if it meets the standard for approval and, additionally, for a biological product that is administered more than once, the risk of safety or diminished efficacy of switching between the reference product and interchangeable is not greater than using the reference product without the switch.

An important element of the legislation is the market exclusivity period for the reference product, which was set at 12 years, i.e., no biosimilar product will be approved before the expiration of the 12 year period. The market exclusivity period starts on the date of first approval of the reference product. There is also a four year data exclusivity period that starts on the date of first approval; no biosimilar application will be accepted by FDA during this period. Ms. Konski noted that innovators may obtain an additional six month pediatric exclusivity period based on similar provisions for small molecule drugs. The six month period is added to the four year filing period, i.e., no biosimilar application may be filed before 4.5 years have elapsed after first approval of the reference product, and to the 12 year data exclusivity period, i.e., no biosimilar application may be

approved before 12.5 years have elapsed after first approval of the reference product.

Regarding the establishment of guidance documents for biosimilars, Ms. Konski stated that the FDA may issue guidance, but it is not required. FDA must allow public comment on any guidance before issuing final guidance. If product class-specific guidance is issued, the FDA must include a description of the criteria they will use to determine whether a product is "highly similar" and the standards they will use to determine interchangeability. She also noted that FDA may indicate in guidance that "science and experience, as of the date of such guidance" will respect to a product or class does not allow approval of an application, but that FDA may subsequently modify or reverse such a guidance document.

Recent regulatory developments pertaining to biosimilar antibodies were reviewed by **Timothy Shea** (Sterne, Kessler, Goldstein & Fox P.L.L.C.). His main topics were the November 2010 FDA public hearing on the BPCI Act and the European Medicine Agency's draft guideline for biosimilar antibodies, which was issued in November 2010.

The FDA public hearing was held November 2–3, 2010 with the stated aim being "to receive information and comments from a broad group of stakeholders… regarding implementation of the BPCI Act". A total of 40 speakers presented prepared statements to a panel of FDA experts and FDA accepted additional submissions on the topic until December 31, 2010. Mr. Shea noted that most of the public comments focused on five issues: (1) clinical trials, (2) an interchangeability standard, (3) naming, (4) extrapolation and (5) foreign studies.

As described by Mr. Shea, the general consensus among presenters at the hearing was that some clinical trials are needed, but they differed in the number and type of studies to be done. The major pharmaceutical firms generally favored larger and more sophisticated studies while the generics firms suggested fewer and more limited studies would be suitable. On the topic of an interchangeability standard, several patient advocacy groups and the Biotechnology Industry Organization (BIO) stated that this was not currently possible. Questions arose around whether the exact same clinical results would be necessary; would switching studies be needed?; would the same standards be applied as those applied when there were changes to innovator product?; is automatic substitution possible? On the topic of naming, discussion at the hearing included what nonproprietary name would be given to biosimilar/interchangeable product, with the Pharmaceutical Research and Manufacturing Association (PhRMA) biopharmaceutical companies and patient advocacy groups arguing for a unique name and generic companies favoring use of the same nonproprietary name for the products. On the question of extrapolating data from clinical study for one approved indication to another, BIO indicated extrapolation may be appropriate if the mechanism of action is well-understood, although a biopharmaceutical firm indicated that extrapolation between diverse indications such as rheumatoid arthritis and cancer was not feasible. A generics firm indicated that data from a single randomized, controlled clinical study should be extrapolated to all indications. Regarding foreign, i.e., non-US, studies, questions arose regarding whether these studies would be acceptable if the "drug substance" was identical; if the same patient population, dosage and route of administration were included; and if the same mechanism of action was involved.

Mr. Shea then discussed the EMA's draft guideline on similar biological medicinal products containing monoclonal antibodies,[31] which was released for consultation on November 18, 2010. EMA will be accepting public comment on the guideline until May 31, 2011. Mr. Shea noted that the guideline states it is intended to complement earlier general guideline for demonstrating biosimilarity of two products, and that "biobetters", i.e., biologicals that are structurally or functionally altered in comparison to already licensed reference products to gain an improved or different clinical performance, are specifically excluded. The biosimilar antibody must not be inferior to the reference antibody. Regarding non-clinical studies, the guideline indicates a risk-based approach to evaluate mAb products on a case-by-case basis will be taken. In vitro studies are to be conducted first, and these would be followed by a decision regarding what, if any, in vivo nonclinical studies are needed. According to Mr. Shea, the guideline states data from a number of comparative in vitro studies should be provided, and these should be designed to exclude all differences of importance in the concentration-activity relationship. Regarding clinical studies, the guideline indicates that the most sensitive clinical model should be used in a homogeneous patient population, and the most sensitive patient population and clinical endpoint is preferred. When multiple therapeutic regimens are licensed, the most sensitive key PK parameters should be assessed, but not all therapeutic dosage regimens need be tested. Clinical safety, including an assessment of immunogenicity, must be demonstrated. In particular, immunogenicity must be carefully assessed when a different expression system is used to produce the antibody product. The guideline states that extrapolation of clinical efficacy and safety data to other indications of reference mAb may be possible based on overall evidence of biosimilarity, but extrapolation will be more challenging if the reference mAb is licensed as an immunomodulator and for cancer, which Mr. Shea noted is the case for rituximab. The final point was that the biosimilar applicant will need to propose pharmacovigilance and risk management activities, which will be similar to those of the reference product but could be more.

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Day 4: December 9, 2010

Antibody Engineering/ Antibody Therapeutics Joint Session

Cameron Dunlop and Michael Huber

Renaissance of Bispecific Antibodies

The focus of the morning session was on bispecific antibodies. These types of molecules were invented 25 years ago, and there is now a resurgence of interest in their development as therapeutics.

Stanley R. Frankel (Micromet) reported on blinatumomab (MT103), a CD19/CD3-bispecific T-cell engaging (BiTE) construct of two linked scFvs that strictly targets effector memory T cells to kill CD19 expressing malignant B cells. In preclinical testing the antibody is potent in the pg/ml range, no T-cell receptor co-stimulation is required and low effector to target cell ratios are sufficient.[1] A Phase 2 study of blinatumomab in relapsed acute lymphoblastic leukemia (ALL) showed a rapid and complete response in 80% of the patients. Minimal residual disease (MRD) was monitored with an elegant method determining the frequency of rearranged immunoglobulin and T-cell receptor genes with patient specific primers. Blinatumomab is also being tested in a dose-escalating Phase 1 study in non-Hodgkin lymphoma; a lower incidence of central nervous system events leading to discontinuation in patients with a high B:T-cell ratio has been observed.

Charlotte McDonaugh (Merrimack Pharmaceuticals) reported on the use of network models to identify key nodes within the ErbB network, and the creation of MM-111; a trimeric complex consisting of an anti-ErbB2 single chain Fv (scFv) and an anti-ErbB3 scFv, with a modified serum albumin molecule linking the two. This construct can displace heregulin from ErbB3 and thereby prevent its phosphorylation, inhibiting tumor growth. Phase 1 clinical studies of MM-111 are currently underway, with and without trastuzumab, with which a synergistic effect was found in both in vivo and in vitro studies. It seems likely that the two therapeutics target two distinct mechanisms of activation within the ErbB network: oncogene activation and ligand-induced activation.

The theme of bispecificity was continued by **Melvyn Little** (Affimed Therapeutics), who discussed TandAbs: tetravalent antibody-derived constructs 105–110 kDa in length capable of binding two epitopes, via two distinct paratopes for each. These lack an Fc domain, a feature designed to prevent non-specific interaction with immune-effector cells. Clinical studies of an anti-CD16A/anti-CD30 TandAb designed to treat Hodgkin lymphoma was initiated in October 2010.

Adnectins as a non-antibody-based approach to bispecificity was discussed by **Eric Furfine** (Adnexus/Bristol-Myers Squibb). The molecules are targeted therapeutics composed of modified human fibronectin subunits. Dr. Furfine described two bispecific Adnectins targeting IGFR + anti-EGFR2 and IGFR + VEGFR2 that are more potent at controlling xenograft tumors expressing the relevant receptors than mono-specific constructs. Balancing

the potency of the paired Adnectins and strategies to enhance pharmacokinetics by binding Adnectins to serum albumin were also discussed

Another potential approach to bispecificity, the CovX-Body, was discussed by **Venkata Ramana Doppalapudi** (Pfizer). This approach involves a scaffold antibody that recognizes a reactive mono- or bispecific linker that can covalently bridge the paratope and one or more pharmacophores. One such construct, CVX-241, utilizes two peptides that recognize Ang-2 and VEGF, respectively. It shows better efficacy than mono-specific CovX-Bodies in tumor xenograft models, and the effect is enhanced when CVX-241 is used in combination with chemotherapy.

The final speaker of the morning session was **Syd Johnson** (MacroGenics), who discussed Dual Affinity Re-Targeting molecules (DART), an Fv-based platform that incorporates disulfide bonds between the C-termini of constructs.[3,4] An anti-CD16$^+$ anti-CD32B DART shows anti-tumor activity, as does an anti-HER2$^+$ anti-TCR DART in a bladder cancer xenograft model. DARTs are stable for weeks in human serum and can be stored for at least two years without loss of viability. Strategies for extending the half-life, including Fc and albumin fusions were also discussed.

Antibody Engineering for Second- and Third-Generation Antibody Therapeutics: Lessons Learned from the First Generation

Paul Carter (Genentech), chairman of the last session of the conference, emphasized that antibody therapeutics have come of age. While their clinical and commercial impact was minimal in the 1990s, it is substantial today and, consequently, competition is intense. The drivers of next-generation therapeutics are diverse: (1) there is a clinical need for higher efficacy and safety; (2) progress in the understanding of biological pathways and networks allows for more and diverse targets; (3) engineering technologies enable the enhancement of intrinsic antibody performance (e.g., conjugates, prolonged half-life, modulation of effector functions); (4) advances have been made in medical technologies (e.g., biomarkers and companion diagnostics, medical device and delivery technologies, use of antibodies for imaging); and finally, (5) the products are commercially promising.

In discussing attempts to find new drug-sensitive targets, **Louis M. Weiner** (Georgetown University Medical Center) presented data from a synthetic lethal screen of the central EGFR pathway. Out of more than 600 targeted genes, 61 hits that define the EGFR "resistance space" were confirmed. This work could eventually lead to the development of new treatment strategies based upon knowledge of the genetic determinants of drug response. Dr. Weiner also presented data showing that in an established HER2$^+$ tumor model high affinity is needed for antibodies that only promote ADCC, and that antibodies promoting ADCC plus signaling perturbation are superior to ADCC alone. Finally, new evidence was shown for the "ADCC-mediated adaptive immunity switch"[5] by a mouse tumor re-challenge study where trastuzumab therapy induced powerful T-cell dependent HER2-specific adaptive immunity.

Mark Sliwkowski (Genentech) provided structural background on the mechanisms of action of the anti-HER2 antibodies trastuzumab and pertuzumab. While trastuzumab disrupts ligand-independent HER2-HER3-PI3K complexes, pertuzumab prevents ligand-induced HER2-HER3 dimerization.[6] Dr. Sliwkowski noted that pertuzumab added meaningful activity comparable to a cytotoxic to trastuzumab in a Phase 2 study of HER2$^+$ breast cancer patients. A Phase 3 study to evaluate the combination of pertuzumab, trastuzumab and docetaxel vs. placebo, trastuzumab and docetaxel in previously untreated HER2-positive metastatic breast cancer is ongoing.[7,8] Dr. Sliwkowski also discussed a promising example of the classic antibody drug conjugate (ADC) concept, trastuzumab emtansine (T-DM1; Genentech/ImmunoGen), which is composed of trastuzumab linked to the cytotoxic drug DM1. The ADC is undergoing evaluation in Phase 3 studies, and has shown efficacy in patients with HER2-positive MBC who had tumor progression after prior treatment with HER2-directed therapy and who had received prior chemotherapy.[7,9]

Hari Hariharan (Biogen Idec) presented examples of tetravalent, bispecific antibodies constructed by fusing scFvs to the C-termini of an IgG. "Hercules" is an anti-LTβR/anti-TRAIL-R2 antibody that agonistically cross-links two TNF family receptors.[10] The bispecific antibody shows higher in vitro tumor cell killing, which is also reflected in superior tumor inhibition in a breast tumor xenograft model. BIIB047, an anti-EGFR/anti-IGF-1R bispecific antibody that simultaneously blocks the cross-talking EGFR and IGF-1R pathways, has been shown to inhibit cellular activation and has a superior efficacy in a pancreatic xenograft model.

As noted by **Anna M. Wu** (University of California, Los Angeles), antibodies are the quintessential molecular-targeted agents, both for therapeutics and imaging. Molecular imaging, especially immuno positron-emission tomography (PET), allows for highly sensitive detection and quantization providing both regional and temporal information.

Professor Wu discussed engineered antibodies for molecular imaging of cancer. In contrast to therapeutic molecules, antibodies for imaging are engineered to reduce half-life to accelerate clearance and remove unwanted background.[11] Other objectives include improved tissue penetration, removal of all effector functions and conjugation to an image moiety (positron-emitting radionuclides such as I-124, Cu-64, F-18). Most suited are rapid targeting/rapid clearing fragments such as diabodies[12] and minibodies, the former even allowing for same day imaging. The possibility of generating diabodies from any specificity and routine microwave-assisted radiolabeling in microfluidic chips makes them a promising class for clinical evaluation. Professor Wu also presented work on diabody/human serum albumin fusion constructs that may have properties closer to the ideal (i.e., small, human, controllable pharmacokinetics).

Christian Klein (Roche Glycart) presented data on GA101 (RO5072759), a type II, glycoengineered humanized anti-CD20 monoclonal antibody.[13] The company's technology inhibits antibody fucosylation through genetic engineering of the expressing cells, resulting in antibodies with higher FcγRIIIa binding, increased ADCC, increased direct cell death and reduced CDC.

Crystallographic studies showed that GA101 binds in a different orientation to CD20 than rituximab, which could be responsible for the observed differences in stoichiometry and tissue distribution. GA101 induced stronger inhibition of tumor growth compared with rituximab in preclinical studies.[14] GA101 is in clinical studies, including two Phase 3 studies in patients with hematological malignancies; it is the most advanced Fc-engineered antibody with increased FcγRIIIa binding currently undergoing evaluation.

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