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IBC's 23rd Annual Antibody Engineering, 10th Annual Antibody Therapeutics International Conferences and the 2012 Annual Meeting of The Antibody Society

December 3–6, 2012, San Diego, CA

Peter-Christian Klöhn,[1] Ulrich Wuellner,[2] Nora Zizlsperger,[3] Yu Zhou,[4] Daniel Tavares,[5] Sven Berger,[6] Kirstin A. Zettlitz,[7] Gabriele Proetzel,[8] May Yong,[9] Richard H.J. Begent,[9] and Janice M. Reichert[10,*]

[1]MRC Prion Unit; Department of Neurodegenerative Diseases; UCL Institute of Neurology; London, UK; [2]Covagen AG; Zurich, Switzerland; [3]Protein Engineering and Antibody Technologies; EMD Serono Research Institute, Inc.; Billerica MA USA; [4]Department of Anesthesia; University of California, San Francisco; San Francisco, CA USA; [5]ImmunoGen, Inc.; Waltham, MA USA; [6]Institut de Recherche, Centre d'Immunologie Pierre Fabre; St Julien en Genevois, France; [7]Crump Institute for Molecular Imaging; Department of Molecular and Medical Pharmacology; David Geffen School of Medicine at UCLA; California NanoSystems Institute; University of California Los Angeles; Los Angeles, CA USA; [8]The Jackson Laboratory; Bar Harbor, ME USA; [9]UCL Cancer Institute; London, UK; [10]Landes Bioscience; Austin, TX USA

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The 23rd Annual Antibody Engineering, 10th Annual Antibody Therapeutics international conferences, and the 2012 Annual Meeting of The Antibody Society, organized by IBC Life Sciences with contributions from The Antibody Society and two Scientific Advisory Boards, were held December 3–6, 2012 in San Diego, CA. The meeting drew over 800 participants who attended sessions on a wide variety of topics relevant to antibody research and development. As a prelude to the main events, a pre-conference workshop held on December 2, 2012 focused on intellectual property issues that impact antibody engineering. The Antibody Engineering Conference was composed of six sessions held December 3–5, 2012: (1) From Receptor Biology to Therapy; (2) Antibodies in a Complex Environment; (3) Antibody Targeted CNS Therapy: Beyond the Blood Brain Barrier; (4) Deep Sequencing in B Cell Biology and Antibody Libraries; (5) Systems Medicine in the Development of Antibody Therapies/Systematic Validation of Novel Antibody Targets; and (6) Antibody Activity and Animal Models. The Antibody Therapeutics conference comprised four sessions held December 4–5, 2012: (1) Clinical and Preclinical Updates of Antibody-Drug Conjugates; (2) Multifunctional Antibodies and Antibody Combinations: Clinical Focus; (3) Development Status of Immunomodulatory Therapeutic Antibodies; and (4) Modulating the Half-Life of Antibody Therapeutics. The Antibody Society's special session on applications for recording and sharing data based on GIATE was held on December 5, 2012, and the conferences concluded with two combined sessions on December 5–6, 2012: (1) Development Status of Early Stage Therapeutic Antibodies; and (2) Immunomodulatory Antibodies for Cancer Therapy.

*Correspondence to: Janice M. Reichert; Email: janice.reichert@landesbioscience.com
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Held on Sunday December 2, 2012, the pre-conference workshop chaired by **John L. Marquardt** (Marquardt Law) drew a capacity crowd interested in learning about intellectual property (IP) issues that affect antibody engineering. **Kevin McCabe** (Sterne, Kessler, Goldstein and Fox P.L.L.C.) discussed provisions of the Biologics Price Competition and Innovation Act of 2009 and the America Invents Act, and he presented recent developments in subject matter patentability as defined in the Supreme Court case Mayo v. Prometheus. **Vicki Norton** (Duane Morris, LLP) guided participants through the complicated and long-running saga of the Cabilly patents and discussed other notable antibody patents. Changes in the requirements for obtaining United States (US) Patents on antibody therapeutics were discussed by **Adriane Antler** (Jones Day) during her presentation on the written description requirement for antibody patents. **Scott Miller** (Life Technologies Corporation) presented methods for identifying IP bottlenecks and discussed practical tools that can assist with understanding, tracking and navigating complex IP landscapes, including patent watching, litigation watching, and IP landscaping services. The final speaker of the session, **Ulrich Storz** (Michalski Hüttermann and Partner), provided a European IP perspective of the therapeutic antibody space and highlighted differences between European and US patent law.

The 23rd Annual Antibody Engineering and 10th Annual Antibody Therapeutics international conferences, and the 2012 Annual Meeting of The Antibody Society, opened December 3, 2012 with a keynote presentation on engineering receptor ligands with powerful cellular responses given by **Andreas Plückthun** (University of Zürich), who was introduced by **James S. Huston** (The Antibody Society, Huston BioConsulting, LLC).

December 3, 2012: Antibody Engineering From Receptor Biology to Therapy

Peter-Christian Klöhn

The first session of the meeting, which was chaired by Professor Plückthun, started with a presentation by **Yosef Yarden** (Weizmann Institute of Science) on moving away from monoclonal antibodies (mAbs) to oligoclonal antibodies that target epidermal growth factor receptor (EGFR) and human growth factor receptor-2 (HER2).

K. Dane Wittrup (Massachusetts Institute of Technology) presented intriguing results on the efficacy of multi-epitopic anti-EGFR antibodies. Activating mutations of EGFR family members, comprising four closely related receptor tyrosine kinases, EGFR (ErbB-1), HER2 (ErbB-2), HER3 (ErbB-3) and HER4 (ErbB-4), have been associated with a number of cancers, including breast, lung, colorectal cancers and glioblastoma. Signal transduction to downstream effectors (e.g., Ras, Raf, Erk) follows after dimerization and autophosphorylation of several critical tyrosine residues in the C-terminal domain of the receptor. EGFR downregulation is the chief therapeutic strategy. Professor Wittrup explained that although statistically efficacious, the potency of existing antibody monotherapies is not overwhelming, with average response rates of ~10% (e.g., 11% for cetuximab, 8% for panitumumab). Combination therapies with two or more antibodies against the same target have proven more effective, and two antibody cocktails, MM-151 (Merrimack) and Sym004 (Symphogen), are now in the clinic.

To test whether bi-epitopic antibodies show increased efficacy for EGFR downregulation, Professor Wittrup's group attached the binding site of the mAb 806 to cetuximab to generate bi-epitopic 806-cetuximab antibodies. mAb 806 recognizes a variant EGFR (EGFRvIII) bearing an internal deletion in its extracellular domain and shows significant antitumor activity.[1] The epitope for 806 is obscured in the self-inhibited version of the molecule and in the activated dimer. Most EGFR-positive cell lines, therefore, do not bind 806. As shown in U87SH cells expressing EGFRvIII, the bi-epitopic 806-cetuximab antibody reduced surface EGFR by 80%, whereas cetuximab, 806 and the combination showed < 20% effect levels. The bi-epitopic antibody BS38LC, a variant with two single chain 806 fragments attached to the C-termini of cetuximab light chains, greatly reduced tumor volumes in the U87SH mouse model and was significantly more effective than the combination of cetuximab and 806. Surprisingly, BS38LC greatly reduced surface EGFR levels in cell lines expressing wt-EGFR, in contrast to cetuximab, 806 or the combination. In similar design strategies, tri-epitopic EGFR antibodies with fibronectin III-based binders were engineered.[2] Tri-epitopics triggered intensive EGFR clustering, in contrast to cetuximab, followed by a fast internalization of aggregated EGFR. Since large EGFR clusters were formed under these conditions, the question arose whether the receptors were activated under these conditions, a result that would exclude these tri-epitopics as therapeutic biologics. The kinetics of tyrosine phosphorylation following incubations with tri-epitopics, however, was indistinguishable from isotype controls, with no indication of receptor activation. In tumor xenograft experiments with distinct cell lines (HT-29, HT-116 and U87), the tri-epitopic antibody was highly effective, whereas cetuximab showed only modest effects.

Tomoyuki Igawa (Chugai Pharmaceutical Co Ltd.) discussed antibody recycling technology, an antibody design strategy to effectively improve the duration of antigen neutralization.[3] Whereas conventional antibodies targeted to surface receptors are rapidly cleared from plasma by cellular uptake and degradation, recycling antibodies show sustained plasma levels. Dr. Igawa explained that these antibodies bind to antigen at neutral pH, are internalized as antibody-antigen complex, and are transported into the endosomal pathway, but rapidly dissociate from the antigen within the acidic environment of endosomes (pH 6). Subsequently, the free antibody binds to the neonatal Fc receptor (FcRn) intercellularly and is released back to the plasma, where it binds to antigen again. In contrast, conventional antibodies remain bound to the antigen as the pH decreases and are finally degraded in lysosomes. Recycling antibodies thus evade lysosomal degradation, a propensity that is based on mutations that confer pH-dependent binding of antigen. To engineer recycling antibodies, amino acids in the heavy chain or light chain variable region of the antibody are mutated to histidine.

To test the efficacy of antibody recycling, a pH-sensitive variant of tocilizumab (SA237), a humanized antibody against the interleukin-6 receptor (IL-6R) was engineered. When administered intravenously (i.v.) into cynomolgus monkeys, SA237 was detectable in the plasma for more than 28 d, whereas unmodified tocilizumab was undetectable after 12 d.

Dr. Igawa also noted that a recycling antibody bound to soluble antigen is taken up by pinocytotis and the free antibody is recycled in analogy to receptor-targeted antibodies, but here the plasma concentration of the antigen can drop dramatically. The antibody therefore actively promotes the degradation of antigen. To further reduce the plasma concentration of antigen, Chugai Pharmaceutical developed the sweeping antibody technology. Sweeping antibodies were engineered to bind to FcRn at plasma pH levels (pH 7.4), a modification that leads to enhanced cellular uptake of antibody-antigen complexes into the cell. Long-acting sweeping antibody can reduce antigen concentration by 50-fold compared with conventional antibody while maintaining antibody pharmacokinetics comparable to conventional antibodies.

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December 3, 2012: Antibody Engineering
Antibodies in a Complex Environment

Ulrich Wuellner

The session on antibodies in a complex environment was chaired by **Kerry Chester** (University College London), who also gave a presentation on developing dual-specific antibodies to tackle tumor heterogeneity. **Roberto Polakiewicz** (Cell Signaling Technology, Inc.) then presented data from a proteomics approach for rapid identification and cloning of monoclonal antibodies from circulation. Dr. Polakiewicz introduced the topic by pointing out that the isolation of monoclonal antibodies from a polyclonal antibody response is challenging mainly because of the complexity of the antibody mixture. A key limitation especially for the isolation of patient derived human antibodies is the availability of sufficiently large numbers of B cells that represent the full repertoire of circulating antibodies. In the proteomics based approach antibodies can be isolated and identified directly from blood plasma, which has the advantage that the circulating antibody pool is reflected and that antibodies can be enriched and selected by function, e.g., antigen binding.

For bottom-up proteomics, however, the resolution of antibody diversity represents a tremendous challenge especially because variable regions and CDRs are not represented in genomic databases. The solution presented by the research teams of Cell Signaling Technology was the use of a custom reference database provided by deep sequencing of the B cell cDNA repertoire and the development of novel bioinformatics for peptide and VR identification by mass spectrometry.[1]

Once peptide sequences of isolated antibodies are obtained via LC-MS/MS they can be matched with sequences derived from custom databases so that Antibody Sequence ID lists can be created. Then functional validation proves that the identified antibodies have the desired target binding properties.

In a proof of concept study it was aimed to generate internalizing antibodies binding to RTK X protein. Therefore, rabbits were immunized with whole cells expressing RTK X. Enrichment and functional binding of the polyclonal serum could be demonstrated. Subsequently clusters of clonal families were identified. For selected antibodies cell binding and selective RTK X mediated internalization was shown.

After presenting these proof-of-concept data, the question of whether the NG-XMTTM technology could be applied to isolate human antibodies from circulation was raised. This would be especially useful for the isolation of native human antibodies with desired properties, e.g., neutralizing or antibodies against auto antigens.

In a first setting antibodies against the hepatitis B virus (HBV) antigen should be isolated from vaccinated healthy donors and in a second approach anti-human cytomegalovirus (HCMV) antibodies were isolated from naturally exposed healthy donors in order to identify neutralizing antibodies. In both studies donors could be identified that showed a good polyclonal response against the antigens of choice. In the HBV setting high affinity antibodies could be identified from a single vaccine recipient and the vaccine response could be tracked at the proteomic level over the course of the immunization schedule. In the HCMV setting in vitro neutralizing antibodies could be identified that demonstrated potencies even superior to the most potent currently known anti-HCMV neutralizing antibodies.[2]

Dr. Polakiewicz summarized by noting that, by using NG-XMTTM technology, it is possible to identify antigen specific circulating antibodies from various sources (rabbit, mouse and human) based on functional properties through specific activity driven purification within a short period of time.

John McCafferty (University of Cambridge) gave a presentation on blocking mediators of the tumor supportive microenvironment with antibodies. He pointed out the important role of tumor necrosis factor (TNF)-converting enzyme (TACE) proteins in the tumor microenvironment. TACE is a critical "sheddase" of many substrates, including epidermal growth factor (EGF) family ligands. It is upregulated in a wide range of cancers including colorectal, hepatic carcinomas and others. TACE expression correlates with disease severity and prognosis and several knockdown studies have indicated a role for TACE in tumor growth. The ectodomain of TACE consists of two distinct domains a highly conserved catalytic domain and a variable Dis-Cys Domain. A strategy was devised that allowed for the selection of inhibitory antibodies that bound to the Dis-Cys domain to achieve TACE specificity. Antibody D1 was shown to efficiently inhibit the proteolytic activity of TACE by binding to its Dis-Cys Domain. Binding of D1 was VH dependent. By performing a chain shuffling selection a VL domain could be identified that directed binding to the catalytic domain. The resulting cross domain inhibitor [D1-(A12)] demonstrated a higher affinity toward full length TACE (0.46 nM vs. 26 nM) and abrogated colorectal xenograft growth in vivo.[3]

Dr. McCafferty then focused on antibodies binding to the Met receptor tyrosine kinase. This receptor is believed to play an important role in the hepatocyte growth factor (HGF)-mediated resistance to several anti-cancer kinase inhibitors.[4] The aim was to select antibodies that would block HGF mediated Met receptor activation. After a primary selection of Met blocking antibodies antibody 7A2 was identified which specifically inhibited HGF mediated SKOV3 cell migration. In the following in vitro affinity maturation campaign antibodies could be identified that showed a 50-fold increased potency in the SKOV3 cell migration assay as compared with the parent antibody. By solving the crystal structure of the complex, it was shown that the lead antibody 107_A07 binds to the first Ig domain of the Met receptor. It does not directly bind to the HGF binding site but efficiently blocks HGF signaling demonstrated by the inhibition of cell migration and growth of tumor xenografts in vivo.

As the first speaker after the networking and refreshment break in the afternoon, **Ann L. White** (University of Southampton) discussed the roles of isotype and Fcγ receptor interactions for immunostimulatory activities of monoclonal antibodies. She classified antibody-antibody Fc receptor interactions into activatory interactions like with the mouse FcγRI (CD64); mouse FcγRIII (CD16) and mouse FcγRIV receptor or inhibitory interactions like observed with mouse FcγRIIB.

The anti-tumor activity of a given monoclonal antibody can be significantly influenced by the antibody subtype and its Fc receptor interactions. These interactions have been best studied for "direct binding antibodies" that specifically recognize tumor antigens, e.g., the anti-CD20 antibody rituximab. These antibodies are most effective when they demonstrate high activatory to inhibitory ratios (e.g., high affinity to activatory receptors and low affinity to inhibitory receptors). This leads to efficient tumor antigen specific cell clearance (ADCC or CDC). In humans, IgG1 and in mice IgG2a represent subtypes with high A/I ratios. For immunomodulatory antibodies that are supposed to stimulate anti-cancer immunity the role of Fc receptor interactions is much less well understood.

Dr. White presented their studies to elucidate the influence of Fc receptor interactions for the bioactivity of an anti CD40 antibody. In preclinical mouse models this particular rat IgG2a anti CD40 antibody showed anti-tumor activity against CD40 positive B-cell lymphomas as well as certain CD40 negative tumors. For their studies they reformatted this antibody into a mouse IgG1 and mouse IgG2a format and could show in a mouse tumor model that only the parent antibody and IgG1 subtype prolonged survival of mice in a dose dependent manner.

When analyzing T cell proliferation after antibody administration it was found that the mIgG1 subtype induced proliferation of $CD8^+$ and $CD4^+$ T cells whereas the mIgG2a subtype had no effect. Subsequently they addressed the question of whether or not these effects depend on FcγRIIB interactions. By comparing $CD8^+$ T cell responses and anti-ovalbumin antibody titers in FcγRIIB wild type and FcγRIIB knockout mice they could demonstrate that FcγRIIB is required for T cell expansion and increased anti-ovalbumin titers. Additional in vitro experiments proved that FcγRIIB interaction with mIgG1 is responsible for B-Cell proliferation. Finally, it was shown that the interaction of mIgG1 with FcγRIIB expressed on, e.g., antigen presenting cells leads to receptor clustering which then triggers the activation signal and proliferation in CD40 expressing cell lines.[5] In concluding, Dr. White noted that the data emphasize that isotype specific FcγR interactions can significantly influence the bioactivity of therapeutic antibodies.

Qi Zeng (A*Star) provided an update of her work on antibody targeting intracellular oncoproteins for cancer therapy. She noted that antibodies have traditionally been thought of as too large to routinely enter the cytosol, and, therefore, therapeutic antibodies could only access molecules that appear on the cell surface. Research from her groups and others has indicated that targeting intracellular oncoproteins may become a new option for the development of anti-cancer antibodies.[6]

Professor Zeng first discussed phosphatase of regenerating liver 3 (PRL-3) protein, which is a very unconventional target for antibody therapies because it is expressed in the cytoplasm of target cells. PRLs are unique phosphatases with a characteristic prenylation motif at their C-termini; they belong to the class of protein tyrosine phosphatases. Overexpression of PRL-3 is associated with multiple human cancers, and, in gastric cancer, the mortality of patients overexpressing PRL-3 has been shown to be higher at all tumor stages.

She then presented data showing that anti-PRL3 antibodies could have a therapeutic effect in vivo even though the antibody target is expressed intracellularly. In an in vivo metastasis formation model used in studies by her group, immunocompromized mice were i.v. injected with GFP-transformed PRL-3 expressing cancer cells and then treated with anti PRL-3 antibody every third day for a period of 17 d. The lungs of animals treated with anti-PRL-3 antibody showed a significantly reduced number of metastasis compared with control groups, thereby demonstrating the anti-tumor activity of anti PRL-3 antibodies. Similar results were obtained in a syngeneic metastasis formation model in immunocompetent mice using an anti-mouse PRL-3 antibody and PRL-3 positive B16F0 cells. In addition, internalization studies revealed that a portion of PRL-3 mAb is taken up by PRL-3 expressing cells.[7]

Three possible models for targeting intracellular oncoproteins were presented by Professor Zeng. First, antibodies could be endocytosed and released from the endosome into the cytoplasm of live cells by mechanisms that are currently not well-understood. Second, the intracellular antigen could be externalized by unconventional secretion mechanisms and subsequent antibody binding could then trigger ADCC or CDC that would kill cancer cells. Third, proteolytic processing could lead to PRL-3 fragments being displayed on MHC class I complexes that are then bound by the anti PRL-3 antibody and targeted for destruction. Combinations or other mechanisms are also possible.

Her group has shown that intracellular proteins such as PRL-3 and mT could be used as antigens to challenge and stimulate immune responses in hosts to produce antibodies for their own anticancer therapies. This unconventional concept suggests that intracellular proteins with selective and high expression in cancer cells are useful targets for mAb-based or vaccination immunotherapies, thus challenging current understanding. In concluding, Professor Zeng noted that their studies provide a proof-of-concept for targeting intracellular oncoproteins using antibody therapy and vaccination, and this can be translated into new treatments for patients in many potential ways. If the myriad of previously unexplored candidate target proteins are investigated, a new era of cancer therapies may soon become a reality.

The last speaker of the session, **John Lambert** (ImmunoGen, Inc.) presented results from their maytansinoid antibody-drug conjugate (ADC) programs. He first pointed out that the first-generation ADCs failed in clinical development mainly because of poor tumor penetration and moderate cytotoxicity of the delivered drug payload. Discovery of the highly potent maytansinoids, however, opened new avenues for ADC development. He exemplified the potential of ADCs by showing the very convincing results of the Phase 3 clinical study of trastuzumab emtansine (T-DM1) in metastatic breast cancer. Several other maytansinoid-based ADCs (also called antibody-maytansinoid conjugates; AMCs) are currently undergoing clinical testing. For each of these molecules, the linker chemistry used for ADC synthesis was tailored toward a specific indication and model system.

The importance of the linker chemistry used for antibody conjugation was demonstrated by showing that different linkers (e.g., cleavable vs. non cleavable) often had the same potency

in vitro but showed substantially different efficacies in in vivo xenograft models. Dr. Lambert presented data showing that a thioether-coupled immunoconjugate was inactive whereas the cleavable disulfide linked version showed potent anti-tumor activity in a COLO 205 xenograft model.[8] An explanation for these effects could be that differently linked maytansinoids are differently metabolized in vivo. For instance, the non-cleavable SMCC-DM1 conjugate produced only one prominent metabolite that had poor bystander killing potential whereas the disulfide linked conjugate SPDB-DM4 was metabolized into three components of which a lysine -N^{ϵ}-SPDB-DM4 demonstrated excellent bystander killing potential.[9] Dr. Lambert noted that tumor heterogeneity represents a challenge for the development of new ADCs, and that, in contrast to a SMCC linked conjugate, the disulfide linked AMCs showed efficacy in a HT29 xenograft model in which the target antigen is expressed heterogeneously.

He then discussed the development of a novel anti-folate receptor 1 antibody (FOLR1). For the initial discovery more than 2000 clones were screened for FOLR1 binding and cross-species reactivity (cynomolgus monkey). Approximately 100 clones were evaluated in a high throughput screen for their payload delivery properties. Finally, after humanization about 10 clones were characterized for their bioactivity in vitro and in vivo. In these assays, different mAbs were tested as SMCC-DM1 conjugates and the antibody M9346A was identified as the novel lead compound based on its superior efficacy in a KB xenograft model. Different linker chemistries were then tested, which showed that a sulfo-SPDB-DM4 conjugate was the most active linker-maytansinoid format for the M9346A antibody. This improved linker was thus incorporated into the novel AMC named IMGN853 which showed efficient dose dependent activity in an ovarian cancer model.

Studies with several linker-maytansinoid formats in another antibody-target system utilizing a multidrug resistant (MDR) and non-multidrug resistant folate receptor in vivo model showed that in the MDR model, a sulfo-SPDB-DM4 conjugate was more active than an SPDB-DM4 AMC, whereas in the non-MDR model both linkers showed similar activity. This result could again be explained by differences in the intracellular processing and increased bystander cell killing activity of the hydrophilic linker Sulfo-SPDB-DM4.

In concluding, Dr. Lambert emphasized that linkers have attributes beyond simply joining a payload to an antibody of choice, and that both the anti-tumor activity and safety of an ADC can be influenced by the linker chemistry. The linker chemistry can influence the product of release (payload charge or hydrophobicity), the rate and site of payload release and the intracellular transport of the released payload. For the development of novel well tolerated and effective ADCs the properties of the antibody, the linker and the payload need to be matched with the biology of the target.

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December 4, 2012: Antibody Engineering
Antibody Targeted CNS Therapy Beyond the Blood Brain Barrier

Nora Zizlsperger

James S. Huston (The Antibody Society; Boston Biomedical Research Institute; Huston BioConsulting, LLC) chaired this session and introduced the speakers. **William M. Pardridge** (University of California, Los Angeles) gave the keynote presentation on targeted delivery of therapeutics across the blood brain barrier (BBB).[1,2] He provided a brief history of the failed attempts in the 1980s to deliver large molecules to the brain. All large molecules and most small molecules do not cross the BBB. He noted that experiments showing molecules uptake in the brain at 0.5% of the injected dose (I.D.) are not exhibiting transport across the BBB, but reflecting the volume of blood in the brain. The key to success for crossing the BBB is engineering a molecular Trojan horse. This approach takes advantage of endogenous receptors that transport across endothelial cells from the blood to the brain. These transcytosis receptors include the insulin receptor (IR) for mono-directional transport from the blood membrane to the brain membrane and the transferrin receptor (TfR) for bi-directional transport between the blood and brain membranes. Antibodies that bind to these receptors were fused to various therapeutic proteins to create the molecular Trojan horses.

Professor Pardridge then presented three examples of approaches to crossing the BBB. In the first example, an antibody to human IR (HIRMAb) was fused to TNFR to treat pro-inflammatory aspects of many neurological diseases.[3] The uptake in monkey brains was shown to be 3% I.D., higher than TNFR-Fc, but similar to the uptake of neuroactive small molecules. The uptake in mouse brains was similar, although a surrogate TfR-TNFR was necessary because no mAbs to mouse IR were available. Neuroprotection with TfR-TNFR was observed

in mouse models for Parkinson disease and stroke. In the second example, HIRMAb was also fused to a single chain variable fragment (scFv) anti-Aβ amyloid to treat Alzheimer disease (AD). Positive pharmacology was observed in transgenic AD mice.[4] In the third example, HIRMAb was fused to α-L-iduronidase (IDUA) to treat the lysosomal storage disorder Hurler syndrome.[5] The uptake in monkey brains was shown to be 1% I.D. A surrogate molecule TfR-IDUA was shown to be effective in a Hurler mouse model. All of these fusion molecules were also shown to be isolated to high purity and retain high binding affinity and/or enzyme activity. **Ruben J. Boado** (ArmaGen Technologies) continued the discussion of crossing the BBB by describing non-viral gene therapy to the brain via antibody-targeted nano-containers.[2]

Danica Stanimirovic (National Research Council of Canada) presented the discovery and engineering of BBB-transmigrating antibodies targeting novel receptors that mediate transcytosis across brain endothelial cells.[6] Two workflows were used to find novel receptors: (1) a proteomics approach isolating the luminal membrane proteins of brain endothelial cells via gradient fractions or in situ capture and identification via mass spectrometry; and (2) functional panning of phage display single domain antibody (sdAb) libraries (llama VHH) against brain vs lung endothelial cells.[7] In addition to the transcytosis receptors mentioned in the prior talks, the membrane protein TMEM30A was identified from both workflows. FC5, a sdAb against TMEM30A, was isolated from workflow 2 and characterized. Pharmacological efficacy was demonstrated with systemically administered FC5 conjugated to different neuroactive peptides in rat chronic pain models. Enhancement in analgesia was observed when FC5 fused with Fc was used as a carrier for analgesic peptides. Most recently, a bispecific sdAb-sdAb was generated linking FC5 with an anti-amyloid-β peptide antibody with promising initial results of entry into the brain parenchyma.[8]

The session concluded with discussion of antibody therapy for several neurodegenerative diseases. **Cynthia A. Lemere** (Brigham and Women's Hospital; Harvard Medical School) gave an overview of pre-clinical and clinical results of passive amyloid-β (Aβ) immunotherapy for Alzheimer disease (AD) from her own and other academic laboratories or companies.[9,10] AD pathogenesis is associated with accrual of amyloid plaques of Aβ peptides that precede neurofibrillary tangles and cognitive decline. Administration of antibodies to different regions and forms of Aβ has been shown to decrease Aβ plaques in the brains of AD mouse models and AD patients in clinical trials; however, a lack of robust cognitive efficacy has been observed in Phase 2 or 3 trials of mild-to-moderate AD patients. Earlier administration may be necessary to prevent the disease. This hypothesis will be tested by treating individuals with dominantly-inherited AD-associated genes with passive Aβ immunotherapy this year in several international trials.[11] Passive immunotherapy against other targets, including pyroglutamate-3 amyloid-β protein (pE3-Aβ), BACE1, and Tau, has been shown to be effective in AD mouse models. In particular, recent work from Professor Lemere's laboratory showed a reduction of amyloid plaques after administration of anti-pE3-Aβ in AD transgenic mice.[12] In addition, no microhemorrhage was observed, unlike prior anti-Aβ therapy in mouse models and humans.

Anne Messer (Wadsworth Center, New York State Department of Health; State University of New York at Albany) presented progress developing intracellular antibody therapy for Parkinson disease.[13] Dr. Messer has worked on developing intrabody therapeutics (gene therapy of scFv or nanobodies against misfolded proteins) for numerous neurodegenerative diseases. For Parkinson disease, intrabodies were selected against peptides of α-synuclein using a human yeast surface display library.[14] The anti-α-synuclein scFvs were shown to bind α-synuclein and protect in an in vitro PD assay with efficacy correlated more with solubility of the scFvs than affinity. Binding alone, however, is not sufficient for a prolonged effect. Following work on anti-huntingtin intrabodies in Huntington disease,[15] the anti-α-synuclein intrabodies were fused to a proteasome targeting (PEST) motif to direct the antigens to the proteasome.[16] The PEST motif fusions not only reduced the amount of α-synuclein, but also increased the solubility of the anti-α-synuclein intrabodies.

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December 4, 2012: Antibody Engineering
Deep Sequencing in B Cell Biology and Antibody Libraries

Yu Zhou

Andrew Bradbury (Los Alamos National Laboratories) chaired the afternoon session, which was organized by Dr. Bradbury and **Jamie K. Scott** (Simon Fraser University). Dr. Bradbury introduced the keynote speaker, **Andrew Fire** (Stanford University), who reviewed the challenges and opportunities offered by deep sequencing of immune responses.

Jacob Glanville (Distributed Bio) discussed how high throughput sequencing influences antibody discovery and development. He presented an observation of significant heritable differences in V-gene profiles between twin pairs using deep sequencing technology,[1] which can be applied to discover problematic allele profiles in specific diseases. He then presented a deep catalog of human genetic variation database of IGHV-gene alleles from the 1000 Genomes project, which confirmed significant variations among human populations, e.g., demographic distributions. Using deep sequencing, a specific IGLV6–57 S43A was identified with 20-fold higher frequency in amyloidosis than in a healthy population.

The human antibody repertoire diversity was first analyzed by deep sequencing of a combinatory antibody library.[2] Further analyses of both unselected antibody libraries and existing functional binders provided additional insight into antibody natural profiles, which guided the design of a synthetic antibody library.[3] The new generation synthetic antibody library was based on the observed natural sequence landscapes, with a higher degree of dispersion, lower degree of redundancy, and more functional sequences.

Csaba Kiss (Los Alamos National Laboratory) presented an overview about deep sequencing analysis of antibody libraries, and the applications in antibody library selections. He compared DNA sequencing methods, Sanger, 454, Ion Torrent, and MiSeq, with regard to the sequencing length, throughput, cost, and accuracy. He also discussed the computational tools to analyze antibody library sequences, VDJFasta and Regular Expression (Regex).

In an analysis of a naïve antibody library using three sequencing technologies (454, Ion Torrent, and MiSeq), Dr. Kiss showed that MiSeq provided the highest number and quality of HCDR3 sequences, while 454 gave the least number of reads and HCDR3s. Comparing VDJFasta and Regex to analyze the 454 data set, Regex found 10% more HCDR3 sequences than VDJFasta though both methods about 84% of common HCDR3s. Dr. Kiss explained why the unique number of HCDR3 sequences decreased with the increase of sequences being analyzed, and demonstrated that the CDR3 length distribution is independent of methods for sequencing and analysis. In conclusion, Dr. Kiss suggested Regex as a valid alternative to VDJFasta because of the faster speed, better sensitivity, more flexible for other libraries, and especially suitability for Ion Torrent and MiSeq.

Dr. Kiss further presented two methods to identify errors and determine the true diversity in antibody library sequence analysis, STOP codons and Hamming distance. The presence of STOP codons is used as an initial sign to remove false HCDR3 sequences, and Hamming distance to detect the redundant sequences. In this discussion, he concluded that sequencing errors do not influence the library size estimation, all three sequencing technologies provide consistent analysis, and the data obtained by the various technologies correlate well with each other.

The deep sequencing of antibody libraries can also be used in antibody selections to isolate antigen-specific antibodies by analyzing the selection output in silico rather than experimental testing of single randomly picked clones in an ELISA assay. He demonstrated that the most abundant clones identified by deep sequencing are antigen-specific binders; however, the correlation between abundance and antibody binding affinity is relatively weak. He suggested modifications, such as more stringent selections combined with deep sequencing of output, might provide a high throughput platform for antibody discovery.

Susan Moir (National Institutes of Health) presented studies using HIV envelope probes to measure HIV-specific B cell responses in infected individuals. Such studies may assist a better design of HIV vaccine candidates. **Ignacio Sanz** (Emory University) talked about how deep sequencing of B cell repertoire and antibody generation from single B cell could expand understanding autoimmune diseases.

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December 4, 2012: Antibody Therapeutics
Clinical and Preclinical Updates of Antibody-Drug Conjugates

Daniel Tavares

The session on antibody-drug conjugates (ADCs), was chaired by **Benjamin P. Chen** (Ignatius Transaction Partners, LLC), and the keynote presentation was provided by **Peter Senter** (Seattle

Genetics, Inc.), who discussed his thoughts on the past, present and future of ADCs. **Paul Polakis** (Genentech) then discussed ADCs for the treatment of cancer, and **Hans-Peter Gerber** (Pfizer) gave an update on the INO-VATE Phase 3 study of inotuzumab ozogamicin plus rituximab as a treatment for relapsed/refractory aggressive non-Hodgkin lymphoma in patients who are not candidates for intensive high-dose chemotherapy. Vedolizumab in patients with moderately to severely active ulcerative colitis and Crohn disease was discussed by **Doina Roman** (Takeda).

William Olson (Progenics Pharmaceuticals) presented an update on PSMA ADC 1301, which is currently in Phase 2 clinical trials for the treatment of metastatic castration-resistant prostate cancer. Prostate-specific membrane-associated antigen (PSMA) is an internalizing membrane protein with a large extracellular domain that is expressed in nearly all cases of prostate cancer, but has limited expression on normal tissues, making it an attractive ADC target for cancer therapy. The Progenics PSMA ADC comprises a fully human IgG1 antibody linked to the tubulin inhibitor monomethylauristatin E (MMAE) by a valine-citrulline dipeptide linker. The conjugate demonstrated picomolar cytotoxic activity in vitro on PSMA-positive C4–2 cells and could selectively target cells with expression levels as low as 10,000 copies of PSMA per cell. Preclinical xenograft studies showed potent and selective anti-tumor activity in a number of human prostate cancer model systems with no evidence of target-related toxicities.

In a Phase 1, open-label, dose-escalation study, PSMA ADC was administered every 3 weeks up to 4 doses in men with castration-resistant metastatic prostate cancer who had previously been treated with docetaxel. A total of 52 patients were treated at doses ranging from 0.4 mg/kg up to 2.8 mg/kg. Dose limiting toxicities, consisting primarily of neutropenia, were observed at 2.8 mg/kg; subsequent doses were reduced to the maximum tolerated dose of 2.5 mg/kg. Pharmacokinetic analysis indicated that the PSMA ADC had a half-life of approximately 2 d at doses over 2.0 mg/kg, and conjugate stability was observed with mean free MMAE concentrations less than 10 ng/mL. Waterfall plots of PSA levels demonstrate that many patients responded to PSMA ADC treatment. Biomarker analysis indicated that reductions in PSA levels usually correlated with reductions in circulating tumor cells (CTCs), particularly at dose levels above 1.8 mg/kg. With Phase 1 studies demonstrating an MTD of 2.5 mg/kg and durable coordinated reductions in PSA and CTCs, the PSMA ADC has advanced to a recently initiated Phase 2 clinical trial. The open-label, single-arm study will assess the anti-tumor activity and tolerability of PSMA ADC dosed at 2.5 mg/kg every 3 weeks for up to 8 doses in about 75 men with metastatic castration-resistant prostate cancer who have received one or two cycles of prior chemotherapy, including one with docetaxel.

Dr. Olson concluded by discussing the use of PSMA itself as a potent predictor of patient responses and a biomarker for companion diagnostics to enrich responsive patient populations. The Dako 3E6 antibody can be used in IHC assays to measure neovascular expression of PSMA in non-prostatic formalin fixed paraffin-embedded tissues. Alternatively, a PSMA+ CTC assay can be used to quantify PSMA-expressing circulating tumors in patients. The PSMA CTC assay qualification and validation studies demonstrated good spike recoveries with low to moderate PSMA expression and no recoveries from non-PSMA expressing cells. The current Phase 2 PSMA ADC clinical trial includes an evaluation of the PSMA biomarker assays.

December 4, 2012: Antibody Therapeutics Multifunctional Antibodies and Antibody Combinations: Clinical Focus

Sven Berger

The afternoon session in the Antibody Therapeutics track was chaired by **Mark R. Alfenito** (EnGen Bio, Inc.), and the focus was on bispecific antibodies and combinations of monoclonal antibodies. The keynote presentation was given by **Gregory Friberg** (Amgen, Inc.), who presented an update on clinical programs for two bispecific T cell engager (BiTE) antibodies, blinatumomab (AMG 103) and the anti-EpCAM/anti-CD3 BiTE antibody AMG 110. Dr. Friberg began with an overview of the BiTE technology. He emphasized the importance of T cells in the anti-tumor response. He further explained that tumor cells have developed a variety of mechanisms to avoid recognition by T cells. BiTE antibodies were developed to harness the body's T cells to lyse tumor cells. Major advantages of these bispecific antibodies include that they are active at femto- to picomolar concentrations, engage both CD4 and CD8 T cells without additional T cell stimulus and can lyse dividing and non-dividing tumor cells.

Blinatumomab specifically targets CD19 with one arm and CD3 with the other. The anti-CD3 Fv regions for subsequent BiTEs have been optimized from a murine single chain variable fragment (scFv) that had no cross-reactivity to cynomolgus monkeys to a fully human scFv with cross-reactive to non-human primates. CD19 is a diagnostic marker for B cell malignancies including acute lymphoblastic leukemia (ALL). The serum half-life of blinatumomab is approximately 2.5 h. It is administered by continuous intravenous (i.v.) infusion using an implanted port and small ambulatory pump. The initial dose in Phase 2 trials was 5 $\mu g/m^2/d$ for one week followed by a higher dose of 15 $\mu g/m^2/d$ for three weeks. For treating patients with minimal residual disease-positive (MRD) ALL, only the higher dose was used. The most common adverse events (AE) were pyrexia, chills, decrease of lymphocytes, and hypokalemia which can be explained by the onset of T cell activation. Blinatumomab showed also activity in pediatric patients with post-transplant relapsed ALL.[1] In a Phase 2 trial in non-Hodgkin lymphoma (NHL) patients, treatment with blinatumomab at 60 $\mu g/m^2/d$ for up to 8 weeks achieved an objective response of 71%. This study included 12 follicular lymphoma patients and five mantle cell lymphoma patients.

Dr. Friberg then discussed the first results of a Phase 1 study of the anti-epithelial cell adhesion molecule (EpCAM) BiTE antibody AMG 110. EpCAM, which is also known as CD326, is a 40-kDa glycoprotein expressed on a wide variety of epithelial

cancers and cancer-initiating cells. Its expression correlates with poor prognosis. Despite the fact that EpCAM is expressed in normal epithelial tissues, its increased accessibility on tumor cells makes it suitable as a target for an EpCAM/CD3-bispecific antibody. In a step-wise intra-patient dose escalation study, patients were treated with a 10 μg/m²/d or a stepwise increase up to 48 μg/m²/d. Higher target doses are currently being tested. The adverse events that defined the dose-limiting toxicity (DLT) were vomiting (15% with grade 3 or more), abdominal pain (30% with grade 3 or more), and diarrhea. While significant liver function test abnormalities were noted, patients seemed to adapt with continued dosing, and no clinical evidence of hepatic dysfunction was observed.

Ken Chang (Immunomedics, Inc.) presented a hexavalent bispecific antibody format (bsHexAbs) that uses the dock-and-lock (DNL) technique. DNL is based on the dimerization-docking domain of protein kinase A (PKA, cyclic AMP–dependent protein kinase) and the anchor domain (AD2) of the A-kinase anchoring protein.[2] This technique has been applied to antibodies by fusing the anchor domain c-terminal to the heavy or, alternatively, to the light chain of an antibody. By genetically fusing a Fab-fragment to the dimerization-docking domain, it can be site-specifically conjugated to either the CH3-domain (CH3-format) or the CL-domain (Cκ-format) of an antibody-anchor domain fusion. This results in a hexavalent bispecific antibody. Dr. Chang discussed preclinical data of a CH3-bsHexAb for the treatment of B cell malignancies. Both anti-CD20/CD22 and anti-CD74/CD20 bsHexAbs inhibit the proliferation of different lymphoma cell lines more effectively than the combination of corresponding parental antibodies.[3] For the treatment of solid tumors, Dr. Chang discussed multivalent antibodies against three targets, IGF-1R, Trop-2 and CEACAM6. An enhanced downregulation of IGF-1R was achieved with a bsHexAb targeting IGF-1R and Trop-2 compared with the parental antibodies. The bsHexAb is also more effective in inhibiting anchorage-independent growth and invasion of cell lines that express both receptors. Interestingly, a bsHexAb against IGF-1R and CEACAM6 is also more effective in downregulating IGF-1R than a corresponding hexavalent antibody targeting IGF-1R alone.

Dr. Chang then discussed the Cκ-format of bsHexAbs. This new format has a significantly longer half-life then the CH3-format. Despite a lower affinity to FcRn (KD = 165 nM) compared with the parental antibody (KD = 16 nM), a similar plasma half-life was determined in animal models. Fc effector functions like ADCC and CDC are maintained or enhanced in this format. First results from xenograft models indicate that a bivalent anti-CD20 and tetravalent anti-CD22 was more effective than the parental anti-CD20 antibody or the corresponding CH3-format bsHexAbs.

Peter Hudson (Victorian Cancer Biologics, Avipep Pty Ltd.) gave an overview of the Avibody™ platform. Lead candidates from this platform are engineered diabodies, which are small bivalent or bispecific antibody fragments. Diabodies are built from two scFvs with a short VH-VL connecting linker that prevents pairing between the two domains on the same chain, so the domains are forced to pair with the complementary domains of another chain.

Dr. Hudson presented the generic design of positioning two cysteine residues within framework regions of the variable domains that are accessible for site-defined surface conjugation of PEG and toxins.[4] This new technique allows conjugation of exactly four payload molecules per diabody. Dr. Hudson then presented data from their lead bivalent, PEGylated, diabody AVP0458, which targets the tumor-associated glycoprotein-72 antigen (TAG72) that is highly expressed in prostate and ovarian cancer tissue. When radiolabeled with 124I, xenograft animal models demonstrated tumor-to-blood ratios superior to scFv or whole antibodies. AVP0458 has entered a first-in-man Phase 1 biodistribution study in ovarian and prostate cancer. In concluding, Dr. Hudson noted that Avibodies can be produced by cost-efficient bacterial GMP fermentation, purified from inclusion bodies and refolded after purification. Yields of up to 1 g/L are achieved with some Avibodies. Site-specific loaded avibody-drug conjugates demonstrated strong anti-tumor activity in xenograft models. Avibodies (Diabodies) thus appear to be an ideal platform either for radiolabeled imaging or drug-loaded antibody therapy.

Ton Logtenberg (Merus B.V.) presented a novel technology platform for the generation of bi- and multi-specific human antibody therapeutics with a common light chain. For this purpose, a transgenic mouse (MeMo) has been developed. Endogenous murine Ig loci were knocked out in the MeMo mouse, and a rearranging human VH locus and a single human light chain were inserted into the genome. The antibody constant regions were kept murine. No significant differences in the number or subpopulations of B cells were determined between wild type and MeMo mice, and isotype switching in MeMo to IgG occurred at normal frequencies. Upon immunization, MeMo mice were shown to mount strong immune responses against a broad variety of antigens tested. MeMo mice, however, express only human antibodies with the single common light chain. The analysis of the sequence of different antibody clones revealed that 80 percent carried only one or no mutations in the light chain after immunization, indicating that the mutation rate in this light chain is very low. Despite the absence of the light chain diversity, MeMo mice have a robust immune response and show high antibody titers after immunization. The resulting monoclonal antibodies have a large sequence and epitope diversity.

Dr. Logtenberg then outlined the characteristics of the Merus bispecific technology Biclonics and the multispecific technology Oligoclonics. Biclonics are derived from engineered CH3-driven heterodimerized antibodies with a common light chain. Two examples of Biclonics were presented. In the first example, two members of the HER family were targeted simultaneously (EGFR × HER3); in another example, one paratope of the bispecific antibody binds to CLEC12A, a marker for acute myeloid leukemia (AML),[5] and the other to CD3. This antibody has been further engineered to abrogate Fc receptor-mediated effector functions without interfering with FcRn binding. Oligoclonics are human antibody mixtures with a common light

chain that can be produced in stable cell clones to ensure a constant ratio of all expressed antibodies.[6]

After introducing the concept of antibody mixtures, **Ivan D. Horak** (Symphogen) presented recent preclinical and clinical results for two of Symphogen's mixtures, rozrolimupab (Sym001) and Sym004. The advantages of antibody mixtures composed of monoclonal antibodies include superior target internalization and degradation, increased Fc mediated effector functions (like ADCC and CDC) and enhanced clearance of soluble targets. Rozrolimupab is a composition of 25 monoclonal antibodies specific for the RhD erythrocyte antigen that are derived from B lymphocytes of RhD-negative female donors with high serum antibody titers against RhD. Rozrolimupab is produced by single batch manufacturing of an equal mixture of 25 CHO cell lines.[7] Dr. Horak noted that the antibody mixture has been tested in an open-label, international, multicenter, exploratory dose-finding study of patients with primary immune thrombocytopenia (ITP). The patients enrolled had a history of primary ITP, an RhD positive serology and previous treatment and response to first line therapy for ITP.[8] A total of 61 ITP patients were treated with a single 75, 100, 125, 150, 200, 250, 300, or 350 μg/kg dose of rozrolimupab. Up to 50 and 60% of the patients responded at 24 and 72 h, respectively, and up to 83% responded at day 7. The most frequently reported adverse events were headache (18%) and pyrexia (13.1%). Most adverse events were of mild or moderate intensity.

Dr. Horak then summarized preclinical results of Sym004, a mixture of two chimeric IgG1 antibodies against the domain III of EGFR. In a cancer cell line, Sym004 had better activity than the combination of cetuximab and trastuzumab, indicating that Sym004 can also inhibit EGFR-HER2 cross-talk.[9] Sym004 induces rapid downregulation of EGFR from cancer cells in vitro and in vivo. In animal models, Sym004 showed sustained tumor suppression where cetuximab or the individual antibodies had only a partial response. Sym004 has recently entered a Phase 2 trial.

The preclinical and clinical progress of the TandAb platform was presented by **Eugene Zhukovsky** (Affimed Therapeutics AG). TandAbs are tetravalent bispecific antibodies with two binding sites for each antigen. One paratope is directed against a tumor-associated antigen and the other against an activating receptor on immune effector cells like cytotoxic T cells or natural killer (NK) cells. TandAbs are approximately two times larger than diabodies or similar bispecific antibody formats. With a molecular weight of 100 to 110 kDa, they are above the threshold for renal clearance. In non-human primates, they showed plasma half-life between 3 and 23 h depending on the amount of the dose and the dosing regimen. This is significantly longer than reported for smaller antibody formats. Dr. Zhukovsky presented examples of both CD3- and CD16A-engaging TandAbs that facilitate either the recruitment of T or NK cells, respectively. The CD16A binding domain of NK cell-recruiting TandAbs has unique properties: it has selective specificity for CD16A with no binding to the CD16B isoform, and it is not influenced by CD16A polymorphism.

AFM11 is a CD19×CD3 TandAb for the treatment of NHL and leukemia. In preclinical studies, AFM11 showed better potency than the corresponding CD19×CD3 BiTE (MT103) with all T cell subtypes. AFM11 was also more potent at lower effector-to-target ratios. In xenograft models, both bispecific antibody formats showed similar levels of protection. AFM11 activates T cells exclusively in the presence of target cells and, in their absence, no T cell proliferation or secretion of cytokines, e.g., IFNγ, is detected. In addition, no off-target cell lysis was observed. AFM13, the most clinically-advanced candidate, is a CD30×CD16A TandAb antibody. In a Phase 1 study, it showed anti-tumor activity in 14 of 27 Hodgkin lymphoma (HL) patients. AFM13 was well tolerated with the most frequent drug-related adverse events being fever and headache. Three of four HL patients who relapsed after prior anti-CD30 treatment with brentuximab vedotin achieved stable disease after AFM13 treatment.

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December 5, 2012: Antibody Engineering Systems Medicine in the Development of Antibody Therapies/Systematic Validation of Novel Antibody Targets

Yu Zhou

Session chair **James D. Marks** (University of California, San Francisco) gave the keynote presentation on a systematic approach to generate tumor targeting and internalizing mAbs.

Such mAbs can deliver payloads into cells to achieve a therapeutic effect. He compared differences between ADCs and antibody-targeted immunoliposomes with respect to the amount of drug delivered, the potency of drugs used, and the size of the molecule. He noted specific features of immunoliposomes, including the impact of targeting on tumor deposition and micro-distribution.[1] Professor Marks then reviewed the generation of an internalizing anti-HER2 antibody F5 by selection of a naïve phage display antibody library on live cells, the construction of F5-targeted immunoliposomal (IL) doxorubicin, and its efficacy in preclinical studies. He discussed the factors that affect IL tumor localization and cellular uptake, including mAb affinity, valency, and epitope.[2,3] He also described a systematic approach to isolate mAbs from phage libraries to any cancer type using the basal subtype of breast cancer as a model. Methods were described to: (1) select internalizing mAbs; (2) identify the antigen bound by the mAb by using immunoprecipitation and LC/MS-MS; (3) predict the mAbs cognate antigen in silico when immunoprecipitation failed by cell line profiling; and (4) direct selections to a specific tumor antigen by combining cell selection followed by selection on yeast displaying the target antigen on their surface. Finally, Professor Marks introduced a project to develop a high-throughput platform to generate mAbs to type 1 and 2 membrane proteins using yeast display of the membrane protein for phage antibody library selection.

Ulrik Nielsen (Merrimack Pharmaceuticals) provided an update on the development of HER2-targeted nanoliposomal doxorubicin (MM-302) using systems optimization of nanotherapeutics. In the Phase 1 clinical study of MM-302, complete regression of tumor lesion was observed after 6-mo treatment of HER2-positive breast cancer, and the drug was well tolerated. Dr. Ulrik Nielsen then reviewed the systems used to optimize nanoliposomal drugs, which include optimization of multiple factors such as the antibody copy number, antibody affinity, the degree of PEGylation, and encapsulated drug concentration. He discussed the computational models used to understand the therapeutic window (e.g., to avoid effects on the heart compared with tumor), explained how to predict the optimal affinity and copy number of antibodies for such therapeutic window, and how the low doxorubicin exposure to the heart DNA from MM-302 compared with the free doxorubicin was achieved. Using the computational simulation, the pharmacokinetics (PK) of liposomal drug can be predicted and carefully controlled, and the drug release rate and the drug responsive patients can also be identified. In addition to the computational modeling, Dr. Ulrik Nielsen introduced an experimental monitor and prediction of nanotherapeutic activity by nanoliposomal imaging reagent MM-Dx-929, ILs-^{64}Cu, to show tumor deposition of immunoliposomes. Such an imaging method showed a high degree of variability in tumor deposition in human tumors and different tumor models. In a mouse model of BT474 tumor, MM-302 responses correlated with MM-Dx-929 deposition in tumors. Both computational and experimental results indicated that micro-distribution and cellular uptake of nanoliposomal drugs play critical roles in nanotherapeutics.

The session co-chair, **Dario Neri** (Swiss Federal Institute of Technology Zurich) presented progress in the drug discovery using DNA-encoded chemical libraries. He first described the principle of the DNA-encoded chemical library, which consists of compounds covalently attached to coding DNA fragments, allowing identification of small molecule ligands to a target in a selection mode rather than screening individual compounds, one molecule at a time. The unique DNA sequences attached serve as amplifiable identification bar codes, which made it possible to identify the binding small molecule after the selection. He also reviewed the history of DNA-encoded chemical library from single compound to multiple compound complexes in the Encoded Self-Assembling Chemical (ESAC) library. In the ESAC library, more than one small molecule can be assembled as a multi-head binding compound complex, and encoded by corresponding DNA sequences incorporated in tandem by complimentary spacing of common DNA fragments. In such manner, the resulting multi-compound molecules that exhibit synergistic binding activity can be identified with enhanced binding specificity and affinity.[4]

Professor Neri showed that multiple potent inhibitors with affinities ranging from 0.2 to 5 μM were identified for the tumor-associated antigen carbonic anhydrase IX (CA IX) and the pro-inflammatory cytokine interleukin-2 (IL-2).[5] In addition to the de novo identification of ligand-like small molecules to interrupt protein–protein interactions, DNA-encoded chemical approach can also be used for "affinity maturation" of sub-optimal ligand binding. By such means, several trypsin inhibitors with over 10,000-fold potency compared with the parental compound, and enhanced selectivity toward other serine proteases, were isolated from a DNA-encoded library based on benzamidine analogs.[6] In summary, the DNA-encoded chemical library offered a powerful and yet flexible approach to identify potent ligand-like small molecules for protein interaction interventions, and potential therapeutic drugs.

Guoqing Chen (Igenica, Inc.) presented an innovative approach to develop anti-tumor antibodies. Dr. Chen began with a discussion of target "crisis" in cancer antibody drug discovery and the lack of reliable methods to identify effective target antigen/antibody pairs. He introduced Igenica's technology platforms that enable a function-orientated approach for target antigen and antibody drug discovery. Igenica's approach consists of both antigen and antibody discovery platforms, sTAg and iTAb. The sTAg (surface-tagged-antigen) discovery platform identified tumor associated cell surface antigens by coupling the pull-down and LC-MS/MS analysis of surface tagged protein from tumor samples. By comparing the surface proteome profile and quantities of multiple tumor samples, the relevant tumor antigen can be identified and used for animal immunization. The iTAb (in vivo anti-tumor antibody) screening platform uses in vivo tumor rejection as a criterion to identify anti-tumor antibody repertoire among the immunized mice, followed by functional antibody isolation and validation.

Using the sTAg and iTAb platforms, both "Herceptin-like" and "Perjeta-like" anti-Her2 mAbs were isolated, indicating the feasibility of this novel approach. Dr. Chen also showed that a novel antigen/antibody pair, AML-01/IGN523, was discovered to treat AML, CLL, MM, and other cancers with stronger

potency than existing antibodies. AML-01 is a single pass, type II transmembrane protein that overexpresses on multiple tumors; such overexpression leads to tumor formation and correlates with tumor progression and metastasis. This antigen, however, has no prior investigational reports, which suggests that Igenica's approach is a unique and powerful tool for tumor associated antigen discovery and potent anti-tumor antibody identification. IGN523 is a humanized IgG1 mAb and Igenica plans to initiate a Phase 1 clinical study for this therapeutic candidate in 2013.

Susanne Gräslund (Structural Genomics Consortium) discussed an effort to provide open access to all data and reagents associated with epigenetic processes. This protein-based consortium currently includes over 1300 PDB entries of 3D structures, protein expression clones, protocols for expression and crystallization, other protein-based protocols, as well as chemical probes and antibodies. First, she discussed the critical questions in epigenetics, such as which proteins and protein domains are responsible to read, write and erase histone marks; how they recognize histone marks specifically and how they assemble into functional multi-protein complexes; which genes are regulated at the transcriptional level by the complexes; how these complexes and functions differ between normal and disease states; and whether these epigenetic targets can be modulated for therapeutics. Then, she presented their progress in identification of epigenetic targets, which include 72 for acetylation, 266 for methylation, and 32 for poly ADP ribosylation.

According to Dr. Gräslund, the specific aim of the project she presented is to generate renewable recombinant antibodies to the identified epigenetic targets. She described the project organization, which consists of multiple research institutes, including Structural Genomics Consortium, University of Toronto, University of Chicago, and Technical University, Braunschweig. The central goal is to generate renewable recombinant antibodies to the selected epigenetic targets. She also described a multi-step workflow that carries antigen production, Fab selection and production, initial validation, full-length IgG production, further validation, to final delivery of antibodies via Life Technology Corporation.

Technically, by cloning the target antigen into vector pNIC-Bio2, which contains a biotinylation sequence and co-expresses BirA ligase, the expressed antigens will be biotinylated in vivo for easy immobilization during selection from the phage-displayed Fab library. A high throughput antigen production system was established using a two-step method (IMAC+Gel filtration) on AKTA Xpress, which allows efficient production of antigen protein in large quantities. The identified Fab sequence was cloned from phage vector into pSFV4 vector, which contains the optimized triple tac promoter, PelB leader for Fab secretion, and heavy chain C-terminal biotinylation tag. The IgG conversion and production use transient transfection of HEK293F cells and pFUSE vector system. The efficient production of target antigen, antibody fragment, and full-length IgG allows for antibody generation in a relative short time period.

The identified antibodies were used to precipitate the target antigens; then mass spectrometry analysis was used to validate the binding specificities and identify the complex proteins. In addition, siRNA knockdown was used to confirm the antigen identity of the target antibody. Furthermore, the chromatin IP/ChIP was used to investigate genome wide protein–DNA interactions. The crystal structure of Fab/antigen complex was resolved to provide insight into structure and function relationships.

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December 5, 2012: Antibody Engineering
Antibody Activity and Animal Models

Kirstin A. Zettlitz

The afternoon's session on antibody activity and animal models was chaired by Professor **Dennis Burton** (The Scripps Research Institute). The first speaker was **Philip Johnson** (The Children's Hospital of Philadelphia), who discussed immunoprophylaxis by gene transfer as a shortcut to a human immunodeficiency virus (HIV) vaccine. He emphasized the need for a HIV vaccine and discussed the question of why, 30 y after the discovery of AIDS, we still don't have one. Considerations for HIV vaccines need to take into account that traditional approaches failed and might never work, and that the HI virus is "smarter" than other infectious agents, meaning that HIV infection (in most humans) does not elicit neutralizing antibodies with broad specificity. Dr. Johnson's approach is to bypass the adaptive immune response by antibody gene transfer, i.e., the gene encoding a rare neutralizing anti-HIV-1 antibody of choice is transferred to the person to be immunized.

Dr. Johnson presented a proof-of-concept experiment using adeno-associated virus (rAAV) gene transfer vector encoding the human IgG1 b12 for the transduction of myofibers in mice. Thereby, ex vivo manipulations are avoided and muscle cells are turned into an antibody factory. IgG1 b12 was endogenously produced and passively distributed into circulation in mice for more than 6 mo.[1] He then discussed the translation of the results into rhesus monkeys using the well-characterized simian immunodeficiency virus (SIV) model. SIV-specific immunoadhesins (4L6 and 5L7) were generated, consisting of SIV gp120-specific variable domains fused to a rhesus IgG2 Fc fragment.[2]

The variable domains were derived from SIV-infected macaques so they would match the challenging virus. Rhesus monkeys were "immunized" by injecting AAV vectors carrying SIV antibody genes into the muscle and antibody presence in the blood was subsequently assessed. The lead candidate 4L6 is expressed for over 4 y and is non-immunogenic. Four weeks after immunization the animals were challenged by injection of live SIV into the blood. Most importantly, "immunized" animals were protected from infection while the challenge virus infected all unimmunized control animals.

For translation into humans, variants of the neutralizing anti-HIV-1 antibody PG9 (immunoadhesin PG9i and whole antibodies in different expression cassettes PG9–2A and PG9-DP) were tested in vitro. Both whole antibody constructs were about 10 times more potent in neutralizing different virus strains then the immunoadhesin and are candidates for a Phase 1 clinical trial. Ultimately, a final product might include two vectors, each containing a potent antibody targeting distinct epitopes in the HIV envelope glycoprotein.

In concluding, Dr. Johnson noted that gene transfer can bypass adaptive immunity; rAAV vectors mediate durable transgene expression in pre-clinical models; they provide the opportunity for designer anti-infective molecules; and they behave similarly in humans compared with animals.

Robert Friesen (Crucell) focused on the mechanism of action of broadly neutralizing antibodies against influenza in the context of the development of a truly universal flu vaccine against influenza A and B. These viruses are responsible for both seasonal flu and influenza pandemics and the high number of lives claimed each year emphasizes the need for new therapies.

Dr. Friesen briefly described the influenza life cycle (HA receptor binding and entry, endocytosis and fusion of the viral envelope with the vacuole's membrane, release of vRNA and viral replication in the nucleus, assembly of new virus particles and egress) before he explained that the humoral immune response targets the head region of the viral surface glycoprotein hemagglutinin (HA), but the vast majority of antibodies produced by the human immune system are relatively strain-specific and bind to parts of the virus that rapidly mutate.

The broadly neutralizing human monoclonal antibodies against group 1 and group 2 influenza A initially discovered, and more recently discovered influenza B antibodies, have protected mice against lethal challenge from influenza viruses. These antibodies were used to identify conserved neutralizing epitopes on the influenza HA and investigate their mechanism of action.

Dr. Friesen then discussed antibodies developed by Crucell that bind to epitopes in the globular head of HA (CR8033, CR8071) lead to cross-binding of viral particles and seem to prevent propagation of viruses without preventing entry and genome replication; thus, the effect might be on formation of viral progeny and egress from infected cells. Fab fragments are not able to inhibit this process, which shows that bivalency is crucial for this mechanism. Antibodies binding to epitopes in the stem region (CR6261, CR8020) block HA cleavage and HA pH-induced conformational changes associated with membrane fusion. These findings establish that both the HA stem and head regions contain broadly protective epitopes. The identification and characterization of mAbs with broad neutralizing activity against influenza A (group 1 and 2) and influenza B might not only lead to the development of antibody-based treatments but may also serve as guide for design of broadly protective vaccines.

Dennis R. Burton (The Scripps Research Institute) discussed different animal models used to evaluate human antibodies against HIV. He started by introducing the concept of reverse engineering for a viral vaccine for viruses, such as HIV, that have evolved mechanisms to evade neutralizing antibody responses. The process involves the isolation of broadly neutralizing monoclonal antibodies (bnMAbs); molecular characterization of Ab-Env (envelope spikes on virus) complexes; structure-based immunogen design to elicit specific bnAb; analysis of antibody responses to the immunogen(s); and eventually the combination of several immunogens to develop a vaccine.

Rapid recent progress has seen the discovery of many new potent bnMAbs to new and previously defined epitopes on HIV, an important development for HIV vaccine design. Examples include the PGT bnMAbs isolated from antibody repertoires of four human HIV-infected donors with remarkably broad and potent neutralizing responses. These novel bnMAbs show ~10- to 100-fold higher neutralization activity when tested on a 162-virus panel, thereby evolving the definition of broad and potent HIV-1 antibodies.[3] One of these antibodies (PGT121) is one of the broadest and potent antibodies isolated to date, and Dr. Burton presented the evaluation of the in vivo protective potency in rhesus macaques.[4] Intravenous administration of PGT121 24 h before a single high dose vaginal challenge of chimeric simian-human immunodeficiency virus (SHIV) resulted in sterilizing immunity in all animals that had received 5 mg/kg or 1 mg/kg, and in 3 of 5 animals administered 0.2 mg/kg. Neutralizing titers in serum and vaginal antibody titers suggest that a protective serum concentration for PGT121 is significantly lower than those observed in previous studies.

After this discussion of in vivo studies performed in monkeys, Dr. Burton took a short digression on the importance of small animal models for HIV infection[5] and presented the BLT mouse model (humanized bone marrow, liver, thymus) that holds great promise to facilitate the in vivo study of human immune response. The dose-dependent protection by b12 against IP JRCSF challenge confirms that in vitro neutralization correlates with protection.

Alternative to antibodies, the antibody-like tetrameric molecule CD4-IgG2 mediates protection against high-dose highly pathogenic mucosal SIV challenge at very low serum neutralizing titers in macaques.[6] Dr. Burton showed in vitro neutralization of SIVmac239 by CD4-IgG2 and CD4-IgG2 protection against SIVmac239 challenge. Because of the molecules short half-life, an osmotic pump was implanted for CD4-IgG2 administration 3 d before intrarectal high dose challenging the animal. Viral load was checked repeatedly up to 8 weeks post infection. Protection was seen in 3/6 and 5/7 animals with 20 mg and 200 mg administration, respectively, and was not correlated with observed macaque anti-human CD4-IgG2 titers. Dr. Burton concluded that protection against virus challenge in macaque and mouse

models generally correlates with neutralizing potency, but there are exceptions.

Dr. Burton then switched from protection to the topic of antibody therapy for HIV infection. Neutralizing antibodies have limited effects on the control of established HIV-1 infection in vivo.[7] Dr. Burton presented the effects of HIV bnMAbs investigated using the huPBL-SCID mouse (SCID mouse reconstitution with human PBMC). After HIV infection, the neutralizing antibody was administered, followed by serial measurement of plasma viral load, HIV recovery and testing for antibody neutralization sensitivity. While a single antibody had no effect on the infection a triple antibody therapy (2F5, 2G12, b12) suppressed the wild type virus for about a week, but an escape variant appeared during high antibody serum concentrations before the wild type virus returned as mAb concentration decreases.[8,9] Trkola and colleagues observed a similar result in humans. Very recently in a mouse model, Nussenzweig and colleagues showed that while a single or even three mAbs was not particularly effective, with five highly potent mAbs (penta-mix) control of virus is consistently observed.

Arturo Casadevall (Albert Einstein College of Medicine) presented a new synthesis for antibody-mediated immunity. He started with an overview about antibody discovery, serum therapy and the bacterial diseases that are treatable. He highlighted the initial success and importance of immunoglobulins and antibody-mediated immunity and discussed reasons for the decline of interest in antibody-mediated immunity, with the focus shifting to other B cell functions, cell-mediated immunity, new emerging pathogens and the perception that antibody-mediated immunity was understood. The first of four themes Dr. Casadevall discussed was the concept of humoral vs. cellular immunity, a paradigm that he considers untenable. One problem is the separation of labor for both subareas. Another problem is the unreliable methods used to test antibody-mediated protection; for example, antibodies that are not protective in assays with polyclonal sera might be inhibited by other mAbs or the antibody may not be present in adequate amounts to mediate protection.

The second theme was summarized as the immunoglobulin made 'whole', and concentrated on the perception that antibodies consist of two domains (Fv and Fc) that do not interact. Contrary to that idea, Dr. Casadevall presented data of mouse-human chimeric antibodies consisting of identical variable domains and different constant human regions. These artificial molecules showed altered specificity and idiotypes that appeared to be due to alterations in the V region and differences of the structure of antigen-antibody complexes.[10] Similar isotype-related changes in fine specificity were observed with mouse IgG switch variants and with V-region identical mAbs that demonstrated different ka for univalent ligands.[11,12] To show that the isotype influences the paratope, the 2D NMR method was used with ^{15}N-labeled antigen peptide in the presence and absence of antibody, and the profiles and rate curves differed between the isotypes.[13] Crystallography and modeling further confirmed that the C_H1 domain affects Fv structure. These results show that the antibody molecule should be viewed as a whole because Fv and Fc are not structurally independent.

Next, Dr. Casadevall focused on new direct functions for immunoglobulins and presented protective antibodies that prevent biofilm formation of *C. neoformans* by cross-linking of fungal capsules. The comparison of IgG and C3 opsonization showed different outcomes in exit dispersion, supporting the hypothesis that antibody binding could alter microbial gene expression and immunoglobulins have direct actions on their microbial targets. His next example was antibody-mediated toxin neutralization, which is thought to be antibody-only action based on the fact that, for many antibodies, Fab fragments can mediate neutralization and that neutralization can occur in vitro without cells. The IgG2a and IgG2b antibodies derived from an IgG1 antibody directed against a protective *B. anthracis* antigen showed identical affinity and fine specificity in vitro, but, surprisingly, their toxin neutralizing activity was isotype-dependent and due to FcR interactions. Dr. Casadevall concluded that the function of antibodies is more than binding and that there is a requirement for FcγR in antibody-mediated toxin neutralization.[14]

The last theme Dr. Casadevall discussed was the problem of emergence, i.e., antibodies with different properties (protective, enhancing and indifferent) that show unpredictable additive or synergistic effects when used in combination. This implies that mixing antibodies results in novel outcomes that are not reducible to effects with the individual antibodies and that are unpredictable even if only two antibodies are combined (S. Chow, unpublished). The protective efficacy of an antibody may not be reducible to that observed with an isolated preparation and determinism needs to be replaced by emergence.

David Hilbert (Zyngenia) introduced the Zybody™ platform technology, which produces single-protein combination therapeutics with a conventional mAb as scaffold fused to optional modular recognition domains (MRDs), i.e., phage display selected target-binding peptides, to form multi-specific, multivalent and multi-epitopic fusion proteins. Because the intact antibody scaffold is not altered, these molecules are expected to retain Fc-mediated effector functions and pharmacokinetic properties. He then discussed how simultaneous targeting of multiple erbB receptors with Zybodies could potentially overcome tumor resistance.

The need for new erbB targeting drugs is explained by the complexity of the erbB receptor family and knowledge of resistance to single agents because of cross-talk and hetero-dimerization between receptors. Additionally, there are restricted patient populations and the complex tumor microenvironment might influence tumor growth.

Dr. Hilbert presented Zybodies based on trastuzumab (Herceptin®) in combination with a HER2 binding MRD with functional attributes of pertuzumab (bispecific, multi-epitopic) and a HER1 binding MRD [trispecific, multi-epitopic HER-egfR(H)-per(H)] and showed that bi- and tri-specific targeting of HER2 and EGFR is more effective in inhibiting signaling of AKT and Erk pathways than trastuzumab, cetuximab, pertuzumab, or combinations thereof. The tri-specific Zybodies also downregulated both EGFR and HER2 more efficiently (4–5-fold) than mixtures of mAbs. Low affinity HER2 peptides that bind to epitopes other than trastuzumab co-operate

with trastuzumab and enhance inhibition of cell proliferation on a panel of epithelial cancer cell lines. For the bispecific Zybody [HER-per(H)] targeting two epitopes on HER2, superior receptor internalization compared with trastuzumab alone could be shown.

Dr. Hilbert then described a project focused on multi-specific targeting of HER1 and HER3 receptors with cetuximab as mAb scaffold and a HER3 binding MRD fused to the heavy chain. This bispecific Zybody [CET-z5417(H)] showed stronger inhibition of tumor cell signaling and proliferation than cetuximab and Genentech's two-in-one antibody DL11F. In a BXPC-1 pancreatic carcinoma xenograft model, bispecific CET-z5417(H) demonstrated superior tumor growth inhibition compared with cetuximab, and comparable activity to DL11F. A trispecific Zybody targeting HER3 and two non-overlapping EGFR epitopes (z5417-CET-ea13) showed even stronger biological activity in inhibiting tumor cell proliferation in vitro.

Another bispecific and multi-valent agonistic Zybody (HER-dr51) is based on trastuzumab fused to a TRAIL-R2 (DR5) binding MRD. Although TRAIL-R agonists are well tolerated none of the clinical trials to date showed clinically significant activity of single agents. A possible reason for the lack of efficacy is insufficient cross-linking of the receptor, which can be overcome by multi-specific targeting of TRAIL-R2 with a Zybody containing two tandem repeats (four binding valencies) of DR-5 targeting MRD. Indeed, HER-dr51 Zybody showed effective tumor cell killing without exogenous cross-linking.

In conclusion the targeting of erbB receptors with Zybodies yields well-differentiated product opportunities.

Allan Bradley (Kymab Limited) introduced the Kymouse platform for human mAb discovery. The combination of a large initial gene repertoire and a normal immune response, including somatic hypermutation, leads to a large potential diversity of unique antibodies in animals. The Kymouse HK™ has a normal serum background and B cell compartment, which allows the initial display of antibodies on B cells while a reduced or deficient B cell repertoire can limit the variety of mAbs. To maximize antibody diversity, the mouse constant regions are used to allow correct signaling and maturation within the B cell, which results in correct Ig isotypes, class switching and somatic hypermutation and makes extensive antibody engineering of variable regions unnecessary. The ability to knock-in (Kymouse KI) or knockout (Kymouse KO) relevant mouse genes makes it possible to remove background antigen and allows an optimal immune response even to highly homologous and conserved disease target antigens.

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December 5, 2012: Antibody Therapeutics Development Status of Immunomodulatory Therapeutic Antibodies

Peter-Christian Klöhn

The morning session of the Antibody Therapeutics track was chaired by **Philip Thorpe** (University of Texas Southwestern). **Dario Neri** (Swiss Federal Institute of Technology Zurich) gave a compelling keynote lecture about the delivery of immune modulators to vascular structures of disease sites. Angiogenesis, the formation of new blood vessels, is a hallmark of tumors and critically required to support tumor growth. To specifically and effectively deliver drugs into tumors, Professor Neri's group aimed to identify unique tumor markers of the vasculature. Tumor marker discovery was driven by mass spectroscopic analysis of in situ biotinylated vascular structures of tumor-bearing mice and led to the identification of tumor-specific protein domains of oncofetal fibronectin and tenascin-C.[1] Antibodies against identified domains of fibronectin (EDA, EDB) and tenascin-C (A1) further attested disease specificity of these splice isoforms and confirmed that EDA was strongly expressed in the vasculature of human lung and liver metastases, and in a large panel of other human tumors with negligible expression in tissues of healthy patients.[1] In normal tissue, oncofetal fibronectin expression is restricted to

the placenta and the endometrium in the proliferative phase.[2] Human antibodies in industrial development include L19 and F8 against the fibronectin domains EDB and EDA, respectively, and F16 against the tenascin-C domain A1. Of note, the EDA domain of fibronectin is also expressed in various inflammatory diseases, thus providing the potential for treatment in non-oncological indications.[2,3]

After establishing a platform for tumor-specific drug delivery, Professor Neri's group developed antibody-drug fusion proteins for the immune modulation of tumors. The L19-tumor necrosis factor (TNF)-fusion protein forms stable trimeric structures and was tested in clinical settings where recombinant TNF was already in use. Patients destined to undergo amputation because of large tumors in a limb can be treated by TNF therapy in a precarious procedure termed isolated limb perfusion (ILP). After isolation of the limb with a compressing device the patient is perfused with TNF at a concentration that would be lethal when given systemically. The targeted version of TNF (L19-TNF) proved therapeutically efficient at 20-fold lower concentrations compared with the non-targeted version and with no requirement for ILP. Intriguingly, large tumors in patients were resolved within six weeks. F8-TNF was effective in a sarcoma mouse model in combination with doxorubicin. Here, doxorubicin showed no effect, F8-TNF alone cured two out of five mice, whereas the combination of both drugs was curative. Surprisingly, it turned out that mice were vaccinated after F8-TNF treatment, since subsequent challenges with cancerous cells did not give rise to tumors. A combination treatment with L19-TNF and doxorubicin has now entered clinical trials.

L19-IL2 (darleukin), which consists of interleukin (IL)-2 (Novartis) fused to the L19 antibody, has now moved to Phase 2a and Phase 2b clinical trials in metastatic melanoma patients. The EDA domain of fibronectin is also expressed in various inflammatory diseases, including endometriosis, arthritis and atherosclerosis. To target anti-inflammatory drugs to affected areas, fusion proteins of the F8 antibody and IL10 were generated. In arthritis mouse models F8-IL10 was curative in combination with methotrexate.[2,4] Professor Neri concluded that vascular targeting of drugs has great potential in cancer treatment and for applications beyond oncology.

Bruce Cree (University of California, San Francisco) focused his presentation on the emerging role of B cells in demyelinating diseases. Strong evidence for a contribution of B cell mediated effects have been reported for a rare inflammatory disease of the central nervous system (CNS), termed neuromyelitis optica (NMO). This disease is characterized by severe attacks of optic neuritis and myelitis, but, unlike multiple sclerosis (MS), the brain is most commonly spared in early disease stages. In more than 90% of cases, NMO is a relapsing disease with severe cumulative disabilities including paraplegia and blindness due to optic neuritis. Remarkable advances were made by the discovery of autoantibodies in NMO patients against aquaporin 4 (AQP4),[5] a water channel that is widely expressed in the optic nerves, the spinal cord and the periventricular regions. This association with a B cell-mediated activity led to the hypothesis that B cell depleting therapies might be effective in reducing relapse occurrence in NMO patients. An open-label preliminary study showed that rituximab was well-tolerated and 7 of 8 patients experienced substantial recovery of neurologic function over one year.[6] In the second part of his talk, Dr. Cree reviewed the current state of therapy in MS and suggested that the B cell-specific antibodies ofatumumab and ocrelizumab in Phase 3 clinical trials would mark a shift of paradigm in the field from T to B cell-targeted treatment.

Eric Fedyk (Millennium Pharmaceuticals Inc.) discussed the mechanism of action of vedolizumab, a promising gut-selective anti-inflammatory biologic in development for the treatment of inflammatory bowel diseases (IBD).[7,8] Crohn disease (CD) and ulcerative colitis are chronic inflammatory conditions of the gastrointestinal tract that typically emerge in young adults and are characterized by symptoms of diarrhea, incontinence, pain, fever and weight loss, leading to a lifetime of disability. IBD is associated with an accumulation of activated helper T (T_H) cells in the gastrointestinal tract. Blocking T_H cell infiltration therefore constitutes a therapeutic paradigm for the treatment of IBD. Memory T_H cells exhibit preferential routes of migration while surveying tissues for the presence of antigen. A distinct subset of gut-homing memory TH cells exists whose infiltration of the gastrointestinal tract requires binding of their α4β7 integrin to MAdCAM-1 expressed on the vascular lumen. Natalizumab is a dual α4β1 and α4β7 integrin antagonist that is approved for treatment of multiple sclerosis (MS) and CD; however, use of this antibody is restricted due to an association with an often fatal opportunistic viral infection of the CNS, termed progressive multifocal leukoencephalopathy (PML). It is believed that antagonizing the α4β1 integrin predisposes patients to PML by impairing immune surveillance of the CNS for reactivated JC virus, the cause of PML.

Millennium Pharmaceuticals is consequently developing the α4β7 integrin antagonist vedolizumab on the premise that it will block gut inflammation without predisposing patients to PML. Vedolizumab binds exclusively to α4β7 integrin and not to the α4β1 and αEβ7 integrins.[9] In human whole blood, vedolizumab binds to a discrete T_H cell subset with $\alpha4^{high}\ \beta1^{low}$ phenotype, but not to the majority of memory T_H cells, neutrophils, and most monocytes.[9] It is a selective antagonist of the α4β7 integrin, which blocks cellular adhesion to MAdCAM-1, but not VCAM-1.[9] In experiments with colitic cotton-top tamarins, a monkey model of human IBD, blocking the α4β7 integrin alleviated chronic diarrhea and gastrointestinal inflammation.[10] A decrease in the frequency of lymphocytes in gastrointestinal tissues corresponded to a significant increase in α4β1 memory T_H cells in peripheral blood.[11] No cases of PML have been reported in clinical trials with vedolizumab to date and the potential effects of vedolizumab on immune surveillance of the CNS was investigated in rhesus experimental autoimmune encephalomyelitis (EAE). Unlike natalizumab which blocks entry of T lymphocytes into the CNS and delays development of EAE, vedolizumab did not inhibit immune surveillance and inflammation of brains of Rhesus macaques and did not inhibit development of EAE. Vedolizumab therefore appears to be a gut-selective anti-inflammatory biologic that may be efficacious in IBD without predisposing patients to PML.

Alan Epstein (University of Southern California Keck School of Medicine) discussed current approaches in cancer

immunotherapy. While concluding that the majority of immunotherapeutic approaches aim to improve immunogenicity of tumors to the host immune system, he advocated a more integrated therapeutic strategy tailored towards reversal of tumor-mediated immunosuppression in addition to antigen-specific activation. Because tumors have evolved several ways to escape immunity (e.g., cellular suppression by regulatory T cells and myeloid-derived suppressor cells, molecular suppression by loss of HLA class I molecules or increased expression of HLA-G), better understanding of the molecular mechanisms of immunosuppression is imperative for an effective tumor therapy. Professor Epstein suggested that mechanisms of immunosuppression in a tumor are similar to those of early immune development where the fetus is protected against immune-destruction in the womb.

As a therapeutic strategy, Professor Epstein's laboratory aims to alter the tumor microenvironment by targeting immunocompetent cells. The chemokine (C-C motif) ligand 16 (CCL16)/LEC, a molecule that interacts with chemokine receptors, attracts leukocytes, monocytes, dendritic cells and lymphocytes. To target tumors, the LEC molecule was fused to an antibody, Tumor Necrosis Therapy targeting (abbreviated TNT), that binds to necrotic areas of tumors. The LEC-TNT fusion protein significantly reduced tumor volumes in several mouse tumor models, including colon 26, Renca renal and MAD 109 lung carcinomas. Surprisingly, a complete cure of tumors in the colon 26 model at an advanced stage was observed with the LEC-TNT fusion protein in addition to CD4 depletion. In contrast, CD4 depletion alone did not show any effects on the tumor volume. LEC-TNT furthermore showed synergistic effects when combined with an IL2-Fc fusion protein. To address toxic side effect of IL2 injections, the gene was mutated at codon 38, an alteration that reduced vasopermeability by 96% compared with unmodified IL2, whereas IL2 activity is marginally affected. To integrate vaccine technology with immunomodulatory reagents, studies are now in progress to test combination regimens. In preliminary studies, LEC-TNT in combination with 5-fluorouracil and cyclophosphamide completely abolished tumor growth in the 4T1 breast carcinoma mouse model. The LEC-TNT fusion protein is currently being tested in spontaneous tumors of dogs and may be tested in patients in the near future, pending support.

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December 5, 2012: Antibody Therapeutics
Modulating the Half-Life of Antibody Therapeutics

Gabriele Proetzel

The chair of the Antibody Therapeutics session on modulating the half-life of antibody therapeutics was **Trudi Feldman** (Abbott Laboratories). The first speaker, **Ray Camphausen** (Adnexus, a Bristol-Myers Squibb R&D Company) discussed approaches to extend the half-life of small modular antibody scaffolds using the example of the Adnectins. Three methods, pegylation, albumin-based approaches and Fc fusion, were presented. He discussed the Adnectin™ molecule CT-322, which is designed to block binding of vascular endothelial growth factor (VEGF) A, C and D to their receptor, VEGFR-2. They showed that, by adding a 40-kDa branched PEG, the half-life was increased to 70 h without losing the molecule's high affinity to human VEGFR-2. In an alternative approach, the albumin binding domain (ABD) of protein G was fused to CT-322. When ABD was linked at the N-terminus, a half-life of 133–199 h was achieved in cynomolgus; however, this was only 67–87 h when ABD was added at the C-terminus. The third approach, Fc-fusion (N-terminal or C-terminal) gave a half-life of ~80 h in cynomolgus.

Additional approaches include use of Adnectins with dual or multiple binding sites. Dr. Camphausen presented the example of FGF21-PKE Adnectin, which uses the Adnectin to increase half-life of FGF21. FGF21 is 20 kD and normally has a half-life of 1–4 h in cynomolgus. When fused to the albumin-targeted Adnectin, the half-life is increased to 96 h in cynomolgus. Adnexus is collaborating with Novozymes in the use of human serum albumin (HSA), optimizing binding to FcRn and reaching 180 h half-life in cynomolgus.

Roland Kontermann (University of Stuttgart) presented concepts for half-life extension strategies. He reviewed the FcRn recycling pathway, showing this can be exploited for half-life extension approaches using albumin or the Fc domain. For single chain diabodies (scDb), current half-life extensions approaches

include pegylation (PEG), glycosylation, albumin (HSA) fusion, fusion with the ABD from streptococcal protein G, Fc fusion, and immunoglobulin-binding domains (IgBD). He showed successful half-life extension with scDB-ABD, scDB-HSA and scDB-PEG_{40K}. For scDB-ABD, Dr. Kontermann also presented data showing that the half-life extension is FcRn-dependent, leading to selective and improved accumulation in antigen-positive xenograft tumors, and more effective than scDB PEG_{40K}. He described a novel approach using IgBDs. They tested domains from Staphylococcus protein A (SpA_B, SpA_B, SpA_B), Staphylococcus protein G (SpG_{c3}) and Staphylococcus protein L (Ppl_{C4+}). SpG_{c3} was the most promising module for half-life extension when testing scDbs and single-chain variable fragments (scFvs), and gave similar results as ABD. Dr. Kontermann's overall conclusion was that there is further room for improvement in half-life extension.

Javier Chaparro-Riggers (Rinat/Pfizer) discussed the concept of pH-sensitive antibodies, focusing on the anti-PCSK9 antibody J16 and it pH-sensitive version J17. The limitation of conventional antibodies is that they only have one binding cycle, and therefore have a short half-life due to target-mediated degradation. To circumvent this, pH-sensitive antibodies have been developed that will release the target at low pH and can then be rescued by the FcRn pathway. The J16 antibody reduces LDL-C levels, and thereby lowers coronary heart disease events. J16 was made pH sensitive by targeting the histidine residues H310, H433 and H435, creating the novel antibody J17. J17 showed prolonged half-life and better efficacy in mouse and cynomolgus. In summary, pH-sensitive antibodies are expanding the therapeutic space and are more effective especially when targeting soluble proteins.

Syd Johnson (MacroGenics) discussed the half-life extension of bispecific dual-affinity re-targeting (DART) proteins. He showed data using ABD-fusion, pegylation and Fc-bearing domains (MP3 containing three gamma chains and MP4). The MP3-DART showed ~2-fold longer half-life than the ABD fusion, and is currently the most favorable format for this system.

Gabriele Proetzel (The Jackson Laboratory) presented data on two humanized FcRn mouse models, Tg276 [B6.Cg-Fcgrt <tm1Dcr> Tg(CAG-FCGRT)276Dcr/DcrJ; JR004919] and Tg32 [B6.Cg-Fcgrt <tm1Dcr> Tg(FCGRT)32Dcr/DcrJ; JR014565]. Both models express human FcRn (hFcRn) while lacking murine FcRn, with Tg276 expressing hFcRn from a ubiquitous promoter and Tg32 from the human FcRn promoter. Expression studies using a monoclonal antibody directed against human FcRn (ADM31) confirmed the overall ubiquitous expression pattern for Tg276. In the case of Tg32, the expression pattern is similar to that described for human. Overall, only low expression levels were detectable in endothelial cells, which was surprising. Both models have been shown to be useful for pharmacokinetic studies and correlate well with cynomolgus data. In Tg276 models, the half-lives measured were shorter compared with Tg32; however, the Tg276 model has proven valuable when comparing antibodies half-life to each other. Dr. Proetzel discussed an example in which use of standard mice could have potentially led to dismissal of antibody candidates that looked inferior in the C57BL/6J wild-type mouse model. In contrast, the humanized FcRn mouse model showed the improvement in PK of the antibody candidate. Both humanized models have been backcrossed to immunodeficient backgrounds (Rag1-null or scid), which allows xenograft studies in the correct hFcRn context.

Patrik Forrer (Molecular Partners) gave an update on DARPin drugs. He discussed the best-in-class VEGF antagonist MP0112, which is licensed to Allergan. MP0112 (AGN150998) is in a Phase 2 study (NCT01397409) of patients with wet age-related macular degeneration (AMD); the active comparator is ranibizumab (Lucentis®; Genentech). The estimated study completion date is September 2013. MP0112 is stable at room temperature for 12 mo, the IC_{50} is 6 pM (ranibizumab IC_{50} = 300 pM), and the half-life is 6.2 d in rabbits. Dosing frequency for MP0112 is 120 d vs. 40 d for ranibizumab. Molecular Partners is developing therapeutic multispecific DARPins inhibiting VEGF and PDGF (MP060). By blocking PDGF in addition to VEGF, the recruitment of pericytes can be stopped and the maturation of neovessels blocked. Proof-of-concept in vitro and in vivo has been established using a HUVEC spheroid xenograft model in scid mice. To prolong half-life of DARPins, pegylation and serum albumin binding approaches were compared. A naked DARPin has a half-life of 10 min in cynomolgus monkeys. Using PEG20, this can be prolonged to 12 h, and, with PEG60, to 2 d. In an alternative approach, DARPins binding serum albumin were developed. Dr. Forrer showed data for three different variants binding different epitopes that achieved half-lives of 1 d, 8 d and 16 d in cynomolgus primates. This result is promising and suggests that it might be possible to for DARPins to achieve the natural half-life of IgGs.

December 5, 2012: Antibody Engineering/Therapeutics The Antibody Society Special Session

May Yong, Richard Begent, Janice M. Reichert

Janice Reichert (The Antibody Society, Reichert Biotechnology Consulting LLC) provided a brief update on the activities of The Antibody Society. Substantial contributions to the Antibody Engineering and Antibody Therapeutics meetings are made by Society members, who serve on the Scientific Advisory Boards, act as session chairs, give presentations, and contribute to preparation of the meeting report that is subsequently published in *mAbs*. She discussed the Society's website (www.antibodysociety.org), which is regularly updated with antibody-related news (e.g., new marketing approvals for therapeutic antibodies), as a valuable resource for the antibody community and she invited participants to also receive news by joining the Society's LinkedIn group or Liking *mAbs* on Facebook.

The use of Guidelines on Information About Translational Experiments (GIATE) in the management of translational research data, an initiative promoted by the Society, was discussed by **May Yong** (University College London). Translational

research is a process that involves turning innovative ideas into new health products. She noted that the presenters at the meeting each work on sections of translational research, including engineering new agents, determining the extent to which these agents interact with other molecules in a complex environment, testing of the agents for efficacy and safety issues, and measuring therapeutic effects in human. Large amounts of data with very diverse origins are generated during translational research. The data needs to be organized to enable complex information from every section of translational research to be linked and placed into context, and ultimately allow a coherent picture of an agent to develop. Data management also ensures that knowledge is not lost, resources are not wasted by unnecessary repetitions, and, most importantly, that data was understood and used as intended.[1]

The recording of data according to a community-agreed format (as exemplified by the uptake of MIAME[2] by the genomics researchers) gives scientists the ability to share data; it means that scientists would create data sets with consistent variable types, allowing them an equal platform with which to compare data. It is in this spirit that she, Richard Begent and other members of The Antibody Society published a list of minimum information types necessary to describe experiments to a useful level.[3] They proposed that these information types should be collected from every experiment, whether the experiment was conducted in the cell line or human tissue. They hope to introduce the practice of recording consistent data sets, which can be related to allow tracking of the decision-making rationale during the development process of an agent. Antibody Society members with different expertise, from physicists to molecular biologists and clinicians were consulted because the challenge in creating this list of minimum information lay in the diversity of data types to be recorded.

Dr. Yong explained that their minimum information list is named Guidelines on Information About Translational Experiments (GIATE). According to GIATE, an experiment is described in terms of the molecular target, the therapy agent and the model in which it is performed. Different information types are collected in different models. For example, information about molecular interactions are collected in experiments in the molecular model, but not in the cell line, animal or human models. Pharmacodynamics data are collected in experiments using the animal and human model, but not in experiments conducted using cell lines. In cell model experiments, therapy effect data are recorded.

There are many existing minimum information lists that describe specific areas such as diseases 4 or proteomic experiments 5 to great detail. GIATE is different because it covers many levels of investigations. The strength of this approach is not in the level of detail but in the way it allows investigation across models. In other words, it allows users to track what is known from cancer biology to the diagnosis and treatment. Dr. Yong noted that the aim is not to collect a high level of detail from each experiment. Instead, the goal is to provide researchers with summaries containing the same type of information. Researchers are also provided with the name and contact details of the data holder, whom they can contact for more information.

In addition to creating consistent data sets, GIATE aims to minimize ambiguity about what experimental data has been recorded. Society members are commonly involved in collaborations with researchers from different groups and countries. In addition, as the development process of a new drug can span many years, it is critical that experimental data and conclusions made by scientists who are no longer working on that project are included appropriately.

In concluding, Dr. Yong noted that, because terminologies vary across groups, countries, expertise and timeframes, scientists who collect the data and the researchers who re-examine them must use a shared vocabulary. Therefore, the meaning of every information type requested by GIATE is clarified with semantics provided by well-known lexicons such as the National Cancer Institute Thesaurus. More information about GIATE can be found at the GIATE section of the Society's website (www.antibodysociety.org/data/datastandards.php). An app for the Mac OSX platform can be downloaded from the site and used to assist in recording experimental data to the GIATE standard.

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December 6, 2012: Antibody Engineering/Therapeutics Development Status of Early Stage Therapeutic Antibodies

Kirstin A. Zettlitz

The morning session of the final day of the meeting, chaired by **Rathin Das** (Synergys Biotherapeutics, Inc.), was dedicated to discussion of early stage therapeutic antibodies. **Alexey A. Lugovskoy** (Merrimack Pharmaceuticals) presented the case study of MM-141, a fully human tetravalent antibody for the treatment of advanced solid tumors. MM-141 targets the PI3K/AKT/mTOR signaling pathway that is activated through IGF-1R and ErbB3 (HER3). This pathway is believed to promote tumor cell survival and is often activated in cancers in response to cytotoxic and targeted therapies. It has been proven clinically that antibody blockade of IGF-1R is ineffective, and Merrimacks data suggest that it is because heregulin (HRG) and ErbB3 provide strong compensation for IGF-1R blockade. Therefore only

the dual IGF-1R/ErbB3 antibody co-inhibitor can completely block the IGF-induced activation of PI3K/AKT/mTOR.

Merrimack used yeast antibody module display approach to select MM-141modules with optimal affinities and stabilities from structure-focused scFv libaries. The clinical molecules, MM-141, binds to IGF-1R and ErbB3 with subnanomolar affinities, block IGF- and heregulin-induced signaling, and induces strong receptor downregulation. MM-141 is stable in solution and manufacturable. While MM-141 showed monotherapy activity in xenograft models of pancreatic cancer, prostate cancer, breast cancer and Ewing's sarcoma, it is likely to be most effective in combination with chemotherapeutics and targeted therapeutics. In fact, everolimus, gemcitabine and docetaxel all activated PI3K/AKT/mTOR and MM-141 inhibited this acquired resistance thus potentiating the activities of these drugs in xenograft models. These results suggest that MM-141 has great potential as an effective therapeutic for treatment of advanced solid tumors.

David P. Humphreys (UCB) discussed improved antibody therapies for the treatment of *Clostridium difficile* infection (CDI). He first explained the disease basics of CDI, which is a serious, but common, nosocomial infection that is costly to treat. CDI is difficult to eradicate from hospitals and affects mostly people older than 65. The current antibiotic therapies target the *C. difficile* organism and only indirectly affect the toxin that causes the symptoms, which include diarrhea, fever and gut inflammation. The symptoms of CDI lead to extended hospital stays and transfers to intensive care units, both of which are major healthcare cost drivers. The mAbs developed by UCB are intended to reduce duration and severity of diarrhea, death rate and recurrence of infection.

Dr. Humphreys emphasized the importance of antibodies in CDI because a correlation exists between the serum concentration of mAbs targeting the toxins TcdA and TcdB and lower disease occurrence, reduced recurrence and asymptomatic carriage.[1,2] Dr. Humphreys then referred to anti-toxin mAbs in clinical development. MBL and Medarex generated neutralizing human antibodies (IgG1) against TcdA (CDA1) and TcdB (MDX-1388). CDA1 alone showed a trend toward delay in recurrence in a Phase 2 trial, and together with MDX-1388 reduced recurrence rates but did not affect the duration and severity of diarrhea. Phase 3 trials of two combinations of mAbs (MODIFY I and II; NCT01241552 and NCT01513239) sponsored by Merck are ongoing.

The UCB drug composition is a mixture of three human IgG1, one anti-TcdA mAb and two anti-TcdB mAbs that all target the C-terminal 'cell-binding domains' of the toxins. The total amount and the ratio of the drug mixture were tested in a hamster primary model of infection that matches that of the Medarex / Merck studies. The mAb mixture was dosed daily via intraperitoneal (i.p.) injection from day -3 to infection (day 0) and survival and body weight was observed for 28 d. UCB mAbs showed better protection of hamsters (higher levels, more durable) compared with MDX. They also demonstrated higher affinity in the low picomolar range, higher potency (low ng/ml) and the UCB mAb mixture was more effective at higher (TcdA) concentration. Additionally the trans-epithelial electrical resistance (TEER) assay was used as an in vitro surrogate for diarrhea due to loss of tight junctions. One UCB anti-TcdA mAb was protective in the TEER assay, CDA-1 was not. Furthermore, the UCB mAbs were potently neutralizing of TcdA derived from strains of ribotype 027 and 078, whereas the MBL/MDX mAbs were substantially non-neutralizing.[3] These results suggest the potential for UCB mAbs to improve patient outcomes by affecting duration and severity of diarrhea, death in addition to recurrence.

The use of human anti-CCR4 mAb immunotherapy for the treatment of cutaneous T cell lymphoma was discussed by **Wayne A. Marasco** (Dana Faber Cancer Institute). The chemokine receptor CCR4 is expressed on skin-homing malignant T cells that form cutaneous T cell lymphoma (CTCL) and is associated with their skin-homing capacity. CCR4 is also expressed on certain T cell subsets like Tregs that can infiltrate the tumor and suppress cellular immunity. For these reasons, CCR4 might be a valuable target for antibody-based therapy for CTLC or other solid tumors.

Dr. Marasco presented a murine anti-CCR4 antibody (mAb1567). Epitope mapping using N-terminal tagged CCR4 with extracellular loops swapped to CCR8 showed that binding occurs N-terminal, but the extracellular loops contribute or interact with the binding antibody as well. MAb1567 inhibits chemotaxis of CCR4-positive CTCL cells and showed potent antitumor effects in a mouse CTCL tumor model. The anti-tumor response was found to be complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by neutrophils. In vitro, NK cell mediated ADCC was also observed. Moreover, mAb1567 effectively inhibits chemotaxis of CD4$^{(+)}$CD25(high) Tregs via CCL22 and abrogates Treg suppression activity in vitro.

Humanization of mAb1567 with 22 changes in the heavy chain frameworks and 12 changes in the light chain framework resulted in 50% loss of affinity, but the effector functions of the humanized antibody were improved. The humanized antibody caused significant tumor shrinkage in vivo and was superior compared with the chimeric version. Affinity maturation focused on light chain CDR3 (variant mAb2–3) and further increased the biological activity. MAb2–3 was selected for further preclinical development.

CCR4 is expressed on T cell subsets, and mAb2–3 increases proliferation of those subsets, but not in the Treg subset. Therefore, the antibody might have signaling function, probably because it binds N-terminal where CCR4 is signaling, but, to use it to block CCR4, the signaling properties need to be carefully observed. Taken together, results suggest that this high-affinity humanized mAb2–3 with potent antitumor effect and a broad range of mechanisms of action may provide a novel immunotherapy for CTCL and other solid tumors.[4]

Stephen F. Carroll (Synergys Biotherapeutics) discussed the development of multifunctional anti-angiogenic antibody therapeutics for cancer and other angiogenesis-dependent diseases. Synergys has in-licensed a therapeutic fully human anti-Ang-1/2 IgG1 from NeoPharm, Inc. Currently marketed anti-angiogenic drugs provide only modest survival benefits for cancer patients and have raised some safety concerns. Therefore, Synergys aims

to generate more efficacious and less toxic therapeutic products by targeting more than one angiogenic factor simultaneously. That is achieved by neutralizing both angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) through human antibodies where each arm can bind and neutralize Ang-1 or Ang-2 (dual-targeted, not bispecific). Ang-1 and Ang-2 are autocrine factors secreted by endothelial cells that bind the same receptor (Tie-2). Both angiopoietins are often overexpressed in cancer tissues and both are involved in tumor-induced vascular remodeling. Ang-1 functions as a Tie-2 agonist and promotes interaction of endothelial cells and pericytes and supports vascular maturation. Simultaneous inhibition of Ang-1 and Ang-2 by AMG-386, a Peptibody targeting Ang-1 and Ang-2 from Amgen, results in blocking of tumor xenograft growth and ovarian follicular angiogenesis. AMG-386 is currently undergoing clinical trials and has shown that the Ang-1/Ang-2/Tie-2 pathway is a suitable target for abrogating tumor angiogenesis.

The anti-Ang-1/2 antibodies being developed by Synergys were isolated from a human scFv phage display library, affinity matured and converted to human IgG. They show significant in vivo activity in colon and pancreatic cancer models and, in combination with gemcitabine, reduced tumor size and blood vessel formation. Dr. Carroll concluded that human anti-Ang-1/2 antibodies would provide similar efficacy and safety profiles as AMG-386 and hold promise as new therapeutics for cancer and other angiogenic diseases.

David Hilbert (Zyngenia) discussed the preclinical development of a TNF/Ang-2 bispecific Zybody for treatment of inflammatory diseases. Zyngenia specializes in the development of modular mAb-based fusion proteins called Zybodies. These consist of a conventional mAb as scaffold and various modular recognition domains (MRDs) fused N- or C-terminal to the heavy or the light chain to generate up to penta-specificity and 2–10 valencies per molecule. Because the mAb scaffold is not changed the molecules retain affinity, binding specificity and valency, as well as FcRn binding and Fc-mediated effector functions. Zybodies have drug-like properties like production and purification yields comparable to mAbs, no post-translational modifications, high stability and low immunogenic potential.

Dr. Hilbert showed simultaneous binding of a Zybody to five independent targets and discussed the diverse therapeutic opportunities that are enabled by multi-specific, multi-valent and simultaneous target binding. The modulation of multiple cellular receptors could enhance cytotoxicity or inhibition of disease-promoting intracellular signaling. Enhanced target cross-linking could lead to enhanced clearance of multiple cytokines, enhanced receptor internalization or agonistic receptor signaling.

In inflammatory bowel disease (IBD; Crohn disease, ulcerative colitis) the antagonism of multiple immune modulators (e.g., TNF, IL-1, IL-6, CD20, CTLA4) often results in increased incidence of opportunistic infections. TNF and Ang-2 are sequential, but functionally distinct inflammatory signals. TNF is a pro-inflammatory cytokine that mediates the release of Ang-2 from vascular endothelial cells. Ang-2 then destabilizes cellular junctions to allow cellular infiltrates and inflammation. The rationale for development of a TNF/Ang-2 dual antagonist is based on the fact that TNF antagonists have been approved for use in both Crohn disease (CD) and ulcerative colitis (UC), and that combination therapies targeting multiple immuno-regulators have resulted in high incidences of infection.

The bispecific Zybody targeting TNF and Ang-2 (HUM-ang2 = Z2.1) consists of adalimumab and the Ang-2 binding MRD fused C-terminal to the heavy chain. In vitro the Zybody inhibits both NFκB and AKT pathways that are activated by TNF and Ang-2, respectively. Z2.1 is able to bind TNF and Ang-2 (and not to Ang-1) simultaneously and independently with low nanomolar affinities. TNF induced cytotoxicity is inhibited with similar IC_{50} as adalimumab and the Fc effector functions of adalimumab are retained. In a hTNFα transgenic mouse model of autoimmune polyarthritis, the lead Hum-ang2 Zybody is superior to adalimumab in both arthritic score and histopathology score.

Dr. Hilbert then presented results from the development of a TNBS-induced model of colitis in hTNF transgenic mice. In this model, the mice were pre-sensitized with TNBS at day -7 and challenged at day 0. The mice were dosed i.p. on days 0, 1, 2 and assessed histopathologically for preservation of the mucosal epithelium. HUM-ang2 Zybody treated mice showed significantly reduced disease severity compared with adalimumab treated mice.

PK/PD studies with HUM-ang2 Zybody were performed in cynomolgus monkeys and PK parameters were measured to understand clearance, exposure and in vivo stability of HUM-ang2, and pharmacokinetic properties for a human mAb were maintained for both specificities. The serum levels of Ang-2 were measured to assess the in vivo biological activity of HUM-ang2 Zybody. In monkeys treated with a single i.v. dose of HUM-ang2, increased serum levels of bound Ang-2 were found, while monkeys treated with multiple doses showed dose dependence and saturation of bound serum Ang-2.

Dr. Hilbert concluded with an overview of the clinical program planned for HUM-Ang2. A Phase 1 study may include evaluation of safety, PK, exploratory biomarkers, as well as response indicators (improved mucosal healing, evidence of biomarker pharmacodynamics changes, improved efficacy compared with placebo and adalimumab).

In the last talk of the session, **Peter Ulrichts** (arGEN-X) presented ARGX-110, an afucosylated, human IgG1 that binds and neutralizes CD70, as an immune checkpoint inhibitor and proliferation blocker. CD70 is the ligand for CD27 and, upon binding, activates the NFκB pathway and results in shedding of sCD27. In healthy individuals, CD70 is transiently expressed on activated B and T cells and mature dendritic cells, but not on resting lymphocytes and non-hematopoietic cells. CD70 is, however, overexpressed on a variety of solid and hematological tumors and is thought to promote proliferation and survival of tumor cells. CD70 also induces Treg activation and concomitant immune escape. Dr. Ulrichts presented a detailed biochemical and functional characterization of ARGX-110 and explained the dual mode of action with very potent blocking of CD27 signaling by high affinity binding of CD70 and the Fc-mediated killing of CD70-expressing cells by CDC/ADCP/ADCC.

The therapeutic potential of ARGX-110 was demonstrated in a Burkitt lymphoma xenograft model where the fucosylated, parental antibody of ARGX-110 significantly increased survival time at low doses. ARGX-110 also showed increased depletion in a whole blood ex vivo depletion assay compared with standard of care mAbs. Pharmacokinetic studies in cynomolgus monkeys resulted in a half-life of 13 d, suggesting a half-life of 23 d in humans according to human PK modeling. Importantly, the antibody showed no sign of toxicity and had no effect on B, T and NK cell numbers. ARGX-110 is scheduled to enter a Phase 1 study in patients with CD70-positive malignancies in January 2013.

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December 6, 2012: Antibody Engineering/Therapeutics Immunomodulatory Antibodies for Cancer Therapy

Sven Berger

Louis M. Weiner (Georgetown University Medical Center) organized and **Kerry Chester** chaired the last session of the conference, which was dedicated to discussion of immunomodulatory antibodies and ADCs as treatments for cancer. **Mark Sliwkowski** (Genentech) reviewed the development of anti-HER2 trastuzumab (Herceptin®), pertuzumab (Perjeta™) and trastuzumab emtansine, which is also known as T-DM1. HER2 is a member of the ErbB family, but, unlike the other members, it is always in an open conformation. Dr. Sliwkowski elucidated that this open conformation permits rapid interaction with other ErbB family members and explains why none of the 11 ErbB ligands binds to HER2. About 18–20% of all breast cancer patients have HER2 gene amplification. Before the approval of trastuzumab in 1998, HER2-positive breast cancer patients had about half the overall survival of HER2-negative patients. Dr. Sliwkowski pointed out that the treatment of early diagnosed breast cancer with trastuzumab increased the four-year disease-free survival (DFS) of patients from 67% to more than 85%. In 2006 the US Food and Drug Administration (FDA) expanded the approved use of trastuzumab to include treatment of early breast cancer.

The antibody works by two fundamentally different mechanisms of action, inhibition of signaling and antibody-dependent cell-mediated cytotoxicity (ADCC). The signaling of HER2 is inhibited by blocking HER2 signal transduction and inhibition of HER2 shedding, which leads to a very active form of HER2. Although trastuzumab increased the survival of HER2-positive breast cancer patients, ~5,000 women die of HER2 positive breast cancer per year. Therefore, Genentech has developed second-generation anti-HER2 drugs. Pertuzumab was created to antagonize the ligand-dependent signaling of HER2 in complex with other ErbB receptors (like HER3) and to inhibit the formation of corresponding dimers. In xenograft models that are resistant to trastuzumab, a combination of pertuzumab and trastuzumab showed strong activity. Consequently, this antibody combination was tested in clinical trials. In the proof of concept trial (NCT01674062; BO17929), the antibody combination was used to treat women that had tumor progression during treatment with trastuzumab plus chemotherapy. In this trial, the antibody combination achieved a 25% objective response rate. Another 25% of treated patients had stable disease after six month. Pertuzumab in combination with trastuzumab plus chemotherapy was then tested as a neoadjuvant treatment in the Phase 2 "NeoSphere" trial. The antibody combination with docetaxel was associated with a significantly higher pathological complete response rate (pCR) than trastuzumab plus docetaxel alone in patients with HER2-positive breast cancer. In this trial, pertuzumab plus docetaxel alone had a pCR of 24% that was statistically not different to the pCR of trastuzumab plus docetaxel (29%). As expected, given the short duration of therapy, the antibody doublet without docetaxel was less effective than the antibody-chemotherapy combinations. Pertuzumab in combination with trastuzumab was approved by the FDA for the treatment of HER2-positive metastatic breast cancer in 2012.

Dr. Sliwkowski then discussed the development of trastuzumab emtansine (T-DM1), which was performed Genentech/Roche in cooperation with ImmunoGen. T-DM1 is an ADC consisting of the cytotoxic agent maytansine attached to trastuzumab. The stability of the linker between the cytotoxic drug and the antibody determines the ADC clearance in animal models. The clearance correlated well with the toxicity of the ADC. While trastuzumab is cytostatic, T-DM1 is cytotoxic and more effective than trastuzumab in xenograft models. In the Phase 2 TDM4450 study (NCT00679341), the objective response rate of T-DM1 was similar to trastuzumab plus docetaxel, but patients treated with T-DM1 experienced much fewer adverse events than those who were treated with trastuzumab plus chemotherapy. Grade 3 or higher adverse events were reduced by almost 50%. In the Phase 3 trial EMILIA (NCT00829166), T-DM1 achieved a significant improvement in progression-free survival (PFS) with an increased median from 6.4 mo in the control group to 9.6 mo in the T-DM1 group. T-DM1 was well tolerated with no unexpected safety signals. A statistically significant overall survival benefit was also observed (30.9 mo for T-DM1 vs. 25.1 mo for lapatinib and capecitabine). Based on this trial, Genentech has submitted a biologics license application for T-DM1 to the FDA; a first decision on the application by FDA is expected in February 2013.

Charles Drake (Johns Hopkins University School of Medicine) detailed recent results of a neutralizing antibody against the receptor programmed death-1 (PD-1). PD-1 is an inhibitory receptor

expressed on T cells, termed an immune 'checkpoint'. Dr. Drake first described the importance of immune checkpoints and summarizing the clinical results of antibodies against CTLA-4, another immune checkpoint. While CTLA-4 knockout mice die at an age of 3–4 weeks due to massive lymphoproliferation, PD-1 knockout mice show no clinical symptoms. The CTLA-4 blocking antibody ipilimumab was approved by the FDA in 2011 for the treatment of patients with metastatic melanoma. Ipilimumab is the first drug that showed a survival benefit in patients with metastatic melanoma, indicating the potential of antibodies neutralizing immune checkpoints.

Interestingly, blocking PD-1 with a monoclonal antibody does not increase the survival of mice in an established melanoma model.[1] The PD-1 blocking antibody MDX-1106, however, produced objective responses in patients with different solid tumors including renal cell carcinoma, melanoma, colorectal cancer and non-small cell lung cancer in Phase 1 studies.[2,3] In its first study, patients received a single intravenous infusion of MDX-1106 and a second and third dose only when a lack of progression was determined.[2] Because the antibody was well tolerated and associated with evidence of antitumor activity, it was next tested in a Phase 1b trial where the mAb was given every 2 weeks in 8 week (4 treatment) cycles. Patients received up to 12 cycles (over approximately 2 y) until disease progression or a complete response occurred. In this study, grade 3 or 4 drug-related adverse events occurred in 14% of patients, with three patients dying of pulmonary toxicity.[3] Objective responses were determined in ~20–25% of treated patients, with many of these responses durable in nature.

In preclinical studies, anti-PD-1 antibodies in combination with radiotherapy increased the long-term survival of mice in a glioblastoma model compared with antibody treatment or radiotherapy alone. The combination of two neutralizing antibodies targeting two different immune checkpoints like PD-1 and LAG-3 (lymphocyte activation gene-3) or PD-1 and CTLA-4 have a synergistic anti-tumor effect in mice. The combination of MDX-1106 and ipilimumab has entered clinical studies for the treatment of unresectable stage III or stage IV metastatic melanoma. Taken together, these data support the notion of using blocking antibodies in combination treatment regimens.

The clinical development of brentuximab vedotin was discussed by **Megan O'Meara** (Seattle Genetics, Inc.). CD30 is a member of the tumor necrosis factor (TNF) receptor family. It was originally discovered on Reed–Sternberg cells in 1982. Dr. O'Meara pointed out that Reed–Sternberg cells are the malignant driver cells for Hodgkin lymphoma (HL). The receptor is also associated with anaplastic large cell lymphoma (ALCL). CD30 has very limited expression in normal tissue, but is expressed on activated lymphocytes. Brentuximab vedotin (ADCETRIS®) is an ADC comprising an anti-CD30 monoclonal antibody attached by a protease-cleavable linker to the microtubule disrupting agent, monomethyl auristatin E (MMAE). The antibody and the cytotoxic agent are separated by an optimized linker that is stable in the serum, but releases MMAE after internalization and degradation of the antibody. MMAE blocks the polymerization of tubulin and inhibits cell division. Dr. O'Meara explained that MMAE is over 200 times more potent than, for example, doxorubicin and therefore cannot be used as a non-targeted chemotherapeutic agent. Brentuximab vedotin was tested in two Phase 1 studies where it induced durable responses with moderate adverse effects. Two Phase 2 pivotal studies were then initiated for relapsed or refractory HL and for relapsed or refractory systemic ALCL. Patients were treated with monotherapy at a concentration of 1.8 mg/kg every three weeks and received up to 16 cycles if they had stable disease or better. In HL a 75% objective response and 34% complete remission rate was achieved. In systemic ALCL, 86% of the patients achieved an objective response, 57% achieved a complete remission and 29% a partial remission. Across both trials, the most common adverse reactions (≥ 20%), regardless of causality, were neutropenia, peripheral sensory neuropathy, fatigue, nausea, anemia, upper respiratory tract infection, diarrhea, pyrexia, rash, thrombocytopenia, cough and vomiting. The product received approval from the FDA for the treatment of relapsed or refractory HL and ALCL in 2011.

Brentuximab vedotin is currently in clinical trials for the treatment of other CD30-positive tumors, including solid tumors, and other non-Hodgkin lymphomas, including diffuse large B-cell lymphoma (DLBCL). Brentuximab vedotin was recently tested in a Phase 1 dose-escalation study in newly-diagnosed advanced stage HL patients as a frontline therapy in combination with adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) or AVD, which omits bleomycin. The maximum tolerated dose was not reached; however, 11/25 patients in the ABVD cohorts had adverse events of pulmonary toxicity that led to discontinuation of bleomycin. No pulmonary toxicity was observed in the AVD cohorts. A 96% complete remission rate was observed at the end of frontline therapy with brentuximab vedotin plus AVD. A Phase 3 study is ongoing to assess treatment with brentuximab vedotin plus AVD vs. ABVD alone in treatment-naïve HL patients. In a recently conducted Phase 1 study for the frontline treatment of patients with mature T cell and NK cell neoplasms, brentuximab vedotin plus CHP showed manageable AE. This combination therapy has reached an objective response rate of 100% and a complete remission rate of 88%. A Phase 3 study comparing CHOP alone to brentuximab vedotin with CHP in the frontline treatment of CD30-positive mature T cell lymphomas will commence soon. In conclusion, brentuximab vedotin showed promising activity in clinical trials as a frontline treatment of different CD30 positive tumors with a manageable safety profile.

Tibor Keler (Celldex Therapeutics, Inc.) discussed an agonist anti-CD27 monoclonal antibody. CD27 is member of the TNF receptor superfamily. It is constitutively expressed on most T cells and a subset of B and NK cells. The only known ligand is CD70. The engagement of CD27 by CD70 promotes a positive costimulatory signaling, resulting in T cell proliferation and survival. Soluble CD70 promotes strong $CD8^+$ cytotoxic T cell responses. Blocking CD27 dependent costimulation of $CD8^+$ T-cell with a neutralizing anti-CD70 antibody completely abrogates antitumor activity of an agonistic anti-CD40 antibody in xenograft models;[4] however, an agonistic antibody against mouse CD27 has strong anti-tumor activity in xenograft models.[4,5] Fully human antibodies against human CD27 have been

generated by immunizing transgenic mice (HuMab-Mouse™) that express human immunoglobulins.[6] The lead candidate 1F5 (CDX-1127) binds to CD27-expressing lymphoma cell lines and blocks CD70 binding. It has a high affinity to the human antigen ($K_D = 1.9 \times 10^{-10}$ M) and a similar binding characteristic to non-human primate CD27. The antibody has been evaluated with isolated human T cells and in transgenic mice expressing human CD27. Immobilized 1F5 has costimulatory activity on TCR stimulated human T cells and T cells from hCD27 transgenic mice. In these mice, 1F5 increases the number of antigen specific INFγ producing T cells after immunization with a model antigen. In hCD27 transgenic mice, 1F5 has anti-tumor activity in disseminated lymphoma and colon carcinoma syngeneic models. The efficacy of 1F5 depends on the tumor burden whereby treatment of established tumors was less effective. The in vivo anti-tumor activity requires the engagement of mouse Fc-receptors presumably to induce cross-linking of CD27 on T cells. This has been demonstrated with an aglycosylated variant (N297S) that showed a complete loss of activity, but no loss in binding to CD27. In addition to T cell activation, 1F5 was shown to mediate ADCC activity against $CD27^+$ human lymphoma cell lines and significant efficacy was observed in xenograft tumor models of human lymphoma cells in SCID mice. In non-human primates, however, no significant depletion of circulating $CD27^+$ lymphocytes was observed.

CDX-1127 has entered a 2-arm Phase 1 study; Arm 1 for the treatment of B cell hematologic malignancies known to express CD27 in which both T cell activation and direct effector function may contribute to the mechanism of action, and arm 2 for solid tumors (none of which express CD27) that may be more likely to be sensitive to immune therapy, including metastatic melanoma, renal cell carcinoma, hormone-refractory prostate adenocarcinoma, ovarian cancer, colorectal adenocarcinoma or non-small cell lung cancer.

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