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IBC's 22nd Annual Antibody Engineering and 9th Annual Antibody Therapeutics International Conferences and the 2011 Annual Meeting of The Antibody Society, December 5–8, 2011, San Diego, CA

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The 22nd Annual Antibody Engineering and 9th Annual Antibody Therapeutics international conferences, and the 2011 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 5–8, 2011 in San Diego, CA. The meeting drew ~800 participants who attended sessions on a wide variety of topics relevant to antibody research and development. As a preview to the main events, a pre-conference workshop held on December 4, 2011 focused on antibodies as probes of structure. The Antibody Engineering Conference comprised eight sessions: (1) structure and dynamics of antibodies and their membrane receptor targets; (2) model-guided generation of binding sites; (3) novel selection strategies; (4) antibodies in a complex environment: targeting intracellular and misfolded proteins; (5) rational vaccine design; (6) viral retargeting with engineered binding molecules; (7) the biology behind potential blockbuster antibodies and (8) antibodies as signaling modifiers: where did we go right, and can we learn from success? The Antibody Therapeutics Conference comprised five sessions: (1) Twenty-five years of therapeutic antibodies: lessons learned and future challenges; (2) preclinical and early stage development of antibody therapeutics; (3) next generation anti-angiogenics; (4) updates of clinical stage antibody therapeutics and (5) antibody drug conjugates and bispecific antibodies.

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Note

Summaries were prepared from PDFs of the presentations provided by speakers after the meeting. When speakers were not able to share their presentations, detailed summaries were not included, although the names, affiliations and presentation titles of all speakers appear in the report.

December 4, 2011: Pre-Conference Workshop
Johan Nilvebrant

Antibodies as Probes of Structure

Sachdev Sidhu (University of Toronto), who co-chaired the pre-conference workshop with **Jamie Scott** (Simon Fraser University), gave a talk on synthetic antibodies. He discussed whether we now have sufficient knowledge about structure and function to take the recombinant technologies one step further and utilize fully synthetic antibody libraries. These libraries would have several advantages over natural repertoires; the sequences and corresponding antibody molecules can be precisely controlled, the antibodies are more efficiently produced and have more predictable behavior. In an example from his time at Genentech, a single framework was used to construct a highly diverse synthetic library. Most of the diversity of this library was efficiently displayed on phage in a functional manner. Currently, the size of synthetic libraries can challenge the magnitude of the natural immune repertoire. Libraries built on a single framework have simplified the development of highly stable antibodies for use in structural biology. The utility of the synthetic approach has been demonstrated by selection of antibodies for numerous targets that represent many diverse classes of proteins.[1]

Dr. Sidhu noted that targeting of highly homologous components of cell signaling pathways is a more challenging task. Ubiquitination is one of the major post-translational modifications, and good inhibitors of this protein-recycling pathway are not yet available. This system consists of so called “writers”, i.e., ubiquitin-conjugating enzymes and ubiquitin protein ligases, which tag proteins with ubiquitin. Deubiquitinating enzymes or “erasers”, catalyze the reverse reaction. Previous studies have shown that different ubiquitin interacting proteins recognize ubiquitin by a large surface area, which is characterized by high specificity but low affinity. To study this complicated network of interactions, a combinatorial library of ubiquitin was designed and expressed on phage. The library was screened for ubiquitin variants that are specifically recognized by certain ubiquitin specific proteases (USPs). Selected variants provided useful tools in structural studies of USPs because they not only mimicked ubiquitin, but also had a higher affinity. Furthermore, the selected ubiquitin variants were shown to be potent and selective inhibitors of specific USPs in various in vitro models, including live cells. The ubiquitin library was also screened for binders to other ubiquitin binding proteins, including the ubiquitin ligases NEDD4 and ITCH, and an ubiquitin interacting motif (UIM) from yeast, VPS27. Highly specific variants without detectable cross-reactivity against a panel of related ubiquitin interacting proteins were identified. In summary, the ubiquitin library has provided useful tools for further studies of the ubiquitin pathway.

Dr. Sidhu also discussed a mind-tickling construction of mirror image binding proteins composed of unnatural D-amino acids. The process starts from a natural target protein made of L-amino acids, e.g., cancer-associated vascular endothelial growth factor (VEGF). First, a mirror image VEGF is synthesized from D-amino acids. A library based on a synthesizable scaffold protein (here Gb1) is next screened for binders to the unnatural mirror image target protein. An identified binder from this selection may subsequently be synthesized from D-amino acids. In the example, the resulting D-binder did in fact recognize the natural form of VEGF. The final aim of this fascinating strategy is to provide more effective protein therapeutics that may be manufactured from metabolically inert and non-immunogenic D-amino acids by chemical synthesis.

In the second talk of the workshop, **Anthony Kossiakoff** (University of Chicago) discussed the technology platform referred to as chaperone-enabled biology-structure (CEBS), where synthetic antibodies are used as chaperones for structural and biological applications. Numerous proteins are difficult to structurally study, especially membrane proteins, multi-domain proteins and protein complexes. Antibody fragments may be useful tools to produce stable crystal packing surfaces to aid structural analysis of proteins and complexes that are recalcitrant to crystallization. Different synthetic binding proteins may be used as crystallization chaperones, e.g., Fab-fragments or monobodies based on a human fibronectin domain. Phage display selection strategies provide means to target synthetic binding proteins to bind at predetermined regions of a protein surface, to trap a desired conformational state and to capture or stabilize a transient protein-protein complex. The same binding protein may also be used to study the functional importance of the targeted region/conformation/complex and thereby provide a direct link between structure and function.

Crystallization chaperones were, for example, useful in the study of the full-length KcsA potassium channel.[2] Structures of a truncated form of this large membrane protein are available, although the C-terminal part had to be cut off to yield crystals. Removal of this cytoplasmic domain affects both the stability and activity of KscA. Therefore, synthetic Fab-fragments against KscA were isolated by phage display using a selection strategy that specifically targeted the C-terminal domain. The isolated antibody fragments were used to generate crystal structures of both the full-length KcsA and the C-terminal domain alone, in complex with two different Fab-fragments. The two structures of the C-terminal domain can be superimposed, which provides evidence that the Fabs do not induce structural changes. Superimposition of the full-length and the truncated KcsA structures also demonstrated that, while the overall structure was not affected by the bound Fab-fragments, the position of the “ion gate” could be assigned more accurately. Furthermore, electrophysiological measurements in the presence of the Fab chaperones did not affect channel function. Hence, the antibody chaperone-assisted strategy yielded new structural insights on the function of this extensively studied and complex ion channel.

Another important challenge is to develop synthetic binding molecules for cell biology research. One aim is to deliver proteins to the cytoplasm of mammalian cells without compromising the integrity of the cell membrane. This may be achieved through the attachment of a cargo to a relevant cell receptor ligand, which is internalized during receptor endocytosis. The cargo may constitute a synthetic binding molecule targeted to an intracellular protein. Upon receptor-mediated internalization of the conjugate, the synthetic binding molecule may exert a specific function in the cell. Such receptor-mediated delivery has been demonstrated for an antibody fragment conjugated to a modified neuropeptide.[3] The cargo-peptide complex was rapidly internalized in live cells upon interaction with the neurokinin-1 receptor. The labeled cargo, antibody fragments that specifically recognized a conformational form of actin, escaped the endosome and was successfully used to image the cytoskeleton. Dr. Kossiakoff also noted that Fab-fragments that cause actin depolymerization or bundling have been developed and may be used to promote cell death in cancer therapy.

The application of antibodies in structural genomics was discussed by **Cheryl Arrowsmith** (SGC-Toronto; Ontario Cancer Institute; University of Toronto). Following the networking break, **James Koerber** (University of California, San Francisco) presented his work on the structure-based engineering of anti-peptide antibodies.

Germaine Fuh (Genentech) presented two examples of bispecific antibodies. In both cases, a monospecific antibody was used as the starting point to engineer a second binding site into the same antigen-binding fragment. In the first example, the well-known human epidermal growth factor receptor 2 (HER2)-specific antibody trastuzumab (Herceptin®) was utilized as a scaffold

to incorporate specificity to VEGF.[4] In trastuzumab, and many other antibodies, most of the key residues for target interaction reside in the heavy chain. Therefore, a complementarity-determining region (CDR)-library of the light chain was constructed and selected for binders to the second target. Examination of the paratopes in the resulting bispecific antibody fragment, bH1, confirmed that both antigens share the same binding surface. Structural and thermodynamic data on the bispecific Fab of bH1 demonstrated that this dual binding is facilitated by an increased structural adaptability.[5] VEGF primarily interacts with the light chain, whereas the heavy chain mediates the main contacts with HER2. The two overlapping binding sites were then subjected to affinity maturation by CDR mutation. This resulted in variant bH1–44, which had about 100 times higher affinity for both HER2 and VEGF. The matured two-in-one antibody can bind HER2 or VEGF in either site of bH1–44. It has also been shown to inhibit tumor progression in vivo in a mouse model.

The concept of a two-in-one antibody has been further developed to target the epidermal growth factor receptor (EGFR) family. A bispecific antibody for EGFR (HER1) and HER3 was developed to achieve a broad inhibitory activity of all important ligand-activated homo- and heterodimers among these receptors.[6] Targeting more than one signaling complex may be more effective and simultaneously decrease the risk of resistance. A similar two-step library approach was used to generate a dual binding molecule from a monospecific template. Binders to EGFR were selected from a Fab-library where the heavy chain CDRs had been randomized. An initial EGFR-binder (D1.5) was further engineered into the HER3-binding D1.5–100 molecule through light chain CDR randomization and selection. Affinity maturation resulted in a molecule with high affinity for both EGFR and HER3 (DL11f or MEHD7945A). Structural data demonstrated that the HER3-interaction involves more light chain interactions and is achieved by an enhanced charge interaction. This binding at the same time retools the heavy chain for a different interaction with HER3. In the structures of the complexes of the bispecific Fab and the different targets, a 13 Å shift between the corresponding epitopes on EGFR and HER3 was observed. Moreover, the two-in-one antibody was more active than each mono-targeting antibody or antibody combination in inhibiting the growth of cancer cells in vitro and more broadly active than each mono-targeting antibody in vivo. Since the cell surface density of both receptors is higher than the density of each receptor alone, the increased activity may be due to increased avidity effects of the two-in-one antibody. These promising findings have motivated ongoing studies of this antibody in the clinic.

In the final presentation of the workshop, **Erica Ollmann Saphire** (The Scripps Research Institute) discussed antibodies against Ebola virus. Dr. Saphire and her colleagues have published the structure of antibody 14G7, which protects against lethal Ebola virus challenge. The antibody recognizes a distinct linear epitope in the prominent mucin-like domain of glycoprotein GP.[7]

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December 5, 2011: Antibody Engineering
D. Cameron Dunlop

Session I: Structure and Dynamics of Antibodies and Their Membrane Receptor Targets

The keynote introductions were given by session moderator **Andreas Plückthun** (University of Zürich). **Ian Wilson** (Scripps Research Institute) began the conference by describing the problem of eliciting a neutralizing anti-influenza antibody response that is effective over multiple years. The recent characterization of antibodies with this capability that are specific for conserved regions of the HA protein provide a useful tool in this regard. Similarly, several new broadly neutralizing antibodies that recognize part of HIV's glycan shield can be added to the burgeoning list of reagents available to researchers should reverse vaccinology for HIV prove a viable strategy.

The multi-pass transmembrane nature of G protein-coupled receptors (GPCRs) has hampered the development of structural biology-based screening methods to aid the search for small molecule and antibody-based therapeutics that target these frequently critical components of cell signaling. **Stanley J. Opella** (University of California, San Diego) outlined developments in NMR methods to determine membrane proteins in a bilayer environment. Conformational changes wrought by the binding of small molecules and antibody fragments could be assessed at atomic resolution in this way, thereby directing and streamlining the development of therapeutics.

MET is a receptor tyrosine kinase that plays key roles in development, tissue regeneration and many cancers. **Ermanno Gherardi** (Medical Research Council Laboratory of Molecular Biology) discussed the use of crystal structures, cryo-electron microscopy and small angle X-ray scattering, with constructs derived from the MET ligand, hepatocyte growth factor/scatter

factor, as well as a bacterial protein, lnl B, with MET, to propose a model in which the ligands are located in the center of the signaling complex and the receptors are found at the periphery. Taken together, this structural information has provided a basis for the creation of MET agonists and antagonists for cancer therapy, and potentially, organ regeneration.

In the final keynote presentation, **Mark Lemmon** (University of Pennsylvania) expanded upon earlier work involving the mechanism whereby the epidermal growth factor receptor (EGFR) is regulated. Studies using other members of the human EGF family, as well as the Drosophila homolog of EGFR, suggest a second potential site for binding and regulation of EGFR. This provides not only a deeper understanding of EGFR regulation, but also new targets for therapeutic intervention.

December 5, 2011: Antibody Engineering
Aroop Sircar

Session II: Model-guided Generation of Binding Sites

James S. Huston (The Antibody Society; Boston Biomedical Research Institute; Huston BioConsulting, LLC) chaired the afternoon session, which was organized by Dr. Huston and **Anthony R. Rees** (MIP Technologies). Dr. Huston explained that the field of structural antibody studies were opened with the availability of fragmentation of IgG into its antigen binding (Fab) and complement binding (Fc) regions, and isolation of the heavy and light chains, which subsequently allowed elucidation of numerous primary sequences of Fab (L and Fd chains) and Fc regions, and the first crystal structures of a homogeneous Fab and a Bence-Jones light chain dimer. He added that initial attempts using the modular force-field program CHARMM (Chemistry at HARvard Macromolecular Mechanics[1]) were an important step toward modeling antibody Fab structures, but binding site prediction remained difficult at best and refractory at worst. This improved significantly in the 1990s, but often the predicted binding site differed from the actual structure. However, in the past decade there has been another quantum leap in computational strategies with the development of the Rosetta Design suite of programs and related algorithms. His perspective is that the future is bright and computation-based structural modeling of antibody binding sites and antigen-antibody complexes is becoming increasingly accessible, as evidenced by the striking presentations in this session.

Andreas Plückthun (University of Zürich) discussed progress toward a modular protein-sequence specific binding code. He emphasized that the key word was "toward" because he felt that while his research has made significant progress toward generating a platform for modular protein sequence-specific binding strategies, there is still a long way to go. He summarized that there is an enormous gap between implicating a target in a disease and truly validating it, and there are no generic tools that can be used to probe each target. While probing DNA is fairly easy due to base complementarity, no techniques are available to probe peptides. Thus, for peptide or protein targets, each project is unique and has to be painstakingly started from scratch.

Professor Plückthun proposed an elegant solution of designing modular armadillo repeats to bind to specific peptides.[2] It was shown that each unit in an armadillo repeat protein binds to a dipeptide. Thus a library of armadillo repeat proteins can be created to screen for binders effectively isolating a specific armadillo repeat protein for binding to a specific dipeptide. For identification of a larger peptide, unique armadillo repeat proteins specific for binding to tandem dipeptides that make up the larger peptide can be assembled in a modular fashion. Such modular armadillo repeats that are specific for a certain peptide can have broad application because they can specifically bind to regions such as unstructured catalytic loops, termini of proteins or modified histone tails. The talk described the first step of generating armadillo repeat units by consensus sequence design followed by force-field based minimization. These designed repeats were highly expressed, stable, soluble and mono-dispersed providing a starting point for armadillo repeat based library design.

Next, Professor Plückthun described the use of designed ankyrin repeat proteins (DARPins) as sensors for folded proteins. Thus, binding of DARPins to folded proteins contrasts the binding of armadillo repeats to extended peptides. DARPins can be specifically designed to identify Extracellular signal-Regulated Kinases (ERK) and phosphorylated ERK (p-ERK) because the binding is specific to the different conformations of the activation loop with and without phosphorylation. Consequently DARPins can serve as reagent-less sensors for the detection of ERK and p-ERK. A brief detail of miniaturization of such DARPin based assays was also discussed.

Peter Hudson (Victorian Cancer Biologics; Avipep Pty Ltd.,) described the first therapeutic use of the diabody[3] antibody format, which are two single chain variable fragments (scFvs) coupled together such that the C-terminal end of the light chain of one Fv is linked to the N-terminal end of the heavy chain of the other Fv. The linker size is 12 residues because shorter linkers impose steric hindrance that prevents proper light chain pairing simultaneously in both scFvs. Experiments demonstrate that the tumor-to-blood ratio of diabodies after injection is the best-in-class, and is considerably higher than classical antibodies and scFvs. The scFvs are small compared with diabodies and are cleared rapidly. Polyethylene glycosylation (PEGylation) of the diabodies prevented uptake by kidneys and effectively increased their serum half-life. While initial forms of diabodies were PEGylated by non-specific lysine conjugation chemistry, the diabodies were computationally engineered to incorporate solvent-accessible cysteine residues that can be subsequently reduced for PEG attachment. Detailed knowledge of the diabody structure has enabled Avipep to engineer two thiols per scFv, i.e., four per diabody. Thiol engineering provided for site-specific PEG conjugation and produced monodisperse proteins that demonstrated exceptional tumor uptake (above 70% ID/g at 24 hrs). The drug payload was then loaded onto the conjugated PEG diabodies and the administration of this antibody-drug conjugate in tumor xenograft studies confirmed no kidney or liver abnormalities.

Diabodies (including Avipep's lead candidate AVP04) can be produced cost-effectively in bacteria using a good manufacturing practice (GMP) production process that yields up to 1 g/L after refolding from bacterial inclusion bodies. AVP04 will enter Phase 1 biodistribution (124-I PET imaging) studies in Q1 2012 in prostate and ovarian cancer patients, with the intention to enter ADC (drug-loaded) Phase 1 therapy trials in 2013. The lead candidate AVP04 targets the cell surface (sialyl) glycoprotein, TAG72, which is upregulated in gastric, ovarian and prostate cancer. The AVP04 diabody staining of prostate cancer cells demonstrates that they are more specific than the parental CC49 antibody, which stained more benign elements. In addition to drug payloads, diabodies can also be armed with imaging payloads for clinical imaging.

Jeffrey J. Gray (Johns Hopkins University) outlined a computational strategy for the prediction of antigen-antibody interaction complexes starting with the sequence of the antibody and the antigen crystal structure. He stated that computational structure prediction provides a fast and inexpensive alternative to experimental structure determination, but cautioned that the structural accuracy is not as good as experimentally obtained structures. Briefly, the antibody is modeled using RosettaAntibody[4] and docked to the antigen using a combination of EnsembleDock[5] and SnugDock.[6] RosettaAntibody selects the light and heavy chain V domain frameworks from its internal database of high-resolution antibody structures curated from the Protein Data Bank (PDB).[7] Subsequently, complementarity determining region (CDR) loop templates are identified by highest sequence similarity to respective CDR databases and not by using Chothia's canonical rules. The CDR H3 loop is built using ab initio computational loop building. Finally, the relative orientation of the light and heavy chain V domain is optimized along with simultaneous minimization of the non-H3 CDR loop backbone structures. Two thousand models are built in response to each query sequence and the ten lowest energy models are output. The RosettaAntibody server allows users to model antibody Fv structures via the website http://antibody.graylab.jhu.edu.[8]

Professor Gray emphasized that the true test of the utility of a homology model is its usability in downstream applications like protein-protein docking and design of interacting interfaces. To demonstrate the practical utility of the RosettaAntibody generated homology models he described SnugDock, a docking protocol tailored for antigen-antibody docking that optimizes the paratope structurally. Such optimizations enabled by the perturbation of the relative orientation of the light and heavy chains, along with CDR backbone minimizations during antigen docking compensate for errors inherent in an antibody homology model and enable sampling of antigen orientations closer to the native structure. The final docking protocol incorporated EnsembleDock to select from the top ten RosettaAntibody models (consistent with the theory of pre-existing equilibrium) and subsequent structural refinement of the paratope by SnugDock (consistent with induced fit). Using a combination of SnugDock and EnsembleDock predicted more accurate orientations of antigen-antibody complexes than with standard RosettaDock alone. SnugDock provided the most accurate prediction in a world-wide double-blind Critical Assessment of PRediction of Interactions (CAPRI),[9] challenge, where SnugDock started with the unbound structure and sampled a loop conformation closer to the bound complex, which demonstrates the enhanced predictive power of SnugDock.[10] Professor Gray concluded with a structural analysis of camelid VHH antibodies that identified key residues responsible for enhanced stability and solubility, and subsequent use of some of these new structural features to extend RosettaAntibody for modeling camelid VHH with very long CDR H3 loops.[11]

Roland L. Dunbrack, Jr. (Fox Chase Cancer Center) described a new clustering analysis of antibody CDR loop conformations.[12] With more than 300 high-resolution antibody structures in the PDB compared with only a few when Chothia created CDR canonical structural classification,[13] Professor Dunbrack highlighted the need to enhance the classification scheme. The current database is 6–10 times that of the previous database, and the 2012 database is expected to be 1.5–2 times that of the current database. To create a new classification scheme: (1) all the light and heavy chain variable regions in the PDB were obtained, (2) the limits of the CDR were identified based on structural features, (3) the CDRs were filtered to remove ones with missing backbone atom density and high B-factors and (4) clustered using a distance measured between the differences in Φ and Ψ angles of each residue in a CDR. This distance measure between angles is more informative for comparing loop topologies as opposed to traditionally used root mean squared deviation (rmsd). The new classification generated 28 CDR length combinations with 15 combinations having multiple members compared with only 20 CDR length combinations and 4 with multiple members identified previously by Chothia and coworkers. Furthermore, previous classification of CDR H3 stems, viz. "bulged" and "non-bulged," no longer hold up in the current data set. This new structural classification of CDR loops will enable better modeling and design of antibody variable domains.

Professor Dunbrack discussed a new way of analyzing variation in cluster conformation using "Diffusion Maps" where, (1) distances are based on paths between data points (representing physically realizable conformational changes, i.e., connected by nearest neighbors), (2) distances between points or objects determined by the number of short paths between them (traversing well populated paths) and (3) diffusion map distances can be used in multi-dimensional scaling to obtain small number of coordinates to express variation in conformations. Such "Diffusion Maps" describe hinge vs. twist motions and the structural diversity generated by such maps are similar to that generated by molecular dynamics (MD) simulations. Professor Dunbrack summarized that previous complicated rules with "Structure-Determining Residues" (SDRs) do not differentiate clusters and are often violated by the more recent data. The new classification additionally provides: (1) frequencies of different conformations, (2) variation in rmsd and dihedral angles within clusters, (3) sufficient counts for sequence propensities in large clusters, (4) simple rules for most cluster assignments and (5) automatic determination of CDR loop classification and effectively loop conformation.

Matthew P. Jacobson (University of California, San Francisco) emphasized that while computational methods are

used in early stage discovery of small molecule drugs, such methods have only been recently used for therapeutic antibodies. He describes his strategy for predicting antibody structure from sequence to be a purist approach to the protein-folding problem.[14] First the conserved immunoglobulin folds are built using comparative modeling. Then the five non-H3 CDR loops are built using knowledge-based canonical loop rules. Finally the CDR H3 conformation is predicted using physics-based loop prediction. For CDR H3 loop predictions, an all-atom force field + "implicit" solvent model is used. Since such energy functions frequently have a quantum mechanics foundation, the computational expense is quite high. Thus knowledge-based potentials are combined with physical force fields to reduce computational expense or, in other words, predict more accurate loop conformations with the same computational resources. Shorter loops (8–9 residues) can be predicted to an accuracy of 2 Å rmsd, but longer loops have worse prediction accuracy necessitating the use of combined potentials. The loop prediction algorithm is unique because it samples low-scoring structures with increasing accuracy in stages.

Professor Jacobson pointed out that the antibody sequence-structure relationships are complicated by a few factors: (1) How much is known about the "canonical" rules, (2) the relative orientation between the heavy and light chains and (3) flexibility of antibody loops. Mutations at key positions can affect the relative orientation of the heavy and light domains, which in turn can affect antigen binding.[15] He showed that the rmsd of the relative orientation of the light and heavy chains decrease with increasing sequence identity. His strategy to predict the relative orientation of the light and heavy chains involves threading the query sequence on to various existing antibody structures, scoring each threaded model and finally choosing the lowest energy conformation. Professor Jacobson feels that the CDRs are flexible and that well-defined loop conformation in a crystal structure is an artifact, and consequently there are multiple conformations that are sampled by each loop. Binding is driven by entropy inherent in the aforementioned loop flexibility, which, though difficult to assess directly, can be sampled well computationally. Affinity maturation rigidifies the CDR loops and lowers the entropic cost of binding.[16]

Brian Kuhlman (University of North Carolina) discussed the goal of his research, which is to use molecular simulations to create novel protein-protein interactions with predefined binding orientation and tight affinity. Potential advantages of computational approach are: (1) specification of exact binding site and orientation, (2) identification of new binders very quickly and (3) use of scaffolds or targets that are not amenable to high throughput screening (HTS). Unfortunately, it is still a very hard problem, and one frequently ends up with weak binding (μM range) or incorrect binding orientation. One can improve the chances of success by (1) making sure of structural motifs predisposed to interact through β-strands, metals or already characterized hot spots and (2) combine computational design with HTS or selection (phage display).

Professor Kuhlman provided an example of each category of design. Beta-strand mediated interactions involve formation of a cooperative hydrogen bond network, identification of exposed β-strand, duplication and rotation to make anti-parallel β-strands, symmetric rigid body docking and finally symmetric interface design/minimization. Designing an example homodimer interface with two separate designs using hydrophobic and polar residues showed that for this example the polar design did not succeed. Crystal structure confirmed that the designed model was very close to the experimentally determined structure, with good agreement in side-chain conformations. This example demonstrated that interacting strands could be used to create interfaces with very high accuracy and designing with hydrophobic side chains was more successful.

The next example involved the use of metals. In this case zinc was engineered to bind at the interface of a human growth factor binding to its receptor because metal coordinate bonds are enthalpically strong and geometrically constrained. Enhancing binding affinity of scaffold proteins involved designing half-sites for zinc divalent cations on each partner, generation of symmetric complex flips of one partner, searching rigid-body alignments consistent with zinc geometry and avoiding backbone clashes and finally symmetrically designing interface side chains and symmetrically minimizing the backbone. The best performing design forms a tight dimer in the presence of zinc as confirmed by gel filtration and multi angle light scattering. The zinc-stabilized complex showed increased stability with the melting temperature increasing from 55 to 95°C and the dissociation constant decreasing from 40 μM to less than 30 nM. Once again the crystal structure was in fairly good agreement with the model. Thus we see that metal binding can promote tight binding and allow specification of binding orientation.

Future studies will explore if metal binding can be used to control pharmacokinetics. A third design involves designing an interface with previously characterized hot spots. In this design, one starts with a crystal structure that already exists, followed by identification of one or two residues that are critical for binding (these have to be linear in sequence). Finally, the linear epitope is built as a loop in the scaffold. Once the loop is grafted, the total interface is formed and subsequently optimized. In this example, the K_d decreased from 1.5 μM to 100 nM.

The final study compared computational design with HTS and combinatorial biology. Some of the advantages of combining computational design with HTS are ability to sample large regions of sequence space and pre-defined binding site and orientation, while the disadvantages are under sampling of conformational space and inaccuracies in the energy function. On the other hand, this approach had the advantages of combinatorial biology or experimental screening in that it works without depending on energy function calculations, but, on the flip side, it can be difficult to determine the binding site and orientation and it is limited by the number of sequences that can be screened. The model system involved redesigning E3 (which usually binds to E2) to bind to Ubc12-NEDD8 E2 (which normally does not bind to E3). The protocol involved: (1) creating one thousand models of the E3 and Ubc12 proteins docked in alternative but very similar conformations (docking

based on known E2-E3 interfaces), (2) for each docked conformation, redesigning residues on E3 that are in contact with Ubc12, (3) picking lowest scoring designs and (4) creating a library based on observed amino acid profiles. This rational library had a size of 8.1 x 10^7 whereas if each residue was completely randomized the library size would be 10^9 times larger. Finally, the directed library is screened using a split DHFR system. Four rounds of selection generated designs that bound with more than 100 nM affinities. Comparing the best binder to the computational results demonstrated good agreement. As a control experiment, a degenerate library was constructed in which the 13 designed residues were randomized. Four rounds of selection with the same amount of DNA as used in the rational library resulted in a best binder with 50 μM binding affinity, i.e., 500 times weaker than that obtained by rational design. The higher affinity binder generated by the rationally designed library clearly shows that the computational design helped.

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December 6, 2011: Antibody Engineering

Johan Nilvebrant

Session III: Novel Selection Strategies

Both antibody engineering and antibody therapeutics sessions were available to participants on the second day of the meeting. The morning's engineering session was moderated by **James D. Marks** (University of California, San Francisco), who was also the first speaker. Professor Marks discussed the selection of internalizing phage antibodies using tumor cells and yeast displayed tumor antigens. **Andrew Bradbury** (Los Alamos National Laboratories) then described his work on combining phage and yeast display.

James Wells (University of California San Francisco) discussed how selection of antibodies for specific conformational states of proteins is achieved by applying powerful protein display and selection technologies in an innovative manner. He also discussed how enzymes with improved activity could be obtained through library selection. Many proteins shift between active and inactive states by allosteric transitions. If a particular state is desired, e.g., to inhibit a particular cellular process, the possibility to trap a state with an antibody could be used to turn proteins on or off at will. The caspases constitute an important class of proteins that transition between active and inactive states. Conformation-specific antibodies to those proteins may provide functional modulators that influence the signaling that is initiated upon activation of those proteases. Caspase-1 can be trapped in an active or inactive form, using either an active-site inhibitor or an allosteric compound. In a previous study, each of these locked conformations were used as targets to select conformation-specific Fab-fragments, using differential phage display selections to specifically enrich binders to either conformational state.[1] The identified Fab-fragments underwent affinity maturation and were shown to be specific for each of the conformational states of caspase-1. The selected antibody fragments could also be used as functional cellular probes to investigate the distribution of caspase-1 conformational states in vitro and also in cells.

In a second example of a novel selection strategy, a selection for catalytic function of subtiligase was developed into a much simpler selection.[2] More recent work aims to improve the catalytic activity of subtiligase by a combination of phage and yeast display. Subtiligase is a double mutant of subtilisin with the ability to catalyze the ligation of two peptides. To screen for improved variants of subtiligase, a combinatorial library of the enzyme was displayed on phage. Initial experiments relied on displayed variants to ligate a labeled peptide onto their own extended N-termini. Only variants that ligated the target peptide to their terminus were captured and amplified in the selection.[2] Improved variants were successfully enriched by this strategy. However, the digital nature of the ligation reaction made the catalytic activity of the identified hits difficult to rank. In other words, the hits could either be the result of an improved enzymatic activity, or of an improved expression. To circumvent this bias, yeast display

is now being employed to provide a more quantitative screen of displayed subtiligase variants.

After the morning break, **Eric Shusta** (University of Wisconsin-Madison) discussed the use of yeast display for identification and engineering of antibodies against membrane protein targets. **Klaus Schwamborn** (Pepscan Therapeutics) then presented an interesting strategy to generate antibodies against G-protein coupled receptors (GPCRs). Constrained peptides are used to functionally mimic a folded protein surface. Peptide-based protein mimicry is a useful method to develop antibodies against problematic proteins or to develop vaccines. Proteins that are composed of conformational or discontinuous sites cannot easily be mimicked by short linear peptides. The CLIPS (**C**hemically **LI**nked **P**eptides onto **S**caffolds) technology can be used to structurally fix linear peptides for experiments in solution as well as on solid phase arrays.[3] Flexible designs to mimic local regions of complex proteins are possible.

GPCRs constitute a major class of potential drug targets, but the number of reported antibodies for therapeutic targeting of them is low. Membrane proteins are generally difficult to isolate as structurally intact proteins and immunization attempts have commonly been unspecific and difficult to reproduce.[4] The aim of Pepscan's strategy is to overcome these problems by designing structural mimics of the protruding extracellular loops of the receptor by peptides fixed on a scaffold. The resulting antigens may be used for antibody generation by hybridoma technology or for screening of phage display libraries. The initial antigen design can be evaluated and refined by screening panels of variants for binding to a labeled natural ligand. To increase the success rate, several immunogens may be selected to represent each complex target protein. In addition, several levels of immunogen design can address increasingly complex aspects of protein mimicry. Therefore, it opens the possibility of tailoring antibodies to certain GPCR domains and to engineer antibodies that fulfill specific biological requirements.

The applicability of the immunogen design for GPCRs has been demonstrated by several examples of intractable targets. As a proof of concept, functional antibodies have been generated toward the chemokine receptors CRCX4, CRCX7 and an undisclosed GPCR target. A further development that is currently being evaluated is an alternating panning concept. Here, the antibody library is challenged with a synthetic mimic of the target receptor or native receptors expressed on cells in alternating rounds of selection. Dr. Schwamborn concluded by noting that Pepscan's peptide based mimicry technology is a promising approach to generate, or map the epitopes of, antibodies toward "difficult-to-target" proteins. Accumulating evidence demonstrate the benefits of this platform.

Franck Perez (Curie Institute) presented methods for antibody selections against native protein conformations. Strategies to select binders to, for instance, target proteins derived from complex subcellular fractions or pre-incubated and modified by cytosolic kinases were described. Those approaches facilitate isolation of antibodies that would be hard or even impossible to obtain by other means. Antibodies identified by these strategies have been used as intracellular probes to track the dynamics of their endogenous targets in real time in live cells.

Recombinant production of target proteins, for example in bacterial hosts, does not yield native post-translational modification patterns. Use of such targets for antibody generation can lead to enrichment of binders against irrelevant forms of the molecule. Hence, there is a demand for new methods for target preparation, which may be better suited to generate versatile antibodies to study protein function in finer detail. A related challenge is to rationalize the labor intense process of antigen generation. For example, a subcellular preparation isolated from rat liver was used as the target in a phage display selection to generate antibodies against components of the Golgi complex.[5] This procedure has the advantage that the target is present in its native form and no identification or purification is required before the selection. The target protein recognized by the antibodies was identified after the selection and the isolated binding molecules were used to track endogenous Golgi protein dynamics in vivo. Another illustrative example was the selection of phospho-specific antibodies for the Golgi phosphoprotein GRASP65.[6] A recombinant form of this target protein was expressed in and purified from bacteria. The protein was then incubated with a mitotic cytosol to allow processing by naturally occurring kinases. This method enabled selection of antibodies specific for the modified form of the protein, without prior knowledge about the localization of the phosphoamino acids.

Rab6 is a small guanosine triphosphatase and a key regulator of intracellular trafficking of proteins to the membrane. Rab6 shifts between an active state with bound GTP and an inactive, GDP-bound, form. A conformation-specific scFv was selected against a non-hydrolyzable GTP-bound form of Rab6.[7] It could later be used as a conformation-specific sensor to follow GFP-labeled Rab6 in live cells. In a similar manner, antibodies for specific forms of tubulin have been developed.[8] They recognized the GTP-cap at the growing end of microtubules and were used to study microtubule dynamics in living cells.

Dr. Perez also briefly mentioned efforts to speed up selection against recombinant targets and to broaden the use of the same scFv by providing it in different formats. In vitro transcription, translation and biotinylation of a recombinant target protein facilitate, for example, direct capture on streptavidin beads that can then be used for subsequent selection of antibodies without any further purification steps. This strategy circumvents several time-consuming steps in the antigen preparation and facilitates selection of antibodies entirely in vitro.[9] To expand the multiplexing possibilities of already identified scFvs in various assays; a series of vectors has also been developed to allow fusion of the binding entity to Fc-fragments from various species.[10] In the final part of his talk, Dr. Perez described the design and construction of a novel synthetic llama VHH-library. The stable nature of these binding molecules makes them particularly suited for intracellular applications.

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December 6, 2011: Antibody Engineering
Thierry Wurch

Session IV: Antibodies in a Complex Environment: Targeting Intracellular and Misfolded Proteins

The afternoon Antibody Engineering session was chaired by **Richard Begent** (University College London) and was dedicated to discussion of the targeting by antibodies of intracellular and misfolded proteins, especially those located in the intracellular compartment. The keynote lecture was given by **Anne Messer** (New York State Department of Health), who reviewed current antibody approaches for the treatment of neurodegenerative diseases, and especially Huntington (HD) and Parkinson (PD) diseases. The major findings of her presentation are reviewed in a recent publication.[1] HD corresponds to an autosomal dominant genetic disorder caused by the expansion of a CAG triplet in the genomic DNA in the gene encoding huntingtin (*htt*), resulting in long stretches of glutamine (Q) residues and leading to misfolded HTT that interacts abnormally. The length of the CAG repeats is linked to the age of onset of the disease.

HTT exon 1 (or HDx-1), containing the extra polyQ stretch, appears as the most interesting domain to target with antibody fragments; thus several anti-HTT scFv were described by Dr. Messer. To select the most efficient scFv fragments, and especially those active in the intracellular compartment ('intrabodies'), a functional screen was set-up to monitor aggregation of a chimeric protein containing the N-terminal end of HTT including the polyQ stretch, fused to a green fluorescent protein (GFP). This construct was expressed after biolistic transfection in coronal mouse brain slices without or with various scFv fragments and aggregate formation was monitored. ScFv fragments directed to the polyQ domain were ineffective and rather stabilized the misfolded reporter GFP construct. A scFv (scFv-C4) targeting the extreme N-terminal end of HTT efficiently inhibited aggregate formation and protected transfected cells against HTT susceptibility to malonate-induced cell-death.[2] A HD pathology model was obtained in Drosophila by expressing in neurons the N-terminal domain of HDx-1 containing a 96 polyQ repeat, causing neurodegeneration and early death in flies. Using adeno-associated virus transfection, the scFv-C4 fragment was expressed in these flies and improved their survival.[2] Another in vivo model—B6.HD6/1—is a C57BL6/J mouse strain, harboring HDx-1 and upstream regulatory DNA and contains about 125 CAG repeats.[3] The mice exhibit nuclear and neuropil aggregates of mHTT within 4 w after birth, and have greatly shortened lifespans.[3] Mice expressing scFv-C4 exhibit a slower progression of aggregate development, but the effect decreases with age and time after treatment.[3] Thus, combination therapies with small molecules or mAbs directed against other HTT epitopes may be needed. Another strategy investigated consists of a fusion of the scFv-C4 sequence to a proteasomal targeting sequence (PEST) to degrade HTT aggregates. First results seem promising.

The second topic developed by Dr. Messer was Parkinson disease, a disorder associated with α -synuclein (α-syn) aggregation following mutations/truncation, overexpression, oxidative stress. A series of scFv intrabodies were isolated from a human scFv library using yeast surface display; they target more precisely the central hydrophobic core of α-syn known as the NAC region (non-β-amyloid component). ST14A neural progenitor cells transfected with mutant A53T α-syn protein inducing aggregates were protected from cell death upon co-transfection with anti-α-syn scFv NAC-32.[4] Increased intracellular solubility of scFv fragments enhanced aggregate clearance.

The presentation by **Amber Southwell** (University of British Columbia) was also focused on the development of intrabodies against HTT. Paul Patterson's group at the California Institute of Technology developed a novel series of anti-HTT exon 1 intrabodies targeting the proline-rich region (Happ1) or the N-terminal end (VL12.3). Both intrabodies reduced toxicity and aggregation of mutant HTT in rat brain slices. Their mode of action was investigated: Happ1 had no effect on HDx-1 localization, but it caused a significant increase in the turnover rate of mutant HDx-1 t, which VL12.3 does not change. In contrast, expression of VL12.3 increases nuclear HDx-1 vs. the cytoplasmic form. The mechanism of mHDx-1 degradation was investigated using various inhibitors of protein degradation pathways. While proteasome and macroautophagy inhibitors reduce turnover of mHDx-1, Happ1 is still able to reduce mHDx-1 under these conditions, indicating Happ1-accelerated mHDx-1 clearance does not rely on these processes.[5] Happ1 also does not increase mHDx-1 ubiquitination. In contrast, a calpain inhibitor and an inhibitor of lysosomal pH block Happ1-mediated acceleration of mHDx-1 clearance, suggesting mHDx-1 is cleaved by calpain, likely followed by lysosomal degradation.[5] Analysis of HTT sequence identified the 15th amino acid as a potential calpain cleavage site, which is part of the VL12.3 epitope.[5] Several HD mouse models were investigated for their sensitivity

to Happ1 and VL12.3 intrabodies, and two models were preferred: YAC128 and BACHD mice. Intrabodies were delivered to mice using intrastriatal adeno-associated viral particles. Classical behavioral assays were run, such as rotarod, beam crossing, climbing, clasping, open-field. Happ1 treatment yielded significant beneficial effects in most of the associated motor and cognitive deficits and strongly improved the histology/neuropathology parameters.[6] In contrast, VL12.3 treatment displayed only marginal or no effect, and even increased the severity of symptoms in an R6/2 mouse model of HD disease.[6] Despite its beneficial activity, Happ1 intrabody is misfolded intracellularly probably due to the presence of unstable disulfide bridges; there is need for protein engineering and removal of Cys bonds.

Rebecca Nisbet (Commonwealth Scientific and Industrial Research Organisation) presented a bio-physical evaluation of the amyloid-β (Aβ) peptide, and especially its crystallization. Structural characterization of Aβ, and notably of oligomers and fibrils, seems difficult and results obtained from different technical approaches, e.g., NMR, atomic force and scanning microscopies, circular dichroism, are controversial. The production of stabilized Aβ peptides may be helpful not only for crystallization, but also for the discovery and development of novel Aβ inhibitors and antibodies. In this study, the amyloïdogenic portion of Aβ (17–42) was crystallized through its fusion to immunity proteins, lm7 and IgNAR. These proteins display, respectively, a loop between two α-helixes and a β-sheet rich structure with a CDR-like β-hairpin;[7] these proteins may therefore be suitable for stabilizing either Ab monomers or oligomers. Various forms of Aβ protein (1–16; 1–42; 17–42) were cloned at the N-terminus of lm7, or in place of the CDR-like structure of V_{NAR} and expressed in *E. coli*. The Ab-lm7 fusions yielded stabilization of the Aβ1–42 and Aβ1–16 monomers and slowed down the aggregation of Aβ17–42-lm7 fusion. The Aβ17–42/V_{NAR} chimera could be successfully crystallized;[8] its structure was solved with a 2 Å resolution. Aβ17–42 forms a 3-strand anti-parallel β-sheet; the oligomeric form was predominant with a tight association of Aβ dimers and paired dimers forming a tetramer, caged by the four V_{NAR} domains in the crystal. The Aβ-lm7 fusions could not be crystallized per se due to high flexibility of the N-terminal end. Crystals could nevertheless be obtained upon stabilization with an anti-Aβ antibody (WO2 Fab). Lastly, the V_{NAR} and lm7 fusion proteins were also applied for the generation of anti-Aβ conformational antibodies by both mouse immunization and phage display technologies. 3C7 Mab binds to an oligomer of Aβ17–42/Im7 of about 150 kDa.

Amyloidosis is a disease caused by amyloid deposits. Its treatment is very challenging since systemic amyloidosis is still usually fatal, and is a major unmet medical need.[9] Use of serum amyloid P component (SAP) as a target to treat amyloidosis was the topic of the presentation by **Julian Gillmore** (University College London Medical School). SAP binds to fibrils in all types of amyloid deposits and contributes to the pathogenesis of amyloidosis. A small molecule [CPHPC] was isolated as a competitive inhibitor of SAP binding to amyloid fibrils.[10] This compound entered a clinical Phase I study; it was well-tolerated and removed >95% of circulating SAP and 90% SAP content from amyloid deposits in target organs. Nevertheless, no regression of amyloid deposits was observed.[11] Various mAbs were generated against human SAP by immunization with isolated pure human SAP.[12] Administration of anti-human SAP antibodies to mice with amyloid deposits containing human SAP, triggered a potent, complement-dependent, macrophage-derived giant cell reaction that swiftly removed massive visceral amyloid deposits without adverse effects.[12] This promising result may translate into an efficacious antibody therapy for amyloidosis. A humanized version of the best anti-SAP mAb exists and preclinical development is finalized. A first-in-man clinical trial is planned in 2012 with GlaxoSmithKline.

Robert Hawkins (University of Manchester) presented his approach to active immune therapy using engineered T cells. Active immune therapy by transfer of T cells from blood or from tumor (tumor-infiltrating lymphocytes) showed promising results under certain circumstances, e.g., in melanoma patients.[13] T cells can be engineered to express chimeric antigen receptors (CARs) such as the archetypal CAR consisting of a scFv fragment specific to a tumor-associated antigen, fused to a component of the T cell receptor complex (typically CD3zeta), which primes the engrafted T cell for anti-tumor activity. Nevertheless, there is no consensus on an optimal CAR structure, and conflicting results are obtained using identical receptors.[14] Human T cells engrafted with a CAR-specific CD19 fused to the CD3zeta receptor (αCD19z) are functional in vitro.[15] T cells expressing αCD19z, and primed with IL-2 combined with cyclophosphamide effectively treated five-day established Raji B-cell lymphoma in an immunocompromised model system with 50% of mice surviving >100 d.[15] This observation strongly supports the combination of antibody-targeted T cells with chemotherapy as a novel approach for the therapy of CD19-positive B-cell malignancies.[15] A similar example was shown based on a chimeric CEA:CD3zeta CAR. Fourteen patients were treated with the CEA-based CAR. Although no toxicity was observed, no sign of clinical efficacy was evident and engineered T cells disappeared seven days after implantation. In the highest dosed cohort, toxicity increased and cytokine release was observed without sign of responses. A lethal adverse effect was described with treatment of autologous T cells expressing an optimized CAR containing CD28, 4–1BB and CD3zeta signaling moieties fused to a HER2 binding moity.[16] The patient quickly experienced respiratory distress and displayed a dramatic pulmonary infiltrate, and died 5 d after treatment. Analysis of serum samples suggested a cytokine storm, probably associated with the large number of administered T cells.[16]

Katherine Vallis (University of Oxford) described the use of radio-immunoconjugates to image DNA damage both in vitro and in vivo. Monitoring of DNA damage responses can be very useful for diagnostics and treatment monitoring. To this end, the protein selected to target was a phosphorylated form of histone H2A variant H2AX (γH2AX), which forms foci at sites of DNA double-strand breaks. Anti-γH2AX antibodies were modified by the addition of either diethylenetriaminepentaacetic acid (DTPA) to allow [^{111}In] labeling or the fluorophore Cy3.[17] A TAT-derived

cell-penetrating peptide was also added to the radio-immunoconjugate to help its nuclear translocation. In irradiated breast cancer cells, confocal microscopy confirmed the expected colocalization of anti-γH2AX-Tat with γH2AX foci and [^{111}In] anti-γH2AX-Tat was retained longer in cells.[17] The same methodology was applied to detect in vivo DNA damage using a mouse xenograft model of human breast cancer MDA-MB-268. After treatment with local X-irradiation or bleomycin to induce DNA breaks, the anti-γH2AX-Tat probes (fluorescent and [^{111}In]) produced specific signals in the tumors that were proportional to the delivered radiation dose and the amount of γH2AX present.[17] The use of radioimmunoconjugates that target γH2AX can be used efficiently as a non-invasive imaging method to monitor DNA damage with many potential applications in preclinical and clinical settings.[17]

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December 6, 2011: Antibody Therapeutics
Emilia Falkowska and Janice M. Reichert

Session I: Twenty-Five Years of Therapeutic Antibodies: Lessons Learned and Future Challenges

The first session devoted to antibody therapeutics was moderated by **Rathin Das** (Synergys Biotherapeutics). The first speaker, **Napoleone Ferrara** (Genentech, Inc.,), described his work on vascular endothelial growth factor (VEGF)-A as a key mediator of tumor angiogenesis and bevacizumab (Avastin®), which targets VEGF-A and is approved as a cancer treatment. While working to find therapies that could increase patient survival beyond what was achieved with bevacizumab, the Bv8 protein was identified. Bv8 was expressed during VEGF-independent tumor growth, and a combination treatment of anti-VEGF and anti-Bv8 significantly inhibited tumor growth in mice compared with each monotherapy. Dr. Ferrara also discussed the role of VEGF in intraocular neovascularization, and the development of ranibizumab (Lucentis®), an anti-VEGF antibody fragment that is approved for use in patients with neovascular age-related macular degeneration (AMD).

In the presentation "From mouse embryos to antibody therapeutics," **Nils Lonberg** (Bristol-Myers Squibb) provided an overview of the technological advances that have solved many of the problems associated with the development of therapeutic antibodies in the 1980s. The problem of immunogenicity was reduced with protein and genetic engineering solutions such as chimeric and CDR-grafted antibodies, and phage display and transgenic mice. Problems associated with specificity and affinity were addressed with improved screening and characterization, transgenic mice and in vitro affinity maturation. Challenges in manufacturing (e.g., homogeneity, formulation, stability) were met through use of CHO cell expression, development of alternate expression platforms (e.g., algae, goats, bacteria, yeast, cell-free systems), and improvement in the understanding of biochemical and biophysical molecular properties. Activities have been improved by glyco- and protein engineering to optimize effector function; use of biological pathway modification approaches; and development of bispecific antibodies, antibody-drug and radio-conjugates, and alternative scaffolds. At least some problems with delivery have been met through improvements in activity and molecule quality that have enabled high concentration formulation and subcutaneous (s.c.) delivery. In concluding, Dr. Lonberg noted that intercellular access, which would expand targets available to mAbs, remains a substantial challenge.

John Lambert (ImmunoGen, Inc.,) spoke about the use of antibodies conjugated to maytansinoids, which are tubulin

polymerization inhibitors, as antibody-drug conjugates (ADCs) that selectively deliver cytotoxic drugs to tumors. Dr. Lambert spoke about considerations that should be taken to make antibody-maytansinoid conjugates (AMCs) more active therapeutics, including basing the selection of the antibody on purpose (not affinity to the target), and basing the selection of the linker on its affect on in vivo activity. Two AMCs in development were described: IMGN529, which is intended for the treatment of non-Hodgkin lymphoma and other B cell malignancies by targeting CD37 and IMGN853, which is intended for the treatment of ovarian cancer and other cancers that overexpress its target, folate receptor 1 (FOLR1). The function of IMGN529, for example, comes from combining the strong pro-apoptotic, CDC and ADCC activity of its anti-CD37 mAb with the potent cytotoxic activity provided by the targeted delivery of the maytansinoid payload.

Paul Parren (Genmab) discussed anti-CD20 antibody therapy, beginning with rituximab (Rituxan®), which interferes with critical B cell function and kills the target cell by engaging effector function. Ofatumumab (Arzerra®), which recognizes a novel membrane-proximal epitope of CD20, has improved properties, e.g., CD20 binding, CDC of cells with low CD20 expression and ADCC, over rituximab. Dr. Parren warned that less is more for optimized dosing as exhaustion of patient cytotoxic effector systems may limit mAb based cancer immunotherapy.

Janice Reichert presented her analyses of trends in the development of therapeutic antibodies by the biopharmaceutical industry. Professor Reichert has tracked the progress and approval of mAb therapeutics, which is now a $50 billion/year industry, for over 10 y.[1] She noted that the commercial clinical pipeline is currently growing at an average rate of ~50–55 new mAbs a year, with anti-cancer molecules making up a total of one-half of all mAb therapeutics. Compared with mAbs for other indications, the anticancer mAbs in the clinic show the greatest molecular diversity. Only ~55% of anticancer mAbs in the clinical pipeline are unmodified IgG; ~16% are antibody-drug conjugates, ~6% are bispecific, ~11% are Fc- or glyco-engineered, and ~10% are antibody fragment or domains. In contrast, ~90% of mAbs undergoing clinical evaluation as treatments for immunological or infectious disease are unmodified IgG molecules. In concluding, she mentioned that success rates for anticancer mAbs are lower than those for mAb therapeutics for immunological disease, and that the reasons for this discrepancy, and for the termination of clinical studies for mAbs, will be explored in the future.

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December 6, 2011: Antibody Therapeutics
Emilia Falkowska

Session II: Preclinical and Early Stage Development of Antibody Therapeutics

The afternoon Antibody Therapeutics session was chaird by Benjamin P. Chen (Burrill and Company). The first speaker was **Jin-San Yoo** (PharmAbcine, Inc.,), who discussed the inhibition of the angiogenic cascade by tanibirumab (TTAC-0001), a human anti-VEGFR2 neutralizing antibody. Tanibirumab may have better efficacy than bevacizumab (Avastin®), the current gold standard angiogenesis inhibitor, because it inhibits binding of VEGF-A, C and D. Dr. Yoo also spoke about dual targeting antibodies that have the tanibirumab backbone, including DIG-KT, which targets VEGFR2 and angiopoietin/Tie-2, and PIG-KM, which targets VEGFR2 and cMET. DIG-KT and PIG-KM showed efficacy in the bevacizumab-resistant U-87 MG (a human glioblastoma-astrocytoma, epithelial-like cell line) GBM model.

Bill Usinger (Trellis Bioscience, Inc.,) described the Trellis discovery platform using Cellspot screening, a multiplexed assay that uses nanoparticle binding of different antigen coated beads conjugated to colored fluorophores to increase the sensitivity, speed and selectivity of human antibodies from PBMCs. This platform allows antibodies to be screened using different parameters at the same time. For instance, isolating neutralizing antibodies that are cross-reactive to hemagglutinin sub-types in influenza infection (found high affinity cross-clade binders that did not give broad neutralization), and affinity and fine epitope specificity in AD-2 peptide binding for cytomegalovirus (CMV) antibody screening.

John Corbin (XOMA LLC) discussed the use of antibodies as allosteric enhancers. Allosteric enhancers increase ligand affinity resulting in greater signal, but do not produce signal in the absence of the ligand. The platform used to isolate such antibodies involves panning a phage display library with the target receptor in the presence of saturating ligand. The antibodies are then screened for preferential binding to the receptor-ligand complex (positive selection), and not binding to free receptor (negative selection). XMetS, a human IgG_2, was isolated in this way. It is an allosteric enhancer of the insulin receptor (INSR) that enhances receptor signaling by increasing insulin binding by 20-fold. In DIO and MLDS/HFD mouse models of diabetes, XMets improved glycemic control, reduced hyperinsulimeia and improved non-HDL cholesterol with no observed hypoglycemia and no disease related weight gain.

Ken Chang (Immunomedics, Inc.,) described a new procedure for forming multivalent or multi-specific proteins, known as the dock-and-lock (DNL) technique, which takes advantage of naturally occurring protein-protein interactions for site-specific conjugation. Dr. Chang discussed the preparation of trispecific antibodies capable of divalent binding to a tumor antigen and monovalent binding to a radiolabeled hapten-peptide for pretargeted imaging and therapy. Dr. Chang also described hexamAbs

(IgG $(Fab)_4$), including an anti-CD20 and an anti-CD22 combination that retained binding specificity, increased avidity and decreased antibody off rates. This hexamAb retained ADCC, but lacked CDC.

Henry Lowman (CytomX Therapeutics, Inc.,) presented the Probody platform. In this platform, the antigen-combining site of an antibody is blocked with a masking peptide, which is removed by endogenous proteases that are preferentially localized or overexpressed in diseased tissue. Disease microenvironments, like those in solid tumors, are rich in protease activity. Probodies or proteolytically-activated antibodies combine the specificity of mAbs with a second level of tissue selectivity, driven by the action of endogenous disease enzymes. The protease-regulation of antigen binding of Probodies offers the potential for reduced toxicities and improved pharmacokinetics.

Josh Xiao (Amgen, Inc.,) discussed the selected lymphocyte antibody method (SLAM) technology and how SLAM "taps into" tumor expressing proteins that have post-translational modifications. Dr. Xiao spoke about the identification of XMAb55, a human neutralizing mAb by SLAM. XMAb55 was isolated from B cells from PBMCs. The B cells were plated into microplates, and selected using FMAT based HTS, screening tumor cells panels vs. normal cell panels. The V genes were then cloned from the isolated B cells of interest.

December 7, 2011: Antibody Engineering
Gustavo Helguera

Session V: Rational Vaccine Design

On the third day of the conferences, both Antibody Engineering and Antibody Therapeutics sessions were available to participants. The morning session of the Antibody Engineering track was chaired by **Dennis R. Burton** (The Scripps Research Institute). In his opening remarks, he explained that previous strategies used in the development of vaccines are not successful against all viruses. Therefore, more knowledge about the interaction between viruses and the immune system and the pathogenesis of the viruses is needed for the rational design of vaccines.

The first lecture was given by **Andrea Carfi** (Novartis Vaccines and Diagnostics), who discussed engineering effective vaccine antigens. Dr. Carfi provided an overview of classical vaccines, including the killed vaccine made from the causative organism processed to an inactive form and the live attenuated vaccines made with strains of the microorganism that are no longer pathogenic and presented more recent types such as recombinant and conjugate vaccines. Dr. Carfi noted that, despite these advances, new vaccines are still needed. For example, there is currently no effective vaccine against respiratory syncytial virus (RSV), the most important respiratory tract pathogen for infants and children. Vaccine candidates were unsuccessful due to vaccine-enhanced disease, poor immunogenicity, problems of stability, tolerability and lack of potency.[1,2] Currently, infants at higher risk can be administered the prophylactic mAb palivizumab (Synagis®). This product and motavizumab, an affinity matured form of palivizumab, target the RSV fusion glycoprotein (F), suggesting that this antigen has a potential as an effective RSV vaccine. The crystal structure of RSV F-derived peptides bound to motavizumab and to the neutralizing mAb 101F provided structural information about the epitopes responsible for the antigenic properties of the F protein.[3,4] The sequence of RSV F shows similarities to the parainfluenza F glycoproteins (Paraflu F). Analysis of the conformational changes occurring from the pre-fusion to the post-fusion states in Paraflu F suggests that it might be more difficult to raise neutralizing antibodies against post-fusion F. Dr. Carfi showed a series of RSV F post-fusion ectodomains constructs, starting with the RSV F wild type, which is regulated in vivo by two furin-cleavage sites that releases a fragment of 27 residues (p27). When the fusion peptide domain and the transmembrane region were deleted, the resulting RSV F protein formed highly stable mono-dispersed trimers that could be easily purified to homogeneity in high yield. Crystallographic studies of the RSV delta FP F protein revealed a structure strikingly similar to post-fusion Paraflu F and that the epitopes for motavizumab and 101F are structurally preserved and exposed. The stable post-fusion RSV F trimers elicited neutralizing response in mice that could protect the animals from RSV infection.[5] Notably, binding studies showed that mice immunization with the post-fusion RSV F elicited antibodies that compete with palivizumab and motavizumab. However, the possibility that additional neutralizing epitopes may be present in the pre-fusion RSV F cannot be discounted. Dr. Carfi concluded that structure-based approaches can guide and improve antigen design, and that understanding the structural basis for immunogenicity and immunodominance will allow us to improve vaccine efficacy and broaden the range of vaccine-preventable diseases.

Bali Pulendran (Emory University) discussed how recent advances in systems biology and in innate immunity are yielding insights into the mechanism of how effective vaccines stimulate a protective immune response. Professor Pulendran began the presentation with an outline of the development of human vaccines and also noted that, despite their great success, the role of innate immunity in the control of acquired immunity in effective vaccines is not fully understood. The highly successful live attenuated yellow fever vaccine YF-17D seems to target innate immune receptors to stimulate a polyvalent immunity. It activates multiple dendritic cell (DC) subsets via Toll like receptors (TLRs) 2, 7, 8 and 9, resulting in a mixed Th1/Th2 cytokine response and in antigen-specific $CD8^+$ T cells. This response highlights the potential of combining different TLR ligands in a vaccine in order to induce polyvalent immune response.[6] Professor Pulendran then discussed a system biology approach to analyze the innate and adaptive immune responses after vaccination with YF-17D and with seasonal influenza. This strategy uses gene expression profiling, multiplex analysis of cytokines and chemokines, and multiparameter flow cytometry, combined with computational modeling, allowing the prediction of the subsequent adaptive immune response to identify innate immune signatures.[7,8]

Professor Pulendran showed how he identified predictive markers of immunogenicity in trivalent inactivated influenza

vaccine (TIV) and live attenuated influenza vaccine (LAIV). In clinical trials, significant variation was observed in the magnitude of human anti-influenza (HAI) titers in response to vaccination, with more than 80% HAI high responses in TIV vaccinees compared with less than 15% of high responses in LAIV vaccinees. This observation was correlated with more than a 10-fold higher presence of antibody secreting cells (ACS) after 7 d of TIV application, compared with LAIV treated subjects. Meta-analysis of microarray data of this set in the TIV differentially expressed genes showed a significant enrichment in genes highly expressed in B cells, particularly in plasma cells, compared with LAIV, which correlates with the magnitude of the HAI titers induced by vaccination with TIV. He also showed studies in mice in which CAMK4 expression on day 3 post-vaccination is negatively correlated with serum antibody response on day 28 post-vaccination, suggesting a role for CAMK4 in the regulation of antibody response to vaccination.

Next, Professor Pulendran discussed a nanoparticle-based synthetic vaccine designed to mimic viral vaccines. The nanoparticles are 0.3–0.4 microns and constituted of PLGA conjugated with the TLR4 ligand MPL, the TLR7 ligand R837, CpG single stranded oligodeoxynucleotide, and the protein antigen HA from H5N1 influenza. In vivo studies in mice with this vaccine showed that the combination of a TLR4-L and a TLR7-L in the same moiety mediate synergistic enhancement of primary and secondary antibody responses. Finally, Professor Pulendran presented data on the combinatorial activation of TLR4 and TLR7/8 that synergistically induces long-lived plasma cells resident in the lymph nodes, which secrete high affinity, neutralizing antibodies. This synergistic effect occurs preferentially on the germinal center pathway of memory B cell development, and not on short lived plasma cells.[9] Professor Pulendran concluded that insights gained from this can guide the rational design of new vaccines against global pandemics and emerging infections, thus allowing the design of synthetic vaccines that mimic the immunogenicity of live viral vaccines.

Alejandro Balazs (California Institute of Technology) presented vectored immunoprophylaxis (VIP) as an alternative to immunization for antibody-based protection against HIV infection. This method consists of the transference of genes in vivo via vectors engineered to secrete broadly neutralizing antibodies into circulation. Dr. Balazs showed data for a model against HIV[10] that took advantage of the identification of numerous antibodies with the capacity to neutralize the majority of the circulating HIV strains. The system was developed in vectors that are non-integrating and non-pathogenic to humans, based on the adeno-associated virus (AAV) serotype 8, with the full-length antibody protein expression driven by a novel, muscle-optimized CASI promoter. After a single intramuscular injection, the optimized AAV vector exhibits long-term luciferase or 4E10 HIV neutralizing antibody expression in immunocompetent mice up to 52 weeks post injection. NSG mice were then challenged with VIP expressing four different HIV neutralizing mAbs (b12, 2G12, 4E10 and 2F5); the animals produced between 20 and 250 μg/ml in serum after 7 weeks. Next, the mice were adoptively populated with human PBMCs, followed by a challenge with the replication competent NL4–3 HIV strain, and sampled every week to quantify the level of CD4 depletion. Among them, the animals transduced with b12 had the most potent protection against HIV challenge and had non-detectable HIV p24 expression in spleens, while those expressing 2G12, 4E10 and 2F5 exhibited partial protection and significant p24-positive staining in spleen cells. Moreover, the AAV encoded vaccine provided robust protection of CD4 cells despite escalating intravenous HIV challenge, with a minimum in vivo protective dose of b12 of 34 μg/ml. In addition, VIP with the VRC01 HIV neutralizing antibody also resulted in dose-dependent protection in vivo. Dr. Balazs concluded by noting that the VIP strategy results in long-lived production of full-length human antibodies in immunocompetent animals and can be applied to therapeutic regimens in which continuous production of mAbs in vivo is required, with potential as an effective prophylactic agent against HIV in humans.

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December 7, 2011: Antibody Engineering
Emily C. Piccione

Session VI: Viral Retargeting with Engineered Binding Molecules

The afternoon session of the Antibody Engineering track, chaired by **Andrew Bradbury** (Los Alamos National Laboratories), was focused on discussion of strategies utilizing engineered binding molecules for viral retargeting. The session opened with **Christian Buchholz**'s (Paul-Ehrlich Institute) presentation on

specific gene delivery to cell types of choice by cell entry targeted lentiviral vectors. He noted that the method is based on single-chain antibodies recognizing cell-surface antigens, which allows gene transfer to be specifically targeted to a variety of cell types.[1]

Robin Parks (Ottawa Hospital Research Institute) discussed the use of genetic fusion of single-chain or single-domain antibodies to capsid protein IX for retargeting of adenovirus vectors. Dr. Parks began by reviewing basic aspects of adenovirus biology including several different strategies currently utilized for retargeting of adenovirus vectors to tumor cells. One strategy for retargeting of adenovirus is to genetically modify the capsid proteins that coat the virus, but this method is prone to lack of specificity.

Dr. Parks described a series of experiments aimed at increasing specificity of adenovirus targeting by using adenovirus capsid protein IX (pIX) as a platform to display polypeptides capable of targeting the virus to a cell surface epitope. Having previously shown that fluorescent virus could be generated by fusing GFP to pIX, Dr. Parks' group sought to add large targeting ligands to pIX.[2] As a test, MR-1, a scFv against tumor-specific EGFRvIII, was fused to pIX for retargeting of adenovirus to EGFRvIII-expressing cells. Despite incorporation of modified pIX into virions, enhanced infection of EGFRvIII-positive cells was not observed. The inability of the pIX-MR1 fusion to bind to EGFRvIII suggested that MR1 was not folded properly when fused to pIX. Since scFv contain disulfide bonds that would not form correctly in the reducing environment of the cytoplasm, they routed pIX-MR1 through the endoplasmic reticulum (ER) through addition of a signal peptide. Routing pIX-MR1 through the ER allowed binding to EGFRvIII, but did not permit its incorporation in the viron because the majority of pIX-MR1 was retained in the ER. By coexpressing native pIX, they were able to enhance incorporation of pIX-MR1 into the adenovirus capsid. However, presentation of MR1 on the adenovirus virion did not enhance infection of EGFRvIII-expressing cells.

As an alternative to scFv, they also considered single-domain antibodies (sdAb) because the monomeric variable domain could be more amenable to addition to the adenovirus capsid. A sdAb against CD66c, an antigen upregulated on many cancer cells, was identified through panning a llama sdAb phage library. This sdAb was fused to pIX and was efficiently incorporated into adenovirus capsids, retained the ability to bind to its ligand, and lead to enhanced infection of target cells. Dr. Parks concluded by stating that these preliminary studies showed that pIX can be used for display of large targeting polypeptides on the surface of the virion and such viruses could be used to direct adenovirus infection to specific cells for treatment of a number of diseases.

Roberto Cattaneo (Mayo Clinic) spoke about his work with measles virus. In the final presentation of the session, **Birgit Dreier** (University of Zurich) discussed the generation of bispecific designed ankyrin repeat proteins (DARPins) adapters for efficient adenoviral gene transfer.

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December 7, 2011: Special Presentation Janice M. Reichert

The Antibody Society

Jamie Scott (Simon Fraser University), President of The Antibody Society (TAbS) in 2011, discussed the recent activities of TAbS, which was formed in 2007 to further the broad interests of antibody engineering and antibody therapeutics. She noted that TAbS organizes the Antibody Engineering and supports the Antibody Therapeutics sessions each year. At this 2011 meeting, TAbS board of directors members Andreas Plückthun, James Huston, James Marks, Richard Begent, Dennis Burton, Andrew Bradbury and Louis Weiner chaired sessions, while Dr. Scott and Sachdev Sidhu co-chaired the pre-conference workshop. Janice Reichert, President-elect for 2012, organizes a report on the Antibody Engineering and Antibody Therapeutics conferences that is published in the March/April issue of *mAbs*,[1] which is the journal of the Society that focuses on topics relevant to antibody research and development. Dr. Scott reminded attendees that TAbS also organizes a special issue in October for *Protein Engineering and Design*, which is the journal of the Society that focuses on topics relating to antibody and protein engineering. Papers for this Antibody Special Issue may be submitted through June 2012 and will be published online as final proofs are completed.

James Huston (The Antibody Society; Boston Biomedical Research Institute; Huston BioConsulting, LLC), Chairman of the Society, discussed the contents of the website, which includes regular updates on news relevant to antibody research and development provided by Janice Reichert and a table of therapeutics antibodies approved in either the United States or Europe that is also updated by Professor Reichert. The website (www.antibodysociety.org/) includes information about the Antibody Engineering/Antibody Therapeutics conferences each year. He noted that benefits of membership in the Society include a 20% discount on the price of meeting registration and free meeting registrations for students selected from those who submit posters. Membership for students is free.

Richard Begent (University College London) discussed Guidelines for Information About Therapy Experiments (GIATE), which is designed to address an unmet need for reporting guidelines for experimental data. He explained that it is a minimum information guideline consisting of a structured list of information elements describing the range of information required, along with a controlled vocabulary to ensure consistency and clarity of meaning. He noted that GIATE provides a consistent information framework that can support data sharing within and between disciplines. It is a guide for the description of experiments that can help to avoid unnecessary duplication, support comparison between experiments, facilitate identification

of incomplete information and identify issues of risk and safety. He encouraged attendees to visit the GIATE sites on the Society webwite, www.antibodysociety.org/data/cde002.php, for more information.

In concluding the presentation, Dr. Scott discussed the importance of participation, which forges bonds between members and facilitates networking, and encouraged attendees to become members and join TAbS committees planned for 2012, including Communications, Sponsorships, Membership and New Initiatives, e.g., workshop on standards for deep sequencing data; an initiative to encourage journals to require deposition of antibody DNA sequence; promotion of adoption of GIATE across the broad spectrum of our members.

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December 7, 2011: Antibody Therapeutics Simon Brack

Session III: Next Generation Anti-angiogenics

The morning session of the Antibody Therapeutics track was opened by chairperson **Philip Thorpe** (University of Texas Southwestern) with a short overview on the prototypic anti-VEGF-A antibody bevacizumab (Avastin®). Even though it is an approved therapy for certain types of colon, lung, kidney and brain cancer (glioblastoma multiforme), its effect on overall survival is marginal at most. Moreover, bevacizumab therapy is associated with substantial toxicity, such as gastrointestinal perforations, bleeding or hypertension. Hence, there is a clear need for better anti-angiogenic agents.

Jennifer Spratlin (Cross Cancer Institute) provided insight into the development of ramucirumab (IMC-1121B), an antagonistic anti-vascular endothelial growth factor receptor 2 (VEGFR-2) antibody. VEGF-A and its receptors, VEGFR-1 and VEGFR-2, are the main players in the regulation of angiogenesis. Since blockade of the VEGF-A/VEGFR-2 interaction inhibits tumor angiogenesis and tumor cell growth in mice, VEGFR-2 remains an attractive target for interfering with VEGF activity. VEGFR-2 is overexpressed in the vasculature of a range of different tumors by endothelial cells that are genetically stable, thus decreasing the potential of adaptive resistance to VEGFR-2 directed therapies.

A proof of concept Phase 1 study with the chimeric anti-VEGFR-2 antibody IMC-1C11 showed that the drug was well-tolerated.[1,2] Three of four colorectal cancer patients receiving the highest dose (4 mg/kg) had stable disease, warranting further development of anti-VEGFR-2 therapies. To reduce the immunogenicity of the therapeutic principle, the human anti-VEGFR-2 IgG1 ramucirumab was generated. A total of 37 patients with solid malignancies were treated with 2 to 16 mg/kg of ramucirumab in a Phase 1 study.[3] After one patient each developed dose limiting hypertension and deep venous thrombosis at the highest dose, the next lower dose (13 mg/kg) was considered the maximal tolerated dose (MTD). Hypertension, often associated with headache, was the most frequent grade 3–5 toxicity, followed by abdominal pain. Patients with partial response or stable disease were found in all dosing cohorts, indicating that the drug was active.

Ramucirumab was evaluated in several Phase 2 studies in hepatocellular carcinoma, metastatic renal cell carcinoma, non-small cell lung cancer, and in metastatic melanoma. In the hepatocellular carcinoma trial, 43 patients received ramucirumab at 8 mg/kg every 2 weeks as first-line monotherapy. Very few drug-related grade 4 and some grade 3 adverse events were observed, of which hypertension was most prominent (12%). Four patients showed a partial response, and 25 patients experienced stable disease, resulting in a moderate disease control rate (RR + SD) of 69%. Preliminary progression-free survival in the patient subset with advanced stage hepatocellular carcinoma (BCLC stage C, Child-Pugh A) was 4.2 mo and overall survival was 17.6 mo.

In the renal cell carcinoma trial, 39 patients with tyrosine kinase inhibitor refractory metastatic renal cell carcinoma were treated with ramucirumab. The results of the trial were similar to the hepatocellular carcinoma study; the drug was well-tolerated but only showed modest activity. Promising activity of ramucirumab was found in stage IIIB/IV non-small cell lung cancer (NSCLC) in combination with paclitaxel and carboplatin. A preliminary efficacy evaluation based on 29 treated patients established one complete response, 14 partial responses and 13 stable diseases. The preliminary median progression-free survival was 5.78 mo.

It is too early to make a comparison of the efficacy of ramucirumab with bevacizumab, but Dr. Spratlin concluded her presentation with a list of several Phase 2 and Phase 3 trials evaluating ramucirumab for metastatic breast, colorectal and gastric cancers that are currently ongoing and may help to find a place for ramucirumab in the anti-angiogenesis therapy landscape.[4]

A different approach to inhibit tumor angiogenesis with anti-Sema4D antibodies was presented by **Maurice Zauderer** (Vaccinex). Sema4D is a member of the semaphorin family of proteins first identified as mediators in axon guidance. Sema4D is involved in the regulation of several different physiological processes, one of which is endothelial and epithelial cell migration. It also enhances CD40-induced immune and inflammatory cell activation, inhibits neurite extension and axon regeneration, and promotes survival and differentiation of oligodendrocyte precursor cells. As a multifunctional target, Sema4D may be of therapeutic relevance in numerous indications, e.g., cancers, rheumatoid arthritis, multiple sclerosis. Results from mouse models suggest that Sema4D antibodies could indeed have wide applicability. For example, a mouse Sema4D-specific antibody, MAb67, was as efficient as the clinically validated anti-TNF therapy etanercept (Enbrel®) in haltling disease progression in an established collagen-induced arthritis model. Besides reducing the inflammatory response, the anti-Sema4D antibody also inhibited bone erosion. This may be explained with recent findings that Sema4D

mediates osteoclast-osteoblast communication. In addition, Sema4D inhibition was also effective in an EAE model, where the clinical score was reduced by 50% by anti-Sema4D antibody treatment initiated during the onset of the disease.

Due to the abundant Sema4D expression on T cells and to a lesser extent on B cells, monocytes and dendritic cells, Vaccinex chose an IgG4 isotype for their humanized anti-Sema4D antibody VX15/2503 to avoid immune cell depletion. VX15/2503 inhibits membrane-bound human Sema4D, as well as a soluble Sema4D that is generated from the membrane-bound form by ectodomain shedding. VS15/2503 was shown to block Sema4D-induced collapse of the actin cytoskeleton and apoptosis in oligodendrocyte precursor cells in vitro. It also neutralized Sema4D mediated inhibition of oligodendrocyte precursor cell maturation into myelin basic protein-producing cells and reverted the inhibition of remyelation following lysophosphatidyl choline induced injury in postnatal brain slice cultures in vitro, thus indicating possible applications in multiple sclerosis patients.

VX15/2503 was able to inhibit primary tumor growth in a number of different tumor xenografts. Interestingly, there was no correlation with the activity of anti-VEGF therapy. Some models were co-dependent on VEGF and Sema4D, whereas others were found to depend solely on Sema4D, but not on VEGF and vice versa. In a head-and-neck xenograft model, HN6, where both Sema4D antibody as well as VEGF antibody slowed tumor progression as monotherapy, the combination of the two agents was additive, suggesting that they act on different pathways. In the orthtopic breast tumor model MAXF-1162, the primary tumor did not respond to VX15/2503, but colonization to distant metastatic sites was reduced impressively. Since small metastases are often not dependent on neoangiogenesis, Dr. Zauderer speculated that VX15/2503 may inhibit intravasation of tumor cells from the primary tumor. VX15/2503 has entered the clinic and is now in Phase 1 studies for advanced solid malignancies. Vaccinex also seeks to establish the antibody for multiple scelrosis, where the project is currently at the preclinical stage.

Dario Neri (ETH Zurich) presented an update on targeting the extracellular matrix of angiogenic vasculature with armed antibodies. Large splice variants of fibronectin, containing the extra-domain A (ED-A) or the extra-domain B (ED-B), and large splice variants of tenascin-C containing domain A1 (TnC-A1) are expressed in the extracellular matrix of tissue undergoing angiogenesis, including a wide variety of different tumors. In contrast to their high abundance in tumors, these proteins are largely absent from normal tissue. Antibody fragments specific to these domains have been shown to accumulate in tumors and in metastatic lesions of cancer patients, which makes them ideal carries for the delivery of toxic payloads.

Over the past 15 y, many different payloads have been fused to anti-ED-A, anti-ED-B or anti-TnC-A1 antibody fragments and tested in mice. Immunocytokine fusions to IL-2, IL-10 and TNF, and radionuclide-conjugated antibodies are most advanced. The choice of the payload depends on the indication, and the format of the antibody fragment may need to be tailored to meet specific requirements. As an example, the 131-iodine labeled anti-ED-B antibody L19 (L19-I131) has been administered to more than 200 patients, but the most promising activity was observed in hematological cancers, mainly in Hodgkin lymphoma. A small immuno-protein format (SIP; scFv-CHε4) was chosen, which showed better tumor:blood ratios than scFv or conventional IgG formats. Dr. Neri presented the impressive case of a young female Hodgkin lymphoma patient who was pretreated with multiple lines of chemotherapy and presented with several metastases before she enrolled in the L19-I131 trial. The patient experienced a complete response, but the tumor grew back eventually. She was treated five times with L19-I131 before she finally succumbed to the disease 2.5 y after study entry.

L19-TNF was investigated as monotherapy or in combination with melphalan in isolated limb perfusion therapy of metastatic melanoma. The disease control in the limbs was found to be durable, indicating that there is value in targeted payload delivery, as Dr. Neri pointed out. Fusion of the scFv antibody fragment L19 to interleukin-2 (L19-IL2) has shown promising anti-tumor activity in a Phase 2a trial conducted in 32 metastatic melanoma patients.[5] The drug is currently in Phase 2b studies that will enroll 90 metastatic melanoma patients in two arms to compare anti-tumor activity of L19-IL2 plus dacarbazine against dacarbazine alone. Furthermore, intralesional injections of L19-IL2 are in development for the treatment of melanoma skin lesions.

The antibody fragments that Dr. Neri discussed may also be useful for the treatment of inflammatory conditions. The anti ED-A antibody F8 accumulates around arthritic blood vessels in mice. An immunocytokine fusion of F8 to interleukin-10 has shown anti-inflammatory activity in a mouse model of arthritis. The drug is currently in Phase 1 studies in rheumatoid arthritis patients. Dr. Neri finished his presentation with imaging data from a proof-of-concept study to establish targeting of artherosclerotic plaques in human patients, offering even more opportunities to these magic bullets.

Philip Thorpe (University of Texas Southwestern) reported on preclinical and clinical studies with the vascular targeting antibody bavituximab and its ability to reactivate tumor immunity. The chimeric IgG1 bavituximab binds to phosphatidylserine (PS), a phospholipid that is confined to the inner leaflet of the plasma membrane in normal cells, but flips to the outer leaflet in tumor blood vessels of various tumor types, thereby becoming accessible to antibodies. Bavituximab has been shown to localize to tumors in vivo and to inhibit tumor growth in several different rodent models. Of particular interest is the observation that bavituximab was synergistic with other treatment regimens, such as chemotherapy, radiation or androgen deprivation, without contributing to toxicity. As an example, the radioresistant NSCLC cell line A541 did not respond to bavituximab monotherapy in vivo, but the antibody sensitized A541 tumors to radiotherapy, leading to complete tumor regression. Possible mechanism of actions include antibody-dependent cellular cytotoxicity (ADCC), which leads to tumor vascular damage, influx of macrophage and shut down of blood flow. However, since PS is also present on tumor cells and tumor cell exosomes, and since PS was found to contribute to immune suppression in the tumor environment, a direct mechanism on immune reactivation

is also likely. Indeed, bavituximab treatment in mice repolarized tumor-associated M2 macrophage into otherwise scarce M1 macrophage. M2 macrophage are pro-angiogenic and immunosuppressive, and express interleukin-10 and TGFβ, while M1 macrophage are pro-inflammatory, express interleukin-1, interleukin-12, TNF, Fc receptors and nitric oxide, and are capable of killing tumor endothelial cells and tumor cells.

In addition, bevatuxuimab induces differentiation of myeloid-derived suppressor cells (MSDC) into macrophages and dendritic cells. In bavituximab treated tumors, dendritic cells mature into antigen-presenting cells with enhanced competence to start a tumor-specific T cell response. The molecular mechanism of how bavituximab affects macrophage and immune cell biology remains to be resolved.

Bavituximab monotherapy was administered in Phase 1 studies to over 80 patients with solid tumors. It was generally safe and well-tolerated. Three single-arm Phase 2 studies of bavituximab in combination with chemotherapy were presented by Dr. Thorpe: (1) combination with paclitaxel and carboplatin as front line therapy in locally advanced or metastatic breast cancer; (2) combination with docetaxel as second line therapy in locally advanced or metastatic breast cancer; and (3) combination with paclitaxel and carboplatin as front line therapy in locally advanced or metastatic NSCLC. These three trials in which 141 patients were treated with bavituximab showed consistent and promising overall response rates that were better than historical data from corresponding trials with the chemotherapeuticals. Randomized trials sponored by Peregrine are now under way in NSCLC and pancreatic cancer. First results from the Phase 2b NSCLC trial of bavituximab + carboplatin + paclitaxel as first line therapy showing that the initial overall response rate was 39% compared with 25% in the chemotherpy control arm were revealed by Dr. Thorpe.

Rolf Brekken (University of Texas Southwestern Medical Center) discussed two avenues to advance anti-VEGF therapies: (1) developing more potent and less toxic drugs and (2) trying to understand resistance to anti-VEGF therapy. He showed data on r84, a novel anti-VEGF-A human IgG1 antibody that specifically inhibits interaction with VEGFR2 but, unlike bevacizumab, does not interfere with binding to VEGFR1.[6-8] The antibody was able to control tumor growth in mouse models, and chronic exposure of mice to r84, which crossreacts with mouse VEGF-A, did not result in observable toxicity, making r84 an attractive therapeutic candidate. A clinical trial application (CTA) for r84 was filed recently by Affitech.

In the second part of his presentation, Dr. Brekken discussed the identification of pathways involved in resistance to VEGF-directed therapy in mouse models of cancer. Resistance may either be intrinsic, i.e., the tumors do not respond to the treatment at all, or it may be evasive, which implies that after an initial response, the tumor becomes refractory to therapy and continues to grow. Dr. Brekken's group set out to identify tumor-derived factors that are associated with intrinsic resistance by studying a panel of 12 different NSCLC lines. Mice were inoculated and treatment with r84, bevacizumab or control antibody was initiated the day after. They found some tumor models that were intrinsically sensitive to both anti-VEGF-A antibodies (e.g., H2073), some that were intrisically resistant to bevacizumab but not to r84 (e.g., A549) and vice versa (e.g., H1395). Microvessel density was reduced by both antibodies to a similar extent in sensitive, but also in resistant, tumors. Also, no obvious patterns in histology, source, gender or oncogenotype in sensitive vs. resistant tumor lines emerged. By expression profiling of bevacizumab-resistant vs. -sensitive cell lines, attention was drawn to Axl as a potential marker of bevacizumab resistance. Axl is a receptor tyrosine kinase that is expressed in various different cancers and is involved in angiogenesis and tumorigenesis. Bevacizumab-sensitive cells lines tended to express higher levels of Axl than resistant cell lines. Genentech has recently published data showing Axl knockdown by RNAi reduced tumor growth and co-treatment with anti-Axl antibodies enhanced the sensitivity of A549 tumors to bevacizumab.[9,10] Dr. Brekken's research group attempted to confirm these results and found that RNAi knockdown of Axl in A549 cells delayed tumor growth, but did not significantly enhance the activity of bevacizumab. Up-coming experiments will try to establish whether Axl inhibition with antibodies or by small organic drugs may render A549 and other tumors sensitive to anti-VEGF therapies.

To study evasive resistance, intrinsically anti-VEGF-sensitive tumors were treated with anti-VEGF until tumors grew large. Upon excision and ex vivo culture, tumor cells were reimplanted and response to anti-VEGF therapy was compared with the parental cell line. By this means, a subline of H1975 that became evasively resistant was obtained; this line will be further characterized with regards to microvessel density and angiogenic growth factor switching.

The last talk of the angiogenesis session was delivered by **Joseph Rosenblatt** (University of Miami) on antibody-endostatin fusion proteins for cancer therapy. Endostatin is a 20 kDa C-terminal fragment of collagen XVIII generated through cleavage by basement membrane degrading enyzmes. Endostatin displays anti-angiogenic activity and affects a multitude of cellular functions by modulating integrin-mediated signaling and by interfering with canonical wnt-signaling. Even though it showed good activity in preclinical models, endostatin achieved dissapointing results in clinical trials. Possible explanations for the discrepancy between prelinical and clinical activity are, among others, very short serum half-life and poor bioavailability.[11] An endostatin variant with improved solubility and serum half-life gained approval by Chinese authorities for NSCLC; however, reported survival benefits are modest.

Based on the observation that multimeric forms of endostatin showed enhanced anti-angiogenic activity,[12] Dr. Rosenblatt's group generated endostatin dimers by fusion of endostatin to the heavy chain C-terminus of an anti-Her2 IgG_3 antibody. The antibody serves as targeting vehicle and is expected to confer improved serum half-life and stability to endostatin. The construct preferentially accumulated in tumors in vivo, and showed signs of activity by extending survival in a Her-2 positive xenograft model. To further enhance the activity, the human endostatin in the antibody fusion construct was replaced by a mutant form, P125A, which showed greater activity than wild-type endostatin

in several assays.[13] The human anti-Her2 IgG_3-endostatin P125A fusion, termed anti-Her2 TEP, was able to induce tumor regression and resulted in 100% survival compared with 40% survival observed with the nonmutant anti-Her2 IgG_3-endostatin fusion in a Her-2 positive xenograft model in vivo.[14] The treatment also reduced tumor vessel density compared with PBS-treated animals. The principle of the anti-Her2-TEP was also applied to an anti-EGFR IgG_3 antibody. This construct was tested in the MDA-MB-231 breast cancer xenograft model. Whereas metastases were found in all of the PBS- and cetuximab-treated mice, treatment with anti-EGFR IgG_3—endostatin P125A fusion reduced the number of mice suffering from metastses in a dose dependent manner. Synergys is aiming to bring the two endostatin P125A antibodies to the clinic; IND filing is expected in 2013 and 2014 for anti-Her2 and anti-EGFR TEPs, respectively.

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December 7, 2011: Antibody Therapeutics
Gustavo Helguera

Session IV: Updates of Clinical Stage Antibody Therapeutics

The afternoon session of the Antibody Therapeutics track was chaired by **Mark R. Alfenito** (EnGen Bio, Inc.,), and the first talk was given by **Patrick A. Baeuerle** (Micromet, Inc.,), who presented an update on blinatumomab (MT103), a bispecific T-cell engaging (BiTE) antibody with murine variable regions targeting CD19 and CD3. This recombinant single-chain bispecific antibody construct recruits unstimulated T cells in the absence of costimuli via the binding to CD3 to target malignant B cells expressing CD19. The BiTE-activated $CD8^+$ and $CD4^+$ T cells induce lysis and apoptosis of CD19-expressing target cells, which involves the release of perforin and granzyme from T cells, and in target cells, the fragmentation of DNA, activation of caspases, and the induction of morphological changes such as blebbing of the target cell membrane.

Blinatumomab is currently being evaluated in several clinical studies, including a Phase 1 study in relapsed/refractory (r/r) non-Hodgkin lymphoma patients, several Phase 2 trials in r/r adult acute lymphocytic leukemia (ALL) patients, and in a pivotal trial in the EU in adult patients with minimal residual disease (MRD) ALL. Blinatumomab has a short serum half-life (1–2 h) and is therefore administered by continuous i.v. infusion via an implanted port and small ambulatory pump. This mode of administration achieves constant drug exposure for weeks and allows patients to leave the hospital after several days. For r/r ALL, stepwise dosing was implemented starting at 5 $\mu g/m^2/d$ for one week followed by a 15 $\mu g/m^2/d$ maintenance dose for three weeks. For MRD ALL, a 15 $\mu g/m^2/d$ maintenance dose without entry dose was used. All patients received steroids upon initiation of infusion to mitigate first-dose reactions. Most common clinical adverse events were transient, flu-like and of grade 1 or 2 (pyrexia, headache, chills, fatigue), presumably caused by the onset of T cell activation. The most common laboratory abnormalities were lymphopenia and leucopenia, which were related to the mode of blinatumomab action involving initial peripheral T cell redistribution and expansion, and sustained targeted B cell depletion. The clinically most relevant adverse events (AEs) were neurological in nature. Most AEs occurred early, and all were transient without causing changes in the CNS by MR imaging. Upon start of treatment, a modest cytokine release could be measured in serum; the effect was transient and did not recur upon a second treatment cycle. In r/r ALL, the intensity of AEs upon start of treatment appeared to correlate with tumor load. It could be managed by introduction of low entry dose for one week, which was not required for MRD ALL patients. There were no reports of treatment-related deaths and no cases of autoimmunity to date, and the frequency of anti-drug antibody response had been very low.

In NHL patients, treatment with blinatumomab at 60 $\mu g/m^2/d$ for up to 8 weeks was highly active.[1] The objective response

according to Cheson criteria and independent review rate across a variety of NHL subtypes is 71%. Dr. Baeuerle presented interim data from a Phase 2 study showing a high response rate in r/r ALL patients where 75% of patients (9 of 12) achieved a complete response (CR or CRh) following treatment with blinatumomab. Strikingly, all patients with a CR/CRh reached a molecular response below the detection limit of 0.01% tumor cells in the bone marrow, which is considered a positive prognostic factor for patient survival. In another Phase 2 study of blinatumomab in patients with chemotherapy-resistant MRD ALL, 80% of subjects achieved a molecular complete response within the first cycle of treatment.[2] Dr. Baeuerle concluded by noting that the therapeutic principle of polyclonal T cell engagement by bispecific antibody is very promising. Monotherapy with the BiTE antibody blinatumomab showed very high response rates of 60–80% in patients with MRD ALL, r/r ALL and with various types of r/r NHL, where the majority of responses reported to date are durable and support a favorable benefit/risk profile.

Vincent Ling (Neurotech) began his presentation with a detailed description of the encapsulated cell technology (ECT). This implantable miniature bioreactor consists of a capsule with an internal scaffold holding a recombinant cell line producing a biologic agent. The capsule is a sealed immuno-protective membrane, which retains the cellular producers inside and allows the release of the secreted compound and the entry of oxygen and nutrients. The NTC-200 cell line was selected as a result of a hardy cell screening, and because it is a non-tumorigenic, naturally immortalized human cell line. In addition, this cell line is very stable and viable under low nutrients and low oxygen conditions and cells proliferate rapidly within the device in vivo. ECT devices can produce mAbs, scFv, Fab, cytokines and other recombinant constructs with the advantage of very stable pharmacodynamics because the compound is released with long-term steady-state kinetics after a single implantation.

As an example, Dr. Ling discussed the ophthalmic ECT Implant. With a cylinder shape, it is nearly 6 mm long and can be easily implanted after a 20 min surgical intravitreal procedure. The device allows the controlled, long-term delivery of biologics directly into the eye. He described some advantages of ECT compared with the standard biologics manufacturing, such as the need for less infrastructure, simplified manufacturing batch records and a simplified path to clinic. Dr. Ling then presented Phase 2 clinical data of their long-term ciliary neurotrophic factor (CNTF) delivery program. He showed data for the treatment of geographic atrophy in dry age-related macular degeneration (dry AMD),[3] from a randomized, double-masked and sham-controlled study that was done at multiple centers in the US. The treatment did not show side effects and resulted in a trend toward visual acuity stabilization compared with sham treated over a period of 12 mo. The next study that he discussed was a Phase 2 for retinitis pigmentosa that lasted almost three years, with no side effects and showing stabilization of the disease compared with sham treated patients.[4] Dr. Ling also presented data on a VEGF antagonist for wet AMD that included the use of ECT with VEGF-receptor fusion proteins. The studies showed no SAE, and in one case, significant reduction in retinal thickening after six months of treatment. Further development in the size and configuration of the ECT devices are projected to increase the output from 10 μg/device/day to >1 mg/device/day, which would be closer to achieving doses necessary for systemic treatment with mAbs.

Roger Palframan (UCB Pharma) provided an update on olokizumab (CDP6038), which is a recombinant mAb that targets IL-6. The mAb is currently in development for the treatment of rheumatoid arthritis. The presentation began with a detailed description of the antibody discovery process at UCB, including a fluorescent foci technique that enables the selection and isolation of single B cells, isolation of the variable region and the construction of the recombinant antibody. Dr. Palframan described the IL-6 receptor assembly and the potential points of intervention in three axes: Axis 1, comprising the IL-6 binding to the IL-6 receptor (1x IL-6:IL-6R); Axis 2, comprising trimer formation with the recruitment of gp130 (1x IL-6:IL-6R:gp130); and Axis 3, comprising the hexameric signaling complex formation (2x IL-6:IL-6R:gp130).[5] This complex can be assembled, resulting in IL-6 cis-signaling via membrane bound IL-6R or in IL-6 trans-signaling via the soluble form of IL-6R.

To determine which approach was the most efficient for the treatment of autoimmune diseases, a series experiments in animal models with affinity-matched anti-murine mAbs targeting either the IL-6 cytokine or the IL-6R was done. Their relative abilities to inhibit the IL-6 signaling pathway were assessed in a range of in vivo experiments, including the inhibition of either murine IL-6 or CFA-induced serum amyloid A response, the inhibition of B cell function via dinitrophenyl-specific antibody production, and the capacity to inhibit collagen-induced arthritis in mice. In all cases, a significantly lower dose of antibody targeting the cytokine IL-6 was required to achieve the same level of response compared with the dose of the antibody targeting IL-6R. These murine in vivo studies suggested that targeting IL-6 cytokine and inhibiting recruitment of the gp130 signaling molecule, rather than targeting IL-6R, was the more efficient therapeutic approach for the treatment of autoimmune diseases such as rheumatoid arthritis. Dr. Palframan presented mathematical modeling of strategies for inhibiting IL-6 signaling in four prototypical intervention strategies: targeting anti-IL-6R, axis 1; anti-IL-6, axis 1; anti-IL-6, axis 2; and anti-IL-6, axis 3. Multiple factors simulating sensitivity of drug responses were evaluated. Overall, anti-IL-6 intervention at axis 3 may be more efficient at inhibiting IL-6 signaling than axis 1 intervention with an anti-IL-6 or anti-IL-6R mAb, in either expected serum- or synovial-like conditions.

Dr. Palframan concluded by showing data corresponding to a Phase 1 clinical study in which olokizumab was well-tolerated. The overall incidence of treatment-emergent adverse events (TEAEs) was higher with placebo (18 subjects [52.9%], 35 events) compared with olokizumab (11 subjects [33.3%], 26 events). No deaths or serious TEAEs were reported during the study, and there were no discontinuations due to TEAEs. Olokizumab had a linear PK profile with a median half-life at the terminal phase of 31.1 d (range 11.3–71.2 d), with bioavailability of the s.c. dose ranging from 84.2 to 92.5%. In addition, there

was no immunogenicity after single doses of up to 3 mg/kg s.c. and 10 mg/kg i.v. in healthy subjects, with no increase in post-dose anti-olokizumab antibodies detected in any subjects. These findings support the on-going clinical evaluation of olokizumab, which is currently in Phase 2b development for the treatment of moderate to severe rheumatoid arthritis.

Mikkel Pedersen (Symphogen) discussed ways to exploit the mechanistic advantages of antibody mixtures to develop novel cancer drugs. He started his presentation with an overview of the technology platform that supports the strategy of developing recombinant antibody mixtures in the context of the next-generation biologics. Cocktails of mAbs that can be set as single target mixtures or multi-targeted mixtures, are being developed under the rationale that natural antibody responses are polyclonal and that, from the perspective of evolution, this way can be more effective. Dr. Pedersen discussed the Symphogen approach, where oligoclonal mixtures containing from 2–6 synergistic recombinant mAbs against one or more targets are produced as one drug product. Using their platform, antibodies secreted from B cells from different origins, e.g., chicken, mouse or human, are identified and cloned in a library with a large and diverse repertoire. Those are later selected and a ranking of antibody mixtures is generated from a functional perspective. The mixtures are evaluated in animal models, and a candidate mixture is selected based on the results. The candidate is manufactured in larger scale in different batches for toxicological evaluation in animals and eventually for clinical evaluation.

Dr. Pedersen gave an overview of Sym004, a mixture of two chimeric IgG_1 antibodies targeting two non-overlapping epitopes on Domain III of EGFR. Compared with cetuximab, this mixture induced a significantly higher inhibition of proliferation in vitro in a human epidermoid carcinoma,[6] superior EGFR internalization and degradation, and reduction in tumor growth in vivo. In proliferation studies in vitro, Sym004 was able to overcome cetuximab resistance due to EGFR-HER2 cross-talk in a cancer cell line with better activity than the combination of cetuximab and trastuzumab.[7] In other tests, antibody mixtures targeting non-overlapping epitopes were shown to enhance complement dependent cytotoxicity against gastric carcinoma cell line and CLL patient samples. Moreover, antibody mixtures seem to have broader inhibitory profiles against cancer cell lines from different origin expressing HER2 compared with antibodies alone, such as trastuzumab and pertuzumab, and they showed superiority against trastuzumab in gastric cancer models in vivo.

Dr. Pedersen also presented data for a mixture of 4 antibodies against two distinct targets showing that this mixture could inhibit horizontal resistance due to compensatory target upregulation in vitro. Dr. Pedersen concluded by noting that mAb mixtures exhibit different advantages compared with standard mAb therapies from a mechanistic perspective, including: superior target internalization and degradation, increased Fc-mediated effector functions, enhanced clearance of soluble targets, delayed development of acquired resistance and synergistic and broader activity by multi-targeting. In addition, this strategy has format advantages, including a controlled and adjustable ratio of mAbs, multiple targets, fully independent target access (unlike bi-specific formats), customized Fc selection, single batch manufacture and reduced cost.

Naomi Hunder (Seattle Genetics) presented data on the clinical development of brentuximab vedotin for relapsed or refractory Hodgkin lymphoma (HL) and systemic anaplastic large cell lymphoma (ALCL). Brentuximab vedotin is an antibody-drug conjugate (ADC) directed against CD30, a transmembrane glycoprotein receptor and member of TNFr superfamily, that is highly expressed on HL Reed-Stemberg cells and ALCL cells. The expression of CD30 on normal cells is limited to activated lymphocytes (B cell, T cell, NK cell), and weak expression on activated monocytes. The anti-CD30 mAb is linked via a protease-cleavable linker to the microtube-disrupting agent monomethyl auristatin E (MMAE). The mechanism of action of brentuximab vedotin consists of the binding of the ADC to CD30 on the surface of the malignant cell, thereby forming a complex that is internalized and traffics to the lysosome, where the MMAE is released, with the subsequent disruption of the microtubule network, cell cycle arrest and induction of apoptosis.

Dr. Hunder showed that the ADC technology improved the efficacy of antibodies by presenting clinical data in relapsed or refractory HL patients showing that at the approved dose brentuximab vedotin exhibited 75% objective response rate, compared with 0% response rate observed in a study of SGN-30, the unmodified anti-CD30 mAb.[8] In a pivotal Phase 2 study of brentuximab vedotin in patients with relapsed or refractory HL, objective responses were observed in 75% of patients, durable complete remissions in 34% of patients, with partial response in 40% of cases after the treatment and 89% of patients achieving 12-mo overall survival. The dose administered was 1.8 mg/kg i.v. every 21 d with median of treatment cycles of 9. Moreover, in a pivotal Phase 2 study of brentuximab vedotin in patients with relapsed or refractory systemic ALCL, objective responses in 86% of patients, with durable complete remissions in 57% of patients and partial responses in 29% of cases was observed. As in the previous study, the dose used was 1.8 mg/kg i.v. every 21 d. The safety profile of both studies combined showed frequent AEs such as peripheral sensory neuropathy (44%), fatigue (42%), nausea (41%), diarrhea (34%) or pyrexia (31%), although in general the events were manageable. Infusion-related reactions were less frequent and included chills, nausea, dyspnea, pruritus, cough, dizziness, erythrema, flushing and pyrexia, among others. Dr. Hunder concluded by noting that durable complete remissions could be achieved with brentuximab vedotin in highly refractory HL and systemic ALCL patients; adverse events include peripheral neuropathy and were manageable; and based on these encouraging trial results, frontline studies in HL and mature T and NK cell lymphomas (including sALCL) are underway (NCT01060904 and NCT01309789). Accelerated approval for brentuximab vedotin was granted for two indications (relapsed or refractory HL and sALCL) earlier in 2011.

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December 8, 2011: Antibody Engineering
Sven Berger

Session VII: The Biology Behind Potential Blockbuster Antibodies

On the fourth day of the conferences, Antibody Engineering and Antibody Therapeutics sessions were held in the morning, while only Antibody Engineering had an afternoon session. The morning sessions in the Antibody Engineering track focused on blockbuster antibodies, while the afternoon session discussed the modification of cell signaling by therapeutic antibodies.

The morning session was opened by a short presentation from the chairman, **Mitchell E. Reff** (Consultant). He pointed out the important contribution to patients' health of antibody blockbusters. He further remarked that none of the present antibody blockbusters were predicted as such. For example, the sales of rituximab were predicted at 250 million dollars, but exceeded 6 billion dollars in 2010. Currently, there are ten antibody blockbusters, each with annual sales greater than one billion dollars. He concluded with the reminder that not all approved antibodies have become blockbusters; 11 approved antibodies failed to return development costs.

Kim A. Campbell (Centocor Research and Development) discussed the development of the anti-IL-12/23 ustekinumab. She began by reviewing characteristics of TNF blocking reagents like infliximab, adalimumab, golimumab, certolizumab and the soluble TNFR2 etanercept. Despite the treatment of more than 10 million patients with these drugs, certain therapeutic drawbacks must be noted. These include an increased risk of infection, autoimmune diseases and other malignancies. Apart from TNF, IL-12 and IL-23 are critical cytokines in inflammation and, therefore, are important therapeutic targets for treating autoimmune diseases. To understand the importance of these cytokines in the onset of autoimmune diseases, the differentiation of naïve CD4 positive T cells into different types of effector helper T cells was presented.

Dr. Campbell then gave an overview of the most important transcription factors, Th1, Th2, Th17 and Treg. While IL-12 drives the differentiation of naïve T cells into Th1, IL-23 leads to the development of Th17 T helper cells. Both Th1 and Th17 cells are strongly associated with immune-mediated diseases. Therefore, Centocor started the development of anti-IL12/IL23 ustekinumab in 1990. It was generated in transgenic mice that express human IgGs. The antibody binds to a neutralizing epitope on the p40 subunit that is present both in IL-12 and IL-23. It inhibits IL-12 binding to the IL-12R 1/2 or IL-23 binding to IL-12R 1/23R functional dual receptor complexes. In toxicology studies conducted in cynomolgus monkeys, ustekinumab was well-tolerated, suggesting a broad therapeutic window for this antibody. It has been tested in Phase 2 studies for the treatment of Crohn disease, multiple sclerosis, psoriatic arthritis and in Phase 3 studies for psoriasis treatment.

To describe the mechanism of action of ustekinumab Dr. Campell gave an overview of the pathophysiology of psoriasis, which is a chronic, inflammatory, immune-mediated skin disease. Although the cause of psoriasis is unknown, activation and differentiation of T helper cells into Th1 and Th17 cells is a critical process. These T cells subsequently activate keratinocytes, which leads to the release of chemokines and the recruitment of neutrophils, monocytes, T cells, Langerhans' cells and immature DCs.[1] This recruitment leads to further activation of T cells, resulting in a "vicious cycle" driving psoriasis pathogenesis. By blocking the differentiation of T cells into Th1 or Th17 cells with ustekinumab, the pathogenesis of psoriasis can be reversed. Other therapies for psoriasis include anti-TNF, anti-LFA-1 and anti-CD2 antibodies and the soluble TNF receptor fusion protein etanercept. However, Dr. Campbell presented clinical data indicating an increased benefit of ustekinumab treatment compared with etanercept.[2] Ustekinumab was approved by the US Food and Drug Administration (FDA) for the treatment of psoriasis in 2009.

The generation and development of a therapeutic soluble gp130 Fc-fusion protein was presented by **Stefan Rose-John** (University of Kiel). Professor Rose-John began with an overview of IL-6 biology. IL-6 activates cells through a cell-surface type I cytokine receptor complex. It first binds to the non-signaling IL-6 receptor α chain (CD126). This complex dimerizes with the signal-transducing component gp130 (CD130), resulting in the activation of receptor-associated kinases (JAK1, JAK2 and Tyk2). In classic signaling, IL-6 binds directly to a membrane-bound IL-6 receptor, while in IL-6 trans-signaling soluble IL-6 receptor bound to IL-6 stimulates cells that only express gp130. The release of the IL-6 receptor from the cell membrane is dependent on the metalloprotease ADAM-17. Interestingly, cells such as smooth muscle cells or endothelial cells, which are important target cells for IL-6 during inflammatory processes, do not express membrane-bound IL-6R. Therefore, these cells can only be activated via IL-6 trans-signaling.

Two fusion proteins were generated for the analysis of IL-6 trans-signaling. One consisted of IL-6 fused to the soluble interleukin-6 receptor by a flexible linker. This fusion protein called Hyper-IL-6, showed much higher activity compared with non-fused IL-6 and soluble IL-6 receptor.[3] It activates several different types of target cells that do not express the IL-6 receptor and therefore cannot be activated by IL-6 alone. The activity of this molecule was demonstrated by blocking the TGFβ mediated induction of FoxP3 in naïve $CD4^{+}CD25^{-}$ T cells. As a further proof of the importance of IL-6 trans-signaling, the soluble gp130 was fused to the Fc-domains of a human IgG. This dimeric fusion protein inhibited IL-6 trans-signaling, but not the classical IL-6 signaling. The fusion protein was evaluated using gp130 dependent proliferation of transfected BAF/3 cells.

In the context of IL-6 signaling, Professor Rose-John summarized results obtained with viable ADAM-17 hypomorphic mice, which were named ADAM-$17^{(ex/ex)}$ mice as an additional exon was inserted into the ADAM-17 gene.[4] These mice showed strongly reduced shedding of ADAM-17 substrates like TNF. They were also more susceptible to inflammation in a dextran sulfate sodium colitis model. This was a result of impaired shedding of EGF-R ligands as shown by the injection of recombinant TGF, which restored the proliferation of intestinal epithelial cells and reduced weight loss of ADAM-$17^{(ex/ex)}$ mice. In this context Professor Rose-John further explained that ADAM-17 is a regulator for both inflammation and tissue regeneration depending on the substrate.

The role of ADAM-17 in inflammation was further described for endothelial cells that are activated by IL-6-trans-signaling. IL-6 is secreted by PMN after phagocytosis of pathogens. When the PMN become apoptotic, ADAM-17 is activated and sheds the IL-6 receptor resulting in activation of endothelial cells by the IL-6/sIL-6 receptor complex. In summary, ADAM-17 can balance the pro- and anti-inflammatory response by shedding IL-6R. Activation of membrane bound IL-6R results in an anti-inflammatory response, while soluble IL-6R leads to a pro-inflammatory response.

Professor Rose-John presented recent data on inflammation-associated cancer, artherosclerosis, sepsis and bacterial infections. In these examples, it became clear that the specific blockade of IL-6 trans-signaling by the sgp130Fc protein was sufficient to block the inflammatory process. Furthermore, in artherosclerosis, sepsis and bacterial infections, inhibition of IL-6 trans-signaling by sgp130Fc seemed to be superior compared with complete inhibition of IL-6 with neutralizing antibodies.

Thi-Sau Migone (Human Genome Sciences) reported on the generation of belimumab, a human antibody targeting B lymphocyte stimulator (BLyS, also termed BAFF). BLyS is a member of the TNF ligand superfamily. The cytokine is a 285-amino acid glycoprotein. It was identified by homology searches based on the sequencing of short expressed sequence tags (ESTs) by Human Genome Sciences. The gene was cloned and expressed in mammalian cells and its activity evaluated on human and murine B cells. Despite induction of a mild effect on B cell proliferation in vitro, BLyS is very important for B cell differentiation, Ig class switching and B cell survival. BLyS binds to and activates the receptors TACI, BCMA and BAFFR. It is expressed as a transmembrane protein on various cell types including monocytes, dendritic cells and bone marrow stromal cells, but is rapidly cleaved by furins from the membrane and released as a soluble trimer. Expression of BLyS is upregulated in response to interferons (IFNs), granulocyte colony-stimulating factor (GCSF) and other factors. Activation of toll-like receptors upregulates the expression of BLyS receptors on B cells. BLyS is a homolog to other TNF superfamily ligands like APRIL, FasL, TNFα and others, but with relatively low identity.

Two of the BLyS receptors, TACI and BCMA, also bind the closest TNF superfamily ligand member APRIL. These receptors are differentially expressed during the course of B cell differentiation. While BAFFR is expressed at high levels on naïve and activated B cells, it is absent on plasma cells and is differentially expressed on memory cells. The TACI receptor is weakly expressed on naïve B cells and strongly expressed on activated B cells, plasma cells and memory cells. The third BLyS receptor, BCMA, is weakly expressed on naïve and activated B cells, intermediate on plasma cells and at low levels on memory cells. The expression of BLyS and APRIL receptors corresponds with the dependence of different B cell subsets on these cytokines.[5] Surface expression of the BAFF-receptor on peripheral B cells and BLyS dependence are important regulatory mechanisms for self-tolerance. Elevated BAFF expression leads to survival of self-reactive B cells. The importance of BLyS in the development and progression of autoimmune disease is further emphasized by the fact that autoimmune prone mice and autoimmune patients have elevated levels of BLyS. Its inhibition leads to slower disease progression in animal models.

A neutralizing antibody to soluble BLyS was generated by Human Genome Sciences in cooperation with Cambridge Antibody Technology (CAT). Three-thousand BLyS binding antibody fragments were isolated from a large phage display library of human scFv and sequenced. Based on their anti-BLyS blocking activity, scFvs were ranked, converted into human IgGs and evaluated in functional assays. The lead candidate, belimumab, blocked binding of BLyS to all three receptors, but did not interfere with binding of APRIL to its corresponding receptors. Belimumab bound soluble BLyS, but not membrane-bound BLyS. Therefore, complement fixation and ADCC are not involved in its mode of action, but it inhibits BLyS-induced B cell proliferation in vitro and BLyS-induced B-cell expansion and Ig secretion in vivo. Belimumab entered clinical trials for the treatment of systemic lupus erythematosus (SLE) in 2000. A double-blind, placebo-controlled, multi-center Phase 2 clinical trial was initiated in 2003. With 449 patients enrolled, this was the largest Phase 2 clinical trial for SLE conducted so far. After a successful Phase 3 clinical trial, belimumab was approved by the FDA in March 2011.

The anti-tumor activity of VEGF-targeted therapy was summarized by **Gavin Thurston** (Regeneron Pharmaceuticals). He noted that continued tumor growth initiates angiogenesis. Tumors secret various growth factors like VEGF to induce the growth of new blood vessels in the tumor. Hypoxia is a key upstream activating component of this process. VEGF induces

endothelial cell sprouting, proliferation and survival, and vascular leakage. Heterozygous deletion or overexpression of VEGF is lethal at an embryonic stage in mice. The pioneering work of Genentech validated VEGF as a therapeutic target and lead to the approval of an anti-VEGF-A antibody (bevacizumab). Despite positive results in many tumor types, current anti-VEGF treatments like bevacizumab, sunitinib, sorafenib and pazopanib remain for many patients non-curative.

Dr. Thurston then presented the development of the novel VEGF inhibitor aflibercept, which, in contrast to bevacizumab, blocks VEGF-A as well as VEGF-B and PIGF. This soluble receptor-Fc fusion protein comprises binding domains from VEGFR-1 and VEGFR-2. Aflibercept blocks tumor angiogenesis and tumor growth in preclinical models. It rapidly induces hypoxia and tumor reorganization in various tumor models. In clinical trials, aflibercept was effective in resolving malignant ascites in ovarian cancer. A formulation of aflibercept (Eylea™) has been approved for the treatment of patients with neovascular age-related macular degeneration, and in a Phase 3 cancer study, aflibercept plus chemotherapy met its primary endpoint of improving overall survival in the second-line treatment of metastatic colorectal cancer (mCRC).

Clinical data indicated that the combination of anti-VEGF therapy with conventional chemotherapy improves survival in cancer patients compared with chemotherapy alone. Certain chemotherapeutic agents appear to work better than others in combination with anti-VEGF treatments. In breast cancer, for example, the combination of bevacizumab/taxanes was more effective than bevacizumab/gemcitabine. This might be explained by tumor vessel normalization mediated by anti-VEGF treatment.[6] Tumor vessel normalization is characterized by attenuation of vascular leakage, reduced intra-tumoral pressure and (potentially) increased delivery of drugs into the tumor. However, other explanations for the enhanced benefit are also possible. Initial concerns regarding anti-VEGF therapy proved unfounded, as most normal vessels do not require ongoing VEGF signals. However, decreased vessel density in endocrine organs after VEGF blockade was determined in animal models, but changes in organ function were very subtle. Dr. Thurston noted that predictive biomarkers for anti-VEGF treatment and a better understanding of mechanisms of resistance to anti-VEGF are still needed.

An overview of the development of RANK ligand inhibitors was presented by **David Lacey** (Amgen). Osteoprotegerin (OPG) a member of the TNFα receptor superfamily represents a decoy receptor for RANK ligand (RANKL) and inhibits RANKL binding to RANK.[7] OPG was identified by Amgen after sequencing large numbers of ESTs. Its function was elucidated using OPG transgenic mice. These mice exhibit a skeletal phenotype of radiodense bones with a normal skeletal radiographic morphology. The magnitude of bone accumulation correlated with OPG serum levels.

RANKL, produced by osteoblasts, binds to RANK on osteoclast precursors, leading to increased osteoclast formation (osteoclastogenesis) that results in increased bone resorption. In humans, OPG deficiency can cause juvenile Paget disease, an autosomal recessive osteopathy. Recombinant OPG inhibits osteoclastogenesis in vitro. Dr. Lacey recapitulated the development of a minimally active domain of OPG. Over 90 different forms of OPG were tested in the in vitro/in vivo assays, including Fc fusion proteins and PEGylated OPG. Full-length non-modified OPG has a very short half-life in rats. However, OPG fused to the Fc domain of an IgG1 is active and shows a large increase in serum half-life. Recombinant OPG was effective in treating different diseases in animal models, including osteoporosis, rheumatoid arthritis and multiple myeloma.

The CHO-produced OPG Fc-fusion protein (AMG-0007) has a high affinity for RANKL (K_D-3 x 10^{-13} M), an IC_{50} of 1 ng/mL in in vitro assays and proved to be more active than OPG produced in *E. coli*. In Phase 1 studies, evidence of possible immune response against the OPG Fc-fusion protein was observed. The molecule was discontinued in favor of an anti-RANKL antibody. Antibodies against RANKL were generated by immunization of transgenic mice that express human IgGs (XenoMouse®). Ultimately, the human antibody denosumab was developed. In comparison to AMG-0007, denosumab has ten times lower affinity, but a longer serum half-life, does not cross react with TRAIL and is more active in monkey models. Having met its primary endpoint in Phase 3 trials, denosumab was approved for treatment of postmenopausal women at risk of osteoporosis and for the prevention of skeletal-related events in patients with bone metastases from solid tumors.

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December 8, 2011: Antibody Engineering Sven Berger

Session VIII: Antibodies as Signaling Modifiers: Where Did We Go Right, and Can We Learn from Success?

The afternoon Antibody Engineering session was chaired by **Louis Weiner** (Georgetown University Medical Center) who

also gave the first lecture. He reviewed the successes and failures in mAb-based cancer therapies. The key points of the antibody blockbusters such as Herceptin®, Rituxan®, Erbitux® were discussed. For trastuzumab (Herceptin®), targeting the central receptor HER2/neu is the key point of the success. The anti-CD20 antibody rituximab (Rituxan®) is a good example of a successful antibody targeting an antigen that is expressed both on tumor cells and cell lineages that can be eliminated on a temporary basis. Cetuximab (Erbitux®) was presented as an example of an antibody drug where patient selection needs to be considered more carefully in the future as only between 10% and 20% of patients clinically responded to Erbitux. A lack of benefit was observed in colorectal cancer patients with activating KRAS mutations.

Professor Weiner remarked that the cost/benefit ratios will be of major importance for future antibody development. Using these antibody blockbusters, he pointed out guiding principles for antibody design, also referring to the pioneering work of Ronald Levy in the treatment of cancer with antibodies. He continued by emphasizing recent success of antibodies that target receptors expressed on T cells, such as CTLA-4 and PD-1. Due to their high specificity and relatively low toxicity, antibodies can be combined with other anti-cancer therapies. Three future trends in antibody development were discussed; the amplification of a pre-existing T cell-mediated immune responses, site-specific immune activation through bispecific antibodies (e.g., Micromet's BiTE technology) and immunoconjugates. Antibody-dependent cell-mediated cytotoxicity (ADCC) was discussed not only in the context of recent Fc-engineering, but also in inducing adaptive immune responses. Many tumors elicit low amplitude pre-existing immunity. Therefore, the amplification of this pre-existing immunity by mAb treatment is a desirable goal in antibody design. The induction of an effective CTL response had been shown before.

In this lecture, Professor Weiner presented new results of human mutated HER2 (hmHER2) transgenic mice to test the immunization potential of human HER2-targeted therapeutics. These results indicated that a host-protective antitumor immunity is triggered by anti-Her2 treatment and that it can be increased by cotreatment with a TLR4 agonist.[1] Furthermore, results of a study using siRNA libraries targeting the EGFR signaling network were presented.[2] In this study, 61 potential regulators of resistance to EGFR-targeted therapies were identified. These genes were further analyzed for their importance in cetuximab-mediated ADCC resistance. These studies identified candidate partner targets for cetuximab therapy.

Mark S. Dennis (Genentech) began his presentation with an image of the vascular system of the human brain to show how many blood vessels are present. He outlined that, despite the fact that a number of molecules like amino acids, caffeine and glucose get into the brain, other large hydrophilic molecules either do not enter the brain or enter only at a very low rate. In early experiments, Edwin Goldmann showed that some chemical dyes injected into the cerebro-spinal fluids of animals stain the brain but not surrounding tissues, leading to the discovery of the blood-brain barrier (BBB). With the first electron microscopic studies, characteristics of the BBB become evident. Endothelial cells around the blood vessels in the brain form very tight junctions that hinder the non-regulated exchange of molecules between the brain and the blood. Although antibodies defuse at a low rate into the brain, the achieved antibody concentrations are too low to be therapeutically relevant. Larger molecules like transferrin are transported across these cells by receptor-mediated transcytosis (RMT). RMT offers a promising mechanism to deliver therapeutic proteins like antibodies or antibody conjugates across the BBB.

Dr. Dennis showed that high affinity antibodies against the transferrin receptor remain trapped in the brain endothelial cells, thereby limiting the therapeutic potential of these antibodies. By lowering the affinity of the anti-TfR antibody, uptake was reduced at low doses (50 μg/kg); however, uptake was increased at a therapeutic dose (20 mg/kg).[3] Further, low affinity antibodies were effectively distributed throughout the brain as indicated by immunohistochemical staining of mouse brain sections 24 h after intravenous injection. The generation of a bispecific antibody (anti-TfR/BACE1) that binds to both TfR and BACE1 (β-site amyloid precursor protein cleaving enzyme 1) was presented. The anti-BACE1 antibody effectively inhibits BACE1 activity and thereby prevents amyloid-β peptide formation. Both antibodies were evaluated in an Alzheimer mouse model. The bispecific antibody led to significantly lower amyloid-β peptide concentration in the mouse brain compared with the monospecific anti-BACE1 antibody. These results indicated that a low affinity anti-TfR antibody can be envisaged for therapy as bispecific antibody for the treatment of CNS diseases.

John McCafferty (University of Cambridge) recapitulated strategies to block receptor signaling with antibodies using the EGF receptor as model.[4] Inhibition of receptor signaling using antibodies can be performed by neutralizing the ligand, binding of the antibody to the ligand binding site, sterical hindrance of receptor dimerization or by stabilizing the receptor in a non-active conformation. Examples for these strategies were presented for antibodies blocking the receptors NOTCH1, NOTCH2, MET and the metalloprotease TNFα converting enzyme (TACE, also named ADAM-17). These antibodies were isolated from a large scFv phage display library. NOTCH receptors regulate cell fate and proliferation during the development of healthy tissue. In cancer, NOTCH receptor activation can lead to the expression of oncogenes and tumor growth. Binding of the ligand triggers a sequence of proteolytic cleavage resulting in the formation of a clipped signaling form of the receptor. Antibodies against the negative regulatory domain were generated that stabilize the receptor in a closed, non-active conformation. Selected antibody clones bind to only one member of the human NOTCH family, either NOTCH1 or NOTCH2, but also cross-react with the murine receptors. Consequently, these antibodies specifically inhibit one of the two receptors.

In a second example of receptor blocking antibodies, anti-TACE antibodies were presented.[5] Previously identified small-molecule inhibitors (SMIs) against matrix metalloprotease (MMP) showed a lack of high specificity. The poor specificity might be a consequence of targeting the catalytic site of MMPs.

A different strategy for inhibiting the matrix metalloprotease TACE was followed for antibody generation, where non-catalytic residues of TACE were targeted and still a strong, but highly specific inhibition of TACE was achieved. Starting from a single anti-TACE antibody that binds exclusively through its variable heavy domain (V_H), a library was generated containing this V_H domain and large number of different variable human light (V_L) chains. Employing phage display, new antibodies were isolated that recognize different epitopes including antibodies that bind the catalytic domain with their V_L domain.

As a third example, Professor McCafferty outlined the development of an anti-Met antibody. A large panel of MET-binders was screened for their inhibitory function in semi-quantitative scatter assays. The functional activity of positive clones was quantified in a cellular migration assay. Despite high antagonist activity, low agonist activity without the ligands was determined for dimeric scFv. The lead candidate was converted into a Fab and affinity maturated by CDR-H3 randomization. In this antibody format, no agonist activity was detectable. Binding was mapped to the stalk region of Met at a site that is distinct from the ligand binding site. Despite this, ligand binding was still inhibited.

Clinical results of an anti-epidermal growth factor receptor (EGFR) antibody combination were presented by **Amita Patnaik** (START Center for Cancer Care). EGFR is a highly validated target for the treatment of several cancers, particularly, metastatic colorectal cancer (mCRC). Although KRAS is a known predictor of lack of response to EGFR-directed mAbs in mCRC, only a small number of patients with wild-type KRAS have benefited. This emphasizes the importance of factors such as the expression of low affinity ligands (e.g., amphiregulin, epiregulin) to predict response to anti-EGFR treatment in CRC in addition to the KRAS status.

Dr. Patnaik noted that antibody combinations may have a more pronounced effect on reducing signaling via EGFR than a single antibody. SYM004 is the mixture of two anti-EGFR antibodies targeting the domain III of the receptor on non-overlapping epitopes. The mechanism of action of SYM004 includes ADCC, CDC, receptor internalization and inhibition of ligand binding, receptor dimerization and downstream signaling. In comparison to approved anti-EGFR mAbs, SYM004 induces more effective and rapid EGFR internalization and degradation. A superior growth inhibition of cancer cell lines such as A431 in vitro and in vivo was determined.

A Phase 1 open-label, multi-center dose escalation study of 20 patients with refractory or recurrent advanced late stage solid tumors without available treatment options was conducted. The primary objective was to determine the safety and tolerability of escalating doses of Sym004 administered intravenously on a weekly basis. The secondary objective was to determine the pharmacokinetic profile, to assess the antitumor activity, to validate the pharmacokinetics and safety profile of multiple doses of Sym004 at the MTD exposure level, and to explore pharmacodynamic markers of activity. In the dose escalation study, doses from 0.4 mg/kg to 12 mg/kg were administered to patients with refractory advanced solid tumors. In the second part of this study, 17 mCRC patients with KRAS wild-type status were treated with 12 mg/kg. Interestingly, both antibodies constituting Sym004 displayed similar serum elimination profiles with a serum half live of approximately 5 d. Efficient removal of EGFR was observed in skin biopsies at Sym004 doses of 6 mg/kg or more. Six of 11 patients who received doses of 6 mg/kg or above showed tumor shrinkage. The most frequent adverse effects (AE) were skin rash, hypomagnesaemia, rash acneiform and infusion-related reactions. Overall, the safety profile of SYM004 is consistent with other anti-EGFR antibody therapies. No human anti-chimeric antibodies were observed. The 12 mg/kg dose is safe and is being explored further in the second part of the dose escalation trial.

A novel format of bi- and trispecific antibodies was described by **K. Dane Wittrup** (Massachusetts Institute of Technology). He noted that the approved anti-EGFR antibodies cetuximab and panitumumab showed only relatively low response rate in clinical trials. This indicates the need for improved anti-EGFR therapy. Antibody combinations have proven to downregulate and inhibit the recycling of EGFR more effectively than a single antibody. No agonistic activity was detected for these antibody combinations.

Professor Wittrup presented new trispecific antibodies against EGFR that were developed with the intention to increase downregulation of EGFR by "branched" clustering. Engineered fibronectin type III domains (Fn3) were generated and isolated by yeast display against different non-overlapping epitopes on the EGF receptor. These epitopes are located on domain I, domain III and at the interface of domain III and IV. Fn3-binders were genetically fused to cetuximab. Among four possible formats, trans-trispecific fusions were the most active format in receptor downregulation. The addition of a fourth binding site did not increase EGFR downregulation.

Treatment with the cetuximab-Fn3 fusion protein does not lead to EGFR phosphorylation. Furthermore, complete inhibition of EGF-mediated phosphorylation was achieved using trispecific cetuximab-Fn3 fusion proteins in cell lines where cetuximab is not effective. This signaling inhibition was also reflected in a complete abrogation of AKT phosphorylation by trispecific cetuximab-Fn3 fusion proteins. The increased blocking of EGFR downstream signals might be of major importance as phosphorylation of AKT and ERK is detectable even at very low EGF concentrations, where EGFR phosphorylation cannot be determined.[6] Activating mutations in BRAF of KRAS clinically correlate with cetuximab resistance. Mouse xenograft models with cell lines harboring these mutations were used to evaluate the trispecific constructs in comparison to cetuximab. Only the trispecific construct showed tumor growth arrest in these models, while cetuximab was ineffective.

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December 8, 2011: Antibody Therapeutics Thierry Wurch

Session V: Drug Conjugates and Bispecific Antibodies

The last session of the Antibody Therapeutics conference was chaired by **Susan Lacy** (Abbott Laboratories) and was dedicated to the growing fields of antibody-drug conjugates (ADCs) and bi-specific antibodies (BsAbs). The first speaker, **William Olson** (Progenics Pharma), discussed a novel ADC targeting prostate specific membrane antigen (PSMA) armed with an auristatin-derived drug obtained via a collaboration with Seattle Genetics. PSMA is abundantly expressed on the surface of prostate cancer cells, as well as cells in the newly formed blood vessels of major solid tumors. The conjugated therapy comprises a human mAb developed by Progenics using the XenoMouse technology from Abgenix (now Amgen) that binds to PSMA and the highly potent cancer drug, monomethyl-auristatin-E (MMAE), which is a derivative of auristatin, a chemotherapeutic agent that inhibits cell proliferation by disrupting microtubules through a valine-citrulline linker.

The anti-tumor activity of PSMA ADC was evaluated against a panel of prostate cancer cell lines in vitro and in a novel in vivo model of taxane-refractory human prostate cancer. In vitro cell killing was potent ($IC_{50} \leq 0.022$ nmol/l) for cells with abundant PSMA expression (>10^5 PSMA/cell), and almost inactive against cells with undetectable PSMA,[1] indicating a correlation between PSMA level and cytotoxicity. Similar in vitro activity was observed against androgen-dependent and -independent cells that had abundant PSMA expression. In vitro activity of PSMA ADC was also dependent on internalization and proper N-glycosylation/folding of PSMA.[1] Less potent and nonselective cytotoxic activity was observed for a control ADC, free monomethylauristatin E, and other microtubule inhibitors.[1] PSMA ADC showed high in vivo activity in treating xenograft tumors that had progressed following an initial course of docetaxel therapy, including tumors that were large (>700 mm^3) before treatment with PSMA ADC.[1]

The preclinical and clinical program on CDX-011, an ADC targeting glycoprotein NMB, was presented by **Tom Davis** (Celldex Therapeutics). The CDX-011 ADC was originally discovered as CR011 and developed by CuraGen, acquired by Celldex in 2009. Glycoprotein NMB (GPNMB) is a tumor-associated protein and potential target to treat melanoma.[2] The GPNMB gene is differentially expressed among melanoma cell lines with high vs. low metastatic potential. GPNMB expression was also described in several other carcinomas, including liver and squamous cell lung, as well as in some normal tissues such as skin. CR011 ADC corresponds to a human parental anti-GPNMB Mab (discovery based on Abgenix's Xenomouse technology and recombinantly produced in CHO-K1 cells) coupled to the cytotoxic drug monomethylauristatin E (MMAE) via a pH-sensitive valine-citrulline linker based on Seattle Genetics' technology.[3]

The ADC derivative CR011-vcMMAE potently and efficiently inhibited growth on GPNMB-positive melanoma cells in vitro.[3] In a melanoma xenograft model of SK-Mel2 tumor fragments, CR011 ADC induced dose-dependent reduction of the tumor size and complete regressions at doses of 1.25 mg/m^{3}. A Phase 1/2 was conducted with CR011-vcMMAE (or CDX-011) in unresectable melanoma (stage 3 or 4) to evaluate its safety, tolerability and pharmacokinetics.[4] The drug was administered intravenously once every 3 weeks to cohorts of 3–6 patients at escalating doses until reaching the maximum tolerated dose (MTD), followed by a Phase 2 trial portion to further evaluate the safety and efficacy of CDX-011.[4] CDX-011 was found to be active in advanced melanoma patients in the study. In the Phase 2 expansion study, CDX-011 was administered at the pre-defined MTD once every 3 weeks. The primary activity endpoint of overall response rate (ORR) in the cohort was achieved with an ORR of 15% (5/34).[4] Median progression free survival (PFS) was 3.9 mo.[4] Preliminary data suggest an increase in PFS in patients with high tumoral GPNMB expression. Skin toxicity with the development of rash, which may be associated with the presence of GPNMB in the skin, correlated with greater PFS.[4] The most frequent treatment-related adverse events included rash, fatigue, hair loss, pruritus, diarrhea and neuropathy.

Another Phase 1/2 study was conducted with CDX-011 in locally-advanced or metastatic breast cancer.[5] As seen in melanoma patients, the 1.88 mg/kg dose was well-tolerated. Dose-limiting toxicity was limited to neuropathy. For all patients treated at the maximum dose level, tumor shrinkage was seen in 62% (16/26) and PFS was 9.1 weeks.[5] A subset of 10 patients had triple-negative disease, a more aggressive breast cancer subtype that carries a high risk of relapse and reduced survival, as well as limited therapeutic options due to the lack of overexpression of HER2/neu, estrogen and progesterone receptors. In these patients, 78% (7/9) had any tumor shrinkage, the 12-week PFS rate was 70% (7/10), and the median PFS was 17.9 weeks.[5]

Berthold Kreft (Bayer Schering Pharma) presented two ADC from the Bayer R&D pipeline. The first molecule was targeting the tumor metabolism-associated target carbonix anhydrase 9 (CA9). CA9 is a cell surface glycoprotein that is expressed in many types of carcinomas such as renal cell, esophagus, cervical, colon and non-small cell lung. Its expression on normal tissues is largely restricted to the apical surface of cells of the stomach, bile duct mucosa and small intestine. The CA9 gene expression is under the direct control of the transcription factor hypoxia-inducible factor-1 (HIF-1) and is significantly upregulated by

tumor hypoxia. The ADC drug BAY-79–4620 corresponds to a fully human parental anti-CA9 Mab (isolated from Morphosys' HuCAL library) coupled to the cytotoxic drug monomethylauristatin E (MMAE) via a pH-sensitive valine-citrulline linker based on Seattle Genetics' technology.[6] In about 10 different xenograft models in mice representing several tumor indications such as cervix, lung, colon, prostate, skin gastric, BAY-79–4620 showed potent anti-tumor efficacy[6] and in some models demonstrated partial and complete tumor shrinkage even following a single dose.[6] The mechanism of action was shown by histology to be typical of anti-tubulin agents.[6] Efficacy in murine preclinical models correlated semi-quantitatively with CA9 expression levels as determined by IHC and ELISA.[6] BAY-79–4620 entered a first-in-humans clinical study and was associated with severe adverse effects. Its clinical development is currently terminated.

The second ADC from Bayer's pipeline was BAY-94–9343, directed against mesothelin. Mesothelin, a glycoprotein expressed in mesothelial cells found in the membrane lining of the peritoneal and pleural cavities, is overexpressed in all mesotheliomas, as well as many ovarian and pancreatic cancers. Due to its limited expression on normal tissues and higher expression in a number of tumor types, mesothelin represents an attractive ADC target. BAY-94–9343 consists of a human anti-mesothelin IgG_1 antibody conjugated to the potent tubulin-binding drug DM4 (ImmunoGen) with an average of 3.2 drug molecules per antibody. The resulting ADC binds to human recombinant mesothelin with high affinity (K_d of 15 nM) leading to antigen-dependent internalization and potent cytotoxicity in tumor cells that express mesothelin, but not in mesothelin-negative cells. In vivo, BAY 94–9343 demonstrated dose-dependent, mesothelin-specific anti-tumor efficacy in subcutaneous and orthotopic xenograft models at doses between 2.5 and 10 mg/kg using a Q3Dx3 schedule. Endogenously expressing mesothelin tumor models included sc and orthotopic OVCAR3 (ovarian), sc BxPC-3 (pancreatic) and sc NCI-H226 (mesothelioma). Furthermore, in mesothelin-positive patient-derived preclinical tumor models of both platinum-resistant ovarian cancer and gemcitabine-resistant pancreatic cancer, BAY 94–9343 exhibited high anti-tumor efficacy leading to partial and complete tumor regressions at doses of 10 mg/kg. BAY 94–9343 was well tolerated in mice at 10 mg/kg (Q3Dx3) without any evidence of body weight loss, compared with either cisplatin or gemcitabine treatments. This compound started a first-in-men study in the last quarter of 2011 in patients with advanced solid tumors to evaluate its safety, tolerability, pharmacokinetics, pharmacodynamics and determination of its maximal tolerated dose.

Ulrik Nielsen (Merrimack Pharmaceuticals) discussed a network systems biology approach to expand on the importance of HER2 and homo- and hetero-dimers engaging it.[7] Network biology is an interdisciplinary approach to drug discovery and development that enables the development of functional and predictive computational models of biological systems based on quantitative, kinetic, multiplexed biological data.[7] Applying such an approach to the epidermal growth factors network, Merrimack Pharmaceuticals, Inc., has developed an albumin fusion protein that targets the epidermal growth factors ErbB2 (HER2) and ErbB3 (HER3).[7] These receptors are part of a complex molecular network whose activation is linked with cancer and can be associated with disease recurrence and poor prognosis for patients.[7] Overexpression or amplification of the epidermal growth factors is known to occur in several cancers, including lung, colon, breast, stomach, head and neck cancer. HER2 is overexpressed in ~25% of breast cancer patients, and primarily causes breast cells to reproduce uncontrollably. Treatment with trastuzumab is mostly associated with the formation of resistance at some stage and is also associated with cardiac dysfunction in 2–7% of cases.

MM-111 is a bispecific biomolecule containing two antibody arms fused to human serum albumin instead of the Fc fragment of an IgG; one arm binds to ErbB2 with high affinity and the other binds to HER3. HER3 is a neuregulin-binding receptor, but its intracellular tyrosine kinase domain is inactive. HER3 forms heterodimers with other EGF receptor family members such as EGFR and HER2. Heterodimerization leads to the activation of pathways which lead to cell proliferation or differentiation. Overexpression of HER3 has been reported in numerous cancers, including prostate, bladder and breast tumors. The bispecific format of MM-111 could be an optimal approach of inhibiting the enhancement of cell proliferation of HER3 in HER2-overexpressing tumors. Preclinical proof of concept has been obtained in xenograft models of HER2-positive tumors such as BT474 breast and N87 gastric cancer cell lines where MM-111 resulted in complete tumor regressions. In June 2009, MM-111 entered clinical development for several Phase 1/2 trials in HER2-positive breast cancer patients either as monotherapy or in combination with trastuzumab, taxanes or lapatinib.

The second topic of Dr. Nielsen's presentation focused on immunoliposomes (IL). IL provide a complementary, and in many instances advantageous, drug delivery strategy to antibody-drug conjugates.[8] Their high loading capacity (20,000–150,000 drug molecules/liposome) allows for the use of a substantially broader range of moderate-to-high potency small molecule drugs compared with the subnanomolar potency of maytansinoid and auristatin-based ADCs.[8] The multivalent display of 5–100 antibody fragments/liposome results in an avidity effect that can make use of even moderate affinity antibodies, as well as cross-linking of cell surface receptors to induce the internalization required for intracellular drug release and subsequent activity.[8] The liposomal drug must be engineered for long circulating pharmacokinetics and stable in vivo drug retention in order to allow the drug to be efficiently delivered to the target tissue.[8] Based on these concepts, MM-302 was designed as a nanotherapeutic encapsulation of doxorubicin (Dox) {1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] or DSPE-PEG with ~20,000 Dox/particle} decorated with antibodies targeting HER2. Computational mode of action studies were performed using Merrimack's network biology approach and predicted tumor deposition of Dox is a rate-limiting step rather than HER2 tumor expression. This IL approach is believed to retain the safety profile of marketed Doxil® (liposomal Dox), in particular its cardiac safety, and achieve better therapeutic efficacy in

HER2-positive tumors. MM-302 is currently in a Phase 1 study in patients with advanced HER2-positive breast cancer.

As the last speaker of this meeting, **Susan Lacy** (Abbott Laboratories) presented Abbott's BsAb platform based on the DVD-Ig scaffold (Dual Variable Domain ImmunoGlobulin). This format combines the antigen binding domains of two mAbs into a single entity by adding an additional binding domain to each variable domain of an Fv arm.[9,10] It preserves the dual functional specificities of the parental mAbs, but behaves like conventional mAbs in many aspects. DVD-Ig molecules can be made to a variety of target pairs (e.g., two soluble ligands, soluble/cell surface antigens, two cell surface antigens) and using different variable domains. The talk was focused on the discovery and preclinical evaluation of a DVD-Ig molecule targeting simultaneously the inflammatory cytokines IL-1α and IL-1β. These ligands are natural agonists of IL-1 receptor. An additional complexity in the IL-1 system is the existence of an endogenous antagonist, IL-1ra, which binds IL-1R without signaling, and therefore negatively regulates IL-1 function.[11] The ideal therapeutic strategy would be to block both IL-1α and IL-1β, but not IL-1ra. However, due to low sequence homology between the three members of the family, it has not been technically feasible to identify potent therapeutic agents with this ability.

To achieve all desired properties, a DVD-Ig approach was set-up to simultaneously inhibit IL-1α and IL-1β.[11] Two parental mouse antibodies directed against human (h) IL-1α (clone 9H10) and hIL-1β (clone 10G11) were used for the construction of several DVD-Ig molecules, by swapping the orientation of IL-1α and IL-1β-specific VH/VL domains, and using different linker lengths.[11] The selected isotype for the first generation of mouse-based DVD-Ig constant portions was mouse IgG2/kappa. Whereas the parental mouse MAbs showed picomolar binding affinities for their cognate antigens, a 5- to 10-fold reduced affinity was obtained within the DVD-Ig format,[11] nevertheless the remaining sub-nanomolar affinity was sufficient to progress the evaluation toward in vivo animal models. Two inflammation models were evaluated, namely an arthritis model (collagen-induced arthritis) and destabilization of the medial meniscus in mouse upon surgery as a pain model in osteoarthritis. Treatment showed strong efficacy, but highly frequent treatment schedule (3 times per week) was necessary to reach maximal target coverage and full efficacy. A humanized equivalent of the bispecific anti-IL1α/1β DVD-Ig was designed as a human IgG_1/kappa isotype. Two different IL1α binders and three different IL-1β binders were combined, as well as different linker motifs at the inner/outer variable domain junction. All molecules were evaluated for their affinity for both cognate targets and simultaneous binding to them using BIAcore analyses. Production of the selected DVD-Ig ABT-981 was scaled-up in CHO-K1; upon purification and formulation, solubility greater than 80 mg/ml was achieved. Pharmacokinetic evaluation was performed in rat and cynomolgus monkeys; serum half-life was 7.6 d and 10.5 d for a single intra-venous injection in rat and monkey, respectively, and was 8.4 d and 8.0 d for the subcutaneous route of administration. According to its favorable preclinical, PK/PD and safety profile, ABT-981 is moving to first-in-human studies.

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