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Antibody Engineering and Therapeutics Conference

The Annual Meeting of the Antibody Society, December 8–12, 2013, Huntington Beach, CA

Juan Carlos Almagro[1], Gary L Gilliland[2], Jamie Scott[3], James W Larrick[4], Andreas Plückthun[5], Trudi Veldman[6], Gregory P Adams[7], Paul WHI Parren[8], Kerry A Chester[9], Andrew Bradbury[10], Janice M Reichert[11,*], and James S Huston[12]

[1]Pfizer; Boston, MA USA; [2]Janssen Research & Development, LLC; Spring House, PA USA; [3]Simon Fraser University; Burnaby, BC Canada; [4]Velocity Pharmaceutical Development; San Francisco, CA USA; [5]Universitaet Zurich; Zurich, Switzerland; [6]AbbVie Bioresearch Center; Worcester, MA USA; [7]Fox Chase Cancer Center; Philadelphia PA USA; [8]Genmab; Utrecht, The Netherlands; [9]University College London; London, UK; [10]Los Alamos National Laboratory; Los Alamos, NM USA; [11]Reichert Biotechnology Consulting LLC; Framingham, MA USA; [12]Huston BioConsulting, LLC; Boston, MA USA

The Antibody Engineering and Therapeutics conference, which serves as the annual meeting of The Antibody Society, will be held in Huntington Beach, CA from Sunday December 8 through Thursday December 12, 2013. The scientific program will cover the full spectrum of challenges in antibody research and development, and provide updates on recent progress in areas from basic science through approval of antibody therapeutics. Keynote presentations will be given by **Leroy Hood** (Institute of System Biology), who will discuss a systems approach for studying disease that is enabled by emerging technology; **Douglas Lauffenburger** (Massachusetts Institute of Technology), who will discuss systems analysis of cell communication network dynamics for therapeutic biologics design; **David Baker** (University of Washington), who will describe computer-based design of smart protein therapeutics; and **William Schief** (The Scripps Research Institute), who will discuss epitope-focused immunogen design.

In this preview of the conference, the workshop and session chairs share their thoughts on what conference participants may learn in sessions on: (1) three-dimensional structure antibody modeling; (2) identifying clonal lineages from next-generation data sets of expressed V_H gene sequences; (3) antibodies in cardiometabolic medicine; (4) the effects of antibody gene variation and usage on the antibody response; (5) directed evolution; (6) antibody pharmacokinetics, distribution and off-target toxicity; (7) use of knowledge-based design to guide development of complementarity-determining regions and epitopes to engineer or elicit the desired antibody; (8) optimizing antibody formats for immunotherapy; 9) antibodies in a complex environment; (10) polyclonal, oligoclonal and bispecific antibodies; (11) antibodies to watch in 2014; and (12) polyreactive antibodies and polyspecificity.

*Correspondence to: Janice M Reichert;
Email: janice.reichert@landesbioscience.com
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Sunday December 8, 2013

Half-day pre-conference workshops on three-dimensional (3D) structure antibody modeling and on identifying clonal lineages from next-generation data sets of expressed V_H gene sequences will be held on Sunday December 8, 2013. The modeling workshop will be moderated by **Juan Carlos Almagro** (Pfizer, Inc and **Gary L Gilliland** (Janssen R&D, Inc. With the success of antibody-based therapeutics, protein engineering efforts are underway throughout the research community to produce efficacious biologics with the appropriate specificities, affinities, cross-reactivity, biological activities and biophysical properties required for developing successful therapies. The requirement for accurate 3D structures of antibodies is a critical aspect of this process. Protein crystallographic efforts are one approach for fulfilling this need, but, if time is short or crystallization is not fruitful, homology modeling is a viable alternative.

The 3D structure antibody modeling workshop will focus on the current state-of-the-art in antibody variable region modeling and the results of a second Antibody Modeling Assessment, following on from the first assessment. For the second assessment, sequences of 11 benchmark antibody F_V regions whose structures were determined at Janssen R&D and Ian Wilson's lab at The Scripps Research Institute, but were not yet deposited in the Protein Data Bank were provided to the modeling participants. These F_V regions were from diverse species and covered a broad range of antigen combining site conformations. The participants included teams from Accelrys Software, Inc Chemical Computing Group, Inc, Johns Hopkins University (Gray Lab), Astellas Pharma, Macromoltek, and Schrödinger. The sequences of the V-regions were also submitted to the Prediction of ImmunoGlobulin Structure (PIGS) web server to generate models for comparison. The resulting models were compared with the unreported crystal

structures by the assessment coordinators, then a second round of modeling of just the CDR-H3 was performed. In this exercise, the modeling groups were provided with the V-region structures without the coordinates for CDR-H3. This second effort was performed to determine if more accurate CDR-H3 models could be generated if the structural context was known. As before, these models were then compared with the crystal structures. The teams and coordinators met in June to review the initial results and plan the coordinated analysis that will be presented at this workshop.

The structure prediction methodologies, their strengths, weaknesses, and future plans will be highlighted and presentations will be given by **Marc Fasnacht** (Accelrys Software, Inc), **Johannes Maier**, (Chemical Computing Group, Inc), **Brian D Weitzner** (Johns Hopkins University), **Hiroki Shirai** (Astellas Pharma/ Osaka University), **Monica Berrondo** (Macromoltek) and **David A Pearlman** (Schrödinger). **Jinquan Luo** and **Alexey Teplyakov** (Janssen R&D, Inc) will provide an overview of the assessment evaluation strategy and a detailed summary of the results of the analyses of the models. Concluding remarks by Juan Carlos Almagro will provide an overview of progress from the first to this second assessment and challenges for the future. A breakout session will follow to allow hands-on demonstrations of the different modeling software systems and discussion. Poster presentations highlighting the assessment results, the structural data and the methods used in the evaluation of the models will be exhibited to help focus the discussion on current and future modeling approaches.

Jamie Scott and **Felix Breden** (Simon Fraser University) will moderate the workshop "Identifying Clonal Lineages from NextGen Data Sets of Expressed VH Gene Sequences," which brings together leaders in this area to discuss their approach to this problem, both in individual presentations and during a group discussion at the end of the session. **Felix Breden** will discuss phylogenetic approaches to analyzing clonal lineages. **Marie-Paule Lefranc** (IMGT, University of Montpellier) will present an algorithm for "cleaning up" NGS data, and new tools for assigning clonal lineage. **Thomas Kepler** (Boston University) will present Baysian statistical methods and software for identifying clonal lineages and the associated uncertainty of these reconstructions. **Paul Kellam** (University College of London) will compare different sequencing approaches and will provide a network approach to determining clonal lineages. **Duane Wesemann** (Harvard Medical School) will discuss his recent finding of B-cell repertoires developing in the gut mucosa and being shaped by the intestinal microbiome. **Jacob Glanville** (Distributed Bio) will discuss antibody lineage assignment using monozygotic twin studies. Finally, **Brian Briney** (The Scripps Research Institute) will present a suite of computational tools that include error-correction and linage-assignment tools. The discussion that follows will include the question, "How can the different approaches be compared to determine the superior one(s)?"; questions will also be taken from the audience.

Monday December 9, 2013

The Antibody Engineering and Therapeutics conference will open on Monday December 9, 2013 with four keynote presentations on system biology, systems medicine and systems immunology in a session chaired by **James Marks** (University of California, San Francisco). **Leroy Hood** (Institute of System Biology) will discuss a systems approach to studying disease enabled by emerging technology. He will present systems approaches to cancer and neurodegeneration, and systems strategies, including family genome sequencing and system-driven blood diagnostics, and emerging technologies that allow exploration of new dimensions of patient data space. Systems analysis of cell communication network dynamics for therapeutic biologics design will be discussed by **Douglas Lauffenburger** (Massachusetts Institute of Technology). Multi-variate analysis of the cells and molecules potentially involved in the execution and regulation of phenotypic behaviors are required to understand the effects of perturbations caused by the interactions of drugs with targets. Professor Lauffenburger will update conference attendees on his lab's pursuit of "in vivo systems biology" approaches to meet this challenge.

After a networking refreshment break, **David Baker** (University of Washington) will describe use of the Rosetta computational design methodology to design ultra-stable protein scaffolds, high affinity small molecules and proteins, and self-assembling nanocages. In the final talk of the session, **William Schief** (The Scripps Research Institute) will discuss epitope-focused immunogen design. He will illustrate how computational, in vitro screening and other methods can be employed to design immunogens intended to induce antibodies against structural epitopes. These strategies may be important for next-generation vaccine and antibody development.

In the afternoon, conference attendees will then have a choice between talks in a session on antibody deep sequencing and repertoires chaired by **Dennis R Burton** (The Scripps Research Institute) and a session on antibodies in cardiometabolic medicine chaired by **James W Larrick** (Panorama Research and Velocity Pharmaceutical Development). In Professor Burton's session, **William Robinson** (Stanford University; Atreca, Inc) will discuss the Immune Repertoire Capture™ process, which uses barcoding and next-generation sequencing to generate paired antibody heavy and light chains expressed by individual B cells. He will present data from the application of this process to the identification of high affinity and functional antibodies produced in infection, vaccination and cancer. Immune sequencing technologies for pairing heavy and light antibody chains will be discussed by **Francois Vigneault** (AbVitro, Inc). Coupling of large-scale antibody repertoire studies with functional analysis of specific antibodies for which the antiviral properties are known can facilitate detailed understanding of naturally-occurring human immune responses following infection or vaccination. **James Crowe Jr** (Vanderbilt University Medical Center) will discuss how high throughput amplicon nucleic acid techniques can be used to provide a window into the complex development of human antibody repertoires.

Following the afternoon networking break, Professor Burton's session will continue with a presentation by **Jiang Zhu** (The Scripps Research Institute). "Antibodyomics," a combination of deep sequencing and bioinformatics, has emerged as a powerful

new approach for antibody analysis. Professor Zhu will discuss how this approach was used to identify broadly neutralizing HIV-1 antibodies from a donor sample with uncharacterized antibody status, and reveal details of temporal antibody response in animal experiments and vaccine trials. An analysis of clonal dynamics, i.e., the ways in which B cell clones expand, contract, diversify and interact, will then be presented by **Thomas Kepler** (Boston University). Professor Kepler will describe mathematical tools for the analysis of data from high throughput immunoglobulin gene sequencing and discuss results from longitudinal studies of clonal dynamics in human vaccines.

Cardiometabolic disorders are among the most prevalent causes of morbidity and mortality in modern medical practice. Although some of the first diagnostic and therapeutic antibodies (e.g., those targeting digoxin or GPIIb/IIIa) addressed problems in this area of medicine, very few successful targets have been identified to date. Dr Larrick's session on antibodies in cardiometabolic medicines will focus on recent work that is may lead to major therapeutic advances.

First, **Simon Jackson** (Amgen) will describe progress with anti-proprotein convertase subtilisin/kexin type 9 (PCSK9) antibodies to treat hypercholesterolemia. PCSK9 is an excellent example of a highly validated target, with human polymorphisms including gain-of-function and loss-of-function mutations that lead to both high and low plasma low density lipoprotein-cholesterol (LDL-C) phenotypes. Early work to understand the mechanism of action of PCSK9, the discovery of antibodies that prevent the LDL receptor interaction of PCSK9 and lower plasma LDL-C and early clinical development of AMG 145 will be discussed.

Alexei Y Bagrov (National Institute on Aging, NIH) will then describe anti-marinobufagenin (MBG) antibodies. MBG, a digitalis-like cardiotonic steroid, is implicated in cardiovascular and renal fibrosis in preeclampsia, chronic renal failure and diabetes mellitus and acts via inhibition of Fli-1, a negative regulator of collagen synthesis and by activation of TGF-β signaling. Monoclonal anti-MBG antibody reverses cardiac and renal fibrosis in experimental diabetes mellitus and chronic renal failure.

Knut Pettersson (Athera Biotechnologies) will update work on phosphorylcholine (PC) antibodies for treatment of cardiovascular disease. PC is a "danger signal" that is recognized by several receptors of the innate immune system, including natural IgM PC antibodies. Low levels of these antibodies are associated with increased risk for cardiovascular disease (CVD). This presentation will describe the development of a fully human monoclonal antibody to be used as secondary prevention in CVD patients with low levels of the natural anti-PC.

Oxidation of LDL is a crucial step in the development of cardiovascular disease. **Shijie "Chris" Li** (Genentech) will describe a novel mechanism of immune complex formation and Fc-γ receptor engagement, and an antibody targeting oxidized LDL (oxLDL). The antibody is anti-inflammatory on innate immune cells via modulation of NFκB activity. Administration of the anti-oxLDL antibody in diet-induced obese monkeys resulted in decreased pro-inflammatory cytokines, increased T-cell responsiveness and improved insulin sensitivity without changing food intake, thus identifying this agent as a potential therapy for reducing inflammation in diabetic conditions.

Kai Y Xu (University of Maryland) has discovered potent inotropic antibodies with powerful physiological effects on cardiac contractility through activation of the (Na^+K)-ATPase, without altering intracellular Na homeostasis and inducing reverse-mode Na/Ca-exchanger activity. Dr Xu's presentation will highlight this novel mechanism, which suggests important advantages of using inotropic antibodies over conventional approaches and may lead to better and safer treatment of heart failure.

Finally, **Ke Cheng** (Cedars-Sinai Medical Center) has developed a novel platform technology whereby two cells can be linked by a nanoparticle composed of an iron core studded with antibodies. The two cells are brought together with therapeutic intent. The therapeutic effect can be amplified by magnetic attraction of the nanoparticle to a target tissue of interest; the iron core also enables visualization by magnetic resonance imaging. The particle can be applied in conjunction with stem cell infusion to enhance exogenous cell homing or applied alone to capture endogenous stem cells in the blood and concentrate them in the injury zone. The particle may also be used as a functional MRI contrast agent.

Tuesday December 10, 2013

The conference's parallel track format continues until Thursday afternoon. On Tuesday December 10, 2013, a morning session on the interface between monoclonal antibody therapy and cellular immunity in cancer will be chaired by **K Dane Wittrup** (Massachusetts Institute of Technology) and a parallel session on the effects of antibody gene variation and usage on the antibody response will be chaired by **Jamie Scott** (Simon Fraser University).

In Professor Wittrup's session, **Glen Dranoff** (Dana Farber Cancer Institute) will focus on analyses that delineate the mechanisms of therapeutic immunity. He will present studies that highlight the coordinated development of potent cytotoxic T cell and functionally important antibody responses for immunotherapy-triggered tumor destruction. Cetuximab has been found to induce cross-talk between natural killer (NK) cells and dendritic cells, which promotes cellular activity that can lead to tumor antigen spreading and T-helper cell (Th1) cytokine release. The ways in which cetuximab-activated NK and dendritic cells collaborate to trigger tumor antigen-specific T-cell immunity in head and neck cancer patients will be the topic of a presentation by **Robert Ferris** (University of Pittsburgh Cancer Institute).

Following the morning networking break, **Jean-Luc Teillaud** (Cordeliers Research Center) will continue the session with a presentation on deciphering adaptive anti-tumor immunity induced by anti-CD20 mAb treatment. Professor Teillaud will show that the balance between Treg and Th1 compartments, which is essential in the control of tumor progression, is fine-tuned by a therapeutic antibody that enables a switch of the

immune response from a pro- to an anti-tumor response. **Yang-Xin Fu** (University of Chicago) will discuss study results for an antibody that has been armed with interferon. This strategy controls antibody resistance by re-activating and re-bridging suppressed innate and adaptive immunity. Manipulating antibody-dependent cell-mediated cytotoxicity (ADCC) to promote anti-tumor responses will be discussed by **Louis Weiner** (Georgetown Medical Center). In particular, he will discuss approaches to effectively manipulate the tumor microenvironment to promote ADCC-initiated antigen presentation and host-protective adaptive immunity.

The alternate session chaired by Professor Scott on Tuesday morning will focus on the effects of antibody gene variation and usage on the antibody response. **Corey Watson** (Icahn School of Medicine at Mt. Sinai) will present an overview of the *IgH* locus (which encodes the antibody germline VH genes and the other components of the immunoglobulin heavy chain), and will discuss the role the huge diversity observed at this locus plays in diversity of the antibody repertoire across populations. **Chunguang Gao** (Harvard Medical School) will discuss the role of a *cis*-acting element in regulating VH-DH-JH recombination, and in balancing VH gene usage. **Ann Feeney** (The Scripps Research Institute) will present mechanisms that may control VH gene usage, discovered by determining the frequency of usage of each germline VH gene by murine pro-B cells (which have a recombined VH genes at the IgH but not a recombined light chain genes at the kappa or lambda loci), and associating these frequencies with the genetic elements surrounding each germline VH gene. **Ignacio Sanz** (Emory University School of Medicine) will discuss the VH4–34 germline gene, which encodes autoreactive antibodies that are censored in healthy individuals; he has used the 9G4 antibody to detect a high frequency of B cells bearing these antibodies among B-cell populations in SLE and HIV infection. **Kevin Henry** (Simon Fraser University) will present an analysis B-cell subsets and the antibodies they produce in HIV infection and SLE, showing differences in the immunogenetic milieu between the two states. Finally, **Wayne Marasco** (Harvard Medical School) will present an analysis of VH gene usage by neutralizing antibodies against the influenza hemagglutinin stem region, the target of a "universal" flu vaccine, discussing novel mechanisms underpinning the observed gene bias.

On Tuesday afternoon, **Andreas Plückthun** (University of Zurich) will chair a session on directed evolution. This session also serves as a tribute to the late Pim Stemmer. Antibody engineering would not be where it is today without directed evolution. In fact, the immune system is the mother of all adaptive systems that use an evolutionary strategy to achieve a goal function: in this case, specific binding. Researchers have first tried to understand, then to imitate, and finally to generalize these strategies in the laboratory. Moreover, the need to work with difficult antigens for therapeutic projects has made it necessary to also apply evolutionary methods to the antigens in order to study and manipulate them.

Nature has exemplified ways and means of creating an initial library with very sparse requirements for the length of DNA needed (the antibody V,J and V,D,J system of "genes" that only together form a full domain, the insertion of diversity at the junction by polymerases and exonucleases, the use of two chains for the final binding site). The B- and T- cell development is a poster child of a Darwinian selection system that works not in historic time scales, but in days and weeks.

But there is more: antibodies have the ability of somatic hypermutation. **Michael Neuberger** (Medical Research Council, Cambridge, UK) will show how he studies this in ways that allow him to develop systems to recapitulate this process in artificial systems to create rapid and flexible protein maturation strategies.

The session as a whole, and particularly its line-up, will pay tribute to Willem ("Pim") PC Stemmer, a pioneer of directed evolution who passed away last year. Of the many contributions he made to the field while working as a research scientist at Affymax, the idea of recombination, or "gene shuffling" in his words, is probably most widely applicable, e.g., to antibodies, novel enzymes and many biocatalytic processes. This approach formed the basis of the subsequent founding of Maxygen. The discovery and success of gene shuffling led to Pim Stemmer being awarded the Charles Stark Draper Prize, the United States' top engineering honor, along with **Frances Arnold** (California Institute of Technology), in 2011. Professor Arnold is equally a pioneer in this field, and she has applied directed evolution to many protein and enzyme systems, achieving feats of molecular improvement that are amazing and useful at the same time, and have yielded deep insight into molecular sciences. It will be exciting to hear the most recent advances in this rapidly moving field, which will be presented in this session.

Dr Stemmer had most recently been CEO of Amunix, a company he co-founded with **Volker Schellenberger** (Amunix). The latter will discuss use of the company's technology to create fusion proteins consisting of a peptide or folded protein fused to a natively unfolded, hydrophilic tail, called XTEN, which increases the half-life of otherwise short-lived protein products. This is an issue well-known to all researchers involved with the in vivo use of antibody fragment and other protein scaffolds.

Some of the most important pharmaceutical targets for drug screening and antibody selection are G-protein-coupled receptors (GPCRs). Their in vitro handling and structure determination by crystallography and NMR, however, are hampered by the inherent instability of most of these targets when solubilized in detergent micelles. The lab of **Andreas Plückthun** (University of Zurich) has recently developed directed evolution technologies that allow generation of GPCR variants with up to 60-fold improved functional expression levels in *E. coli* and superior stability in detergent solution. The approach has allowed determination of several X-ray structures of evolved signaling-competent constructs directly from *E. coli*, and may form the basis of greatly facilitating selection of agonistic or antagonistic ligands, proteins and antibodies

The ultimate dream is to have evolution run continuously, as long as needed for achieving the set goal. **David R Liu** (Harvard University) will present his elegant system, termed Phage-Assisted Continuous Evolution (PACE), which is an important step along this way. Professor Liu's work shows the

interdisciplinary nature of innovations in this field. In total, Professor Plückthun's session brings together very advanced protein engineering concepts, and will outline where the field stands and where it is heading.

Also on Tuesday afternoon, **Trudi Veldman** (AbbVie) will chair a session on antibody pharmacokinetics (PK), distribution and off-target toxicity. Tailoring PK, efficacy and mechanism of action is critical for biologics differentiation. Whether the aim is to target tumors with naked antibodies or antibody-drug conjugates, and whether the target is in the periphery or in the brain, this session provides insights into different approaches to improve exposure of the protein therapeutic while engaging the target with optimized efficacy and specificity. In addition, the success of a therapeutic protein drug depends on multiple features, including target affinity, specificity, mechanism of action and, as has become more the focus recently, good drug-like properties and in vivo behavior. Understanding which features contribute to the PK properties of a protein drug and learning ways to modulate these properties through engineering is the topic of this relevant session.

Lubna Abuqayyas (Amgen) will present investigations of the PK, tissue and tumor distribution of therapeutic IgG, including the role of FcR and FcRn on IgG uptake in the brain and the effects of co-administration of agents that alter the tissue vasculature on PK and tumor uptake of anti-cancer antibodies. **Jean Lachowicz** (Angiochem Inc) will discuss data from studies with a peptide-antibody conjugate that uses receptor-mediated transcytosis across the blood-brain barrier for the treatment of brain tumors.

The antibody and antigen interaction in the environment of the endosomal compartment is a determining factor in the fate of both the antibody and the antigen. Recycling antibody with pH-dependent antigen binding results in extended serum exposure. **Naoka Hiromiwa** (Chugai Pharmaceutical Co Ltd) will discuss calcium-dependent antigen binding and its effect on antibody recycling in vivo. Several studies have shown that the fast turnover of certain target antigens can be altered by binding to antibodies. These long-lived antigen/antibody complexes accumulate and can form a depot of antigen that can cause exacerbation of disease. **Srinath Kasturirangan** (MedImmune) will describe a bispecific antibody to IL-6 that effectively clears the cytokine from the circulation. Antibody-drug conjugates (ADCs) have emerged as an efficient modality to deliver cytotoxic drugs to tumor cells. Understanding the disposition and PK profile of ADCs is crucial to their development. **Brooke Vandenbrink** (Amgen) will present data on the effect of linker structure and chemistry on in vivo ADC integrity and stability.

The exquisite specificity of antibodies is generally considered an advantage with respect to safety; however, several antibodies that cross-react with apparently structurally unrelated plasma proteins have been reported. **Stefan Barghorn** (AbbVie) will describe the biochemical identification of an unexpected cross-reactivity of an anti-amyloid β antibody that resulted in preclinical toxicity in monkeys.

Wednesday December 11, 2013

On the morning of Wednesday December 11, 2013, attendees will have a difficult choice between a session on knowledge-based design, which will be chaired by **Gregory P Adams** (Fox Chase Cancer Center), and a session on optimizing antibody formats for immunotherapy, which will be chaired by **Paul WHI Parren** (Genmab).

In Dr Adams' session, discussion will center on guided development of CDRs and epitopes to engineer or elicit the desired antibody. To be effective clinical agents, antibodies must bind to epitopes on target proteins where they can block or mimic interactions with ligands, engage immune effector cells and immune responses or trigger a change in the status of the target, such as inducing internalization to decrease the concentration of a critical receptor or facilitate the delivery of a cytotoxic agent. While numerous antibodies have been developed using classic immunization strategies or more recently developed approaches such as selections from phage or yeast-based libraries, many fail to target the critical functional epitopes and therefore fail to realize their full potential. This session, coupled with the keynote presentations by William Schief and David Baker, will explore emerging strategies that may enable the development, or induction, of antibodies or related molecules that are specific for desired epitopes that are highly conserved or poorly immunogenic.

In the first presentation, Dr Adams will use a project from his laboratory focused on the goal of developing functional antibodies specific for the Müllerian Inhibiting Substance Type II Receptor to illustrate the hurdles of developing antibodies specific for highly conserved targets using existing technologies, and introduce how these limitations may be overcome using knowledge-based design.

Roland Dunbrack (Fox Chase Cancer Center), who collaborated with Dr. Adams in the work described in the first talk, will then describe his group's recent efforts to study the conformation of antibody CDRs and organize them into conformational clusters that can be predicted from sequence and CDR length. The resulting database is an extremely useful tool that can be employed in the design and development of antibodies with highly predictable structures, thereby bringing us closer to the ultimate goal of ab initio antibody design.

The latest developments in Rosetta-based approaches for antibody structure prediction and docking will then be presented by **Jeffrey Gray** (Johns Hopkins University). His talk will focus on CDR H3 loop structure, comparing H3 loops with those of other proteins and describing how H3 properties help predict their structure. New loop prediction methods will be discussed, including the use of homology models to accurately guide the design and development of CDR loops that dock to desired target epitopes.

Stephen Demarest (Lilly Biotechnology Center) will present the use of a strategy combining computational and rational design approaches with directed engineering to develop novel bispecific and multispecific antibody platforms that have defined geometries and valencies and exhibit high stability. Applications

enabled by these designed molecules will also be discussed. **David Pearlman** (Schrödinger) will focus on approaches for improved antibody design using structure-based analysis and computation. This approach has been particularly successful in predicting the structure of the critical CDR H3 loop from its amino acid sequence when an acceptable model of the remainder of the CDR is available. Recent studies that validate this approach and identify residues that represent mutational hot spots will be described.

Dr Parren's session brings together a number of experts who will discuss their exciting new solutions for improving antibody products and clinical utility by the application of distinct antibody biology, engineering and translational science approaches. The optimization of antibody formats to increase potency or enhance specific functions represents a rapidly evolving area in the therapeutic antibody field. Despite the great success of antibodies in a wide range of therapeutic areas, there is a continuous drive for improvement. From the perspectives of patients and clinicians, there is a strong need for new therapies that induce more durable clinical responses. From the regulatory authorities' and payers' perspectives, there is pressure for the development of new therapeutics that give meaningful improvements over existing treatments. From the industry perspective, many therapeutic areas are becoming very crowded, resulting in reduced market share that is exacerbated by competition with biosimilar products following patents expiry. To face this future, the antibody field needs to develop ideas and strategies for new products with leapfrog potential.

William Strohl (Janssen R&D) will discuss the inherent protease sensitivity of IgG1 antibodies, which represents the isotype of most antibodies approved for human use. It has been found that the IgG1 hinge can be cleaved by proteases produced by microorganisms or invasive tumors, leading to loss of effector function while retaining antigen binding with suboptimal in vivo activity as a result. Knowledge gained from IgG2 biology elegantly combined with Fc function engineering yielded a novel protease-resistant IgG platform with great potential for increasing antibody activity in proteolytic microenvironments.

Peter Sondermann (SuppreMol GmbH) will describe several strategies to exploit the immune modulatory role of IgG Fc receptors for the treatment for autoimmune diseases. An antibody targeting FcγRIIb, the inhibitory IgG Fc receptor, demonstrated reduced immune activation in a mouse systemic lupus erythematosus model. Alternatively, the concept of using soluble FcγRIIb to compete with activating IgG Fc receptors for immune complex binding was assessed in a clinical Phase 1b/2a study in immune thrombocytopenic purpura.

George Georgiou (University of Texas at Austin) pioneered the display of whole aglycosylated antibodies in *Escherichia coli*, which enabled the engineering of IgGs with an ability to efficiently and selectively interact with IgG Fc receptors. Inceased phagocytosis of opsonized tumor cells was demonstrated for an aglycosylated IgG1 mutant with a strongly increased FcγRIIa/FcγRIIb binding ratio. The platform opens novel possibilities for selecting therapeutic antibody variants with highly specific tailored functions.

Ronald Taylor (The University of Virginia School of Medicine) will provide important insights from studies on therapeutic type I CD20 antibodies that utilize Fc-mediated functions as their principal mechanisms of action. The data strongly suggest that excessive engagement of such mechanisms could lead to the exhaustion of effector reservoirs, resulting in suboptimal cell killing. Notably, this cautions against the more-is-better and maximally-tolerated-dose regimens often sought in clinical development. The findings provide a general foundation for the design of optimal treatment regimens for antibody formats that rely on recruiting innate immune functions for therapy.

Janine Schuurman (Genmab) will discuss a study that reveals the molecular mechanism of complement activation by IgG antibodies at the surface of cells. This knowledge was exploited to inhibit, as well as to enhance, the ability of IgG antibodies to induce complement-mediated tumor cell lysis. This led to the development of a broadly applicable antibody platform, termed HexaBody, which allows the creation of novel therapeutics with increased potency.

Tariq Ghayur (AbbVie) will discuss the discovery and development of an IL1α/β bispecific antibody from the dual-variable domain immunoglobulin (DVD-Ig) platform, which contains two antigen-binding units in each antibody arm with a potential for multivalent binding. Bispecific antibodies provide a challenge and opportunity to identify applications where the simultaneous engagement of two antigens synergize to induce superior effects.

Dr Parren's session can provide only a small sample of the seemingly endless opportunities provided by antibody formats with increased function. The explosion of antibody biology, discovery and development knowledge that accompanies them provides confidence that this work will contribute to the ultimate goal of further increasing clinical efficacy and utility of antibody therapies.

In the afternoon of Wednesday December 11, 2013, **Kerry A. Chester** (University College London) will chair a session on antibodies in a complex environment, and **Andrew Bradbury** (Los Alamos National Laboratories) will chair a session on polyclonal, oligoclonal and bispecific antibodies.

Professor Chester's session will explore the roles of antibodies acting in a complex environment, with emphasis on antibody interactions with receptors. She will begin with a tribute to Phillip Thorpe, Professor of Pharmacology at the University of Texas Southwestern Medical Center, who died earlier this year. A brilliant translational scientist, developing antibody-based therapeutics for the clinic, Phillip had many achievements and insight, including the discovery that phosphatidylserine can serve as a target in cancer blood vessels.

The first speaker, **Sally Ward** (University of Texas Southwestern Medical Center), will present her latest work in unravelling complexity and developing understanding in the field of neonatal Fc receptor (FcRn) targeting. FcRn serves multiple functions through its ability to transport antibodies within and across cells. The presentation will elucidate the dynamic behavior of FcRn within cells using fluorescence microscopy. The studies are combined with the development of FcRn inhibitors for the treatment of antibody-mediated autoimmune diseases.

Sergio Quezada (University College London) will present novel mechanistic insights on the role of antibody/Fc receptor interactions. He will explain how the delicate immunological balance in cancer, characterized by the dominant infiltration of regulatory T cells (Treg), is modified by antibodies against CTLA-4, a key immune modulatory receptor. Subsequent tumor rejection depends on the depletion of tumor-infiltrating Treg cells expressing high levels of CTLA-4. The presentation will demonstrate that this depletion is driven by FcγRIV expression on tumor infiltrating myeloid cells. The work highlights how antibody/Fc Receptor interactions within the complex tumor environment can influence the final outcome of antibody-based immune-modulatory therapies.

Holbrook Kohrt (Stanford University) will explain how stimulation of natural killer (NK) cells within the tumor milieu can influence the efficacy of established antibodies such as rituximab, trastuzumab, and cetuximab. Professor Kohrt's presentation demonstrates how the antitumor efficacy of these mAbs, due in part to antibody-dependent cell-mediated cytotoxicity (ADCC), can be enhanced by stimulation of NK cells with an anti-CD137 agonistic mAb. CD137 is a costimulatory molecule expressed on a variety of immune cells, including NK cells, following activation. Understanding these systems could lead to significantly improved cancer therapies.

The development of antibodies to peptide/MHC complexes offers significant therapeutic options because this unique class of markers presents peptides from intracellular proteins on the cell surface; theoretically making the whole proteome amenable to treatment with antibodies and offering unsurpassed specificity for targeting cancerous and infected cells. **Jon Weidanz** (Texas Tech University Health Sciences Center) will present his work in developing a T cell receptor mimic antibody (TCRm) targeting the Her2-HLA-A2 complex and other peptide-MHC complexes. Data will be reported that describes an integrated approach to immunotherapy, combining innovative technologies to discover and target disease-specific peptide/HLA class I complexes.

The modulation of immune responses by therapeutic antibodies has shown increasing therapeutic potential since the first clinical proof of concept was achieved using antibodies targeting check-point modulators. Other breakthrough approaches using bispecific antibody-like molecules engaging T cells also show great promise in the clinic. **Thierry Wurch** (Institut de Recherches Servier) will illustrate this theme of the immune regulatory properties of therapeutic antibodies with two case studies: one dealing with the discovery and preclinical evaluation of a novel antibody targeting the checkpoint ligand B7-H3; the second featuring a novel format of bispecific antibody-like molecules termed DARTs.

In the final presentation of Professor Chester's session, **Carl June** (University of Pennsylvania) will demonstrate the clinical power and complexity of adoptive T cell transfer, using engineered T cells with chimeric antigen receptors (CARs). Adoptive T cell transfer for cancer and chronic infection is an emerging field that shows promise in recent trials. Professor June will show how synthetic biology-based engineering can overcome immune tolerance, which has been a major limitation of immunotherapy-based strategies. He will explain how advances in cell engineering and cell culture approaches have facilitated broader evaluation of this technology, moving adoptive transfer from a "boutique" to the cusp of a mainstream technology.

Natural IgG antibodies have two binding sites, and the immune response to infection is polyclonal, whereas most recombinant therapeutic antibodies are monoclonal. Dr. Bradbury's session will explore the advantages of increasing the numbers of antibody binding sites in different contexts. In the first presentation, **Andrew Bradbury** (Los Alamos National Laboratories) will discuss the generation and use of recombinant renewable polyclonal antibodies as research reagents. These combine the advantages of polyclonals (multiple antibodies recognizing different epitopes usable in different assays) with those of monoclonals (renewable, specificity). The use of phage and yeast display allows properties of the polyclonals to be easily improved, including the subtraction of undesirable specificities or the addition of desirable ones. The advantages of defined oligoclonal antibody mixtures in therapy for the treatment of toxin poisoning caused by *Clostridium botulinum* and *Clostridium difficile* will be discussed by **James Marks** (University of California, San Francisco) and **David Humphreys** (UCB), respectively. In both cases, the use of defined antibody mixtures is more effective than monoclonal treatments, and in the case of botulism, also more effective than the currently used anti-toxin produced in horses. In bispecific antibodies, different specificities are encoded within the same molecule. **Robert Mabry** (Adimab), **Nicolas Fischer** (Novimmune) and **Johan Rainey** (MedImmune) will describe different variants, uses and formats of bispecific antibodies. Dr. Mabry will describe the use of yeast display to generate functional bispecific antibodies with clinical utility. Dr. Fischer will describe an approach in which the heavy chain is varied, but the light chain is kept constant. This approach allows the creation of bispecifics that use two different heavy chains, but identical light chains. Finally, Dr. Rainey will describe the use of a novel scFv-based bispecific antibody format for the treatment of *Pseudomonas aeruginosa*.

Wednesday's program will conclude with The Antibody Society's special session. The Society serves to further the broad interests of the antibody and novel binder community by organizing meetings, pursuing antibody-related therapeutic initiatives, and supporting our next generation of scientists. In particular, the Society serves as scientific sponsor of the Antibody Engineering and Therapeutics conference. The transitions of antibody therapeutics to late-stage clinical development, regulatory review and the market occurred at a rapid pace in 2013. In her presentation "Which are the Antibodies to Watch in 2014?," Society President **Janice M. Reichert** (Reichert Biotechnology Consulting LLC, Editor-in-Chief of *mAbs*) will provide an overview of recently approved antibodies, and discuss candidates that may be approved in 2014 and those currently in Phase 3 studies. Dr. Reichert will also update attendees on the Society's activities.

Thursday December 12, 2013

On the final day of the meeting, concurrent sessions will be held in the morning, but a single session will be featured in the afternoon. To start the day, **James S Huston** (Chairman, The Antibody Society; Huston BioConsulting, LLC) will chair a session on polyreactive antibodies and polyspecificity, and **Ian M Tomlinson** (GlaxoSmithKline) will chair a session on great antibody discovery platforms of the past, present and future.

In only a few years, the revolution in creation of affordable large-scale proteomics and genomics, coupled with progress in systems immunobiology has turned this area of humoral immunity into one of great activity. Dr Huston's session will feature leaders in the field of polyreactive and polyspecific antibodies, whose research illuminates their fundamental properties. This research is generating remarkable insights into the immune system and creating opportunities for their application to fundamental problems in medicine and antibody-based diagnostics and therapeutics.

Binding sites of very low affinity can still retain specificity, but the immune system functions with a vast array of antibody binding sites, comprising a sizeable population of binding sites with both low affinity and diverse but weak cross-reactivity with different antigens. This combination of properties creates technical challenges and opportunities for antibody engineering based on their biophysical and thermodynamic properties. In the first presentation, **James S Huston** will address these aspects of variable domains, F_v regions, the single-chain F_v, and designed single scaffold binder platforms.

Antibody polyspecificity and autoreactivity pose inherently difficult problems for characterization and for understanding their implications in immunology and medicine. **Jamie K Scott** (Simon Fraser University) will place these issues in the context of immunology and medicine, as well as discuss her research on how details of polyspecificity assays can affect the validity of their results. Her discussion places the other talks of this session in perspective, as the following presentations expand upon the immunogentics of these antibodies, and how microarray technology/systems immunobiology are being applied to polyspecific and polyreactive antibodies.

The combination of deep sequencing with computational and structural biology has allowed **James Crowe Jr** (Vanderbilt University Medical Center) and his colleagues to discover that polyspecific antibodies are encoded by human germline antibody genes. Professor Crowe will discuss how the genetic and structural basis for polyspecificity leads to understanding the process of creating antibody diversity in the mature antibody repertoire, based in part on unexpected principles. For the immune system, polyreactive antibodies emerge as key vehicles for building the antibodies of high affinity and exquisite specificity that have been the focus of research for the past century.

We often draw parallels between the human immune system and large antibody display libraries; however, as therapeutic antibody research has accelerated, the practical and fundamental problem of developability has become a serious issue. The complexity of polyspecific antibodies can mask poor properties until late in the development process of a therapeutic antibody. Such issues are very costly in resources needed for process development and may cause termination of a therapeutic candidate, even late in preparations for clinical studies. **Eric Krauland** (Adimab) has led a large team at Adimab to solve the problems created by polyspecific antibodies in display library selections. Using yeast-IgG display libraries, where selections are conducted by fluorescent-activated cell sorting (FACS), they have devised an elegant upstream approach that provides for sensitive assay and quantitative FACS depletion of polyspecific antibodies from libraries that allows the FACS selection process to produce significantly greater yields of IgG antibody candidates with superior developability.

Peptide binding arrays have been widely used as a method of characterizing polyspecificity. **Stephen Albert Johnston** (Arizona State University) is a leader in creating microarray-based immunosignatures, which he has now extended from immunology to medicine. He has created a computer-chip based system to produce microarrays that provide a universal diagnostic platform. It holds promise for systems medicine and provides a continuous monitoring platform for early disease detection that could lead to improved outcomes and reduced medical costs.

Eshel Ben-Jacob (Tel Aviv University) is a physicist whose pioneering work in network theory led him to the challenge of the immune system and network systems immunobiology. He has used large- scale peptide array data to characterize human immune networks. Professor Ben-Jacob will describe his research with microarray data subjected to refined mathematical analysis to monitor the features of the polyspecificity in immune networks. He will also discuss the implications of using this approach to dissect the interplay between a patient's immune repertoire and carcinogenesis.

As an alternative to microarrays, **Fridtjof Lund-Johansen** (Oslo University Hospital) has developed antibody array analysis using labeled antibody microspheres, coupled with measurement of protein size, to define specificity. The use of flow cytometry is known to be significantly more sensitive than ELISA detection methods, and this approach consequently offers an accessible alternative for the definition of immunosignatures that may prove of special value.

The parallel session chaired by Dr Tomlinson on Thursday morning will focus on antibody discovery platforms used to produce a substantial number of the antibodies currently in clinical development, e.g., display technologies and transgenic mice, and those that may serve this purpose in the future. **Jane Osbourn** (MedImmune) will describe phage display and how phage selection was used to produce adalimumab (Humira®), which had global sales over $9 billion in 2012. Yeast display, which has evolved from an academic research tool to an integrated drug discovery platform, will be discussed by **K Dane Wittrup** (Massachusetts Institute of Technology). **Nils Lonberg** (Bristol-Myers Squibb) will describe how transgenic mice with germline-configuration human Ig genes were developed and used for drug discovery. The selection and formatting of domain antibodies for therapeutic use will be discussed by **Laurent Jespers** (GlaxoSmithKline). **Bo Yu** (Larix Bioscience) will then inform attendees how antibody membrane switch technology can streamline library screening

and cell line development. In the final presentation of the session, **Geoff Yarranton** (KalosBios Pharmaceuticals, Inc) will discuss optimizing antibody therapeutics using target/epitope selection, Humaneering® and in vivo biology.

The 2013 Antibody Engineering and Therapeutics conference will conclude with a session on ADCs chaired by **Louis Weiner** (Georgetown University Medical Center). **Peter Senter** (Seattle Genetics) will provide an overview of advances in the ADC field e.g., novel classes of potent small molecules, alternate conjugation methodologies, that can be used to generate homogeneous and highly active ADCs. Perspectives on expanding the reach of ADCs through new linker and effector chemistry will be shared by **John Lambert** (ImmunoGen, Inc). **Tom Hawthorne** (Celldex Therapeutics) will provide a case study on the development of CDX-011 for the treatment of breast cancer and melanoma. Following a networking refreshment break, **Edward Reilly** (AbbVie) will discuss ABT-414, an anti-EGFR ADC in early phase clinical studies of patients with EGFR-expressing refractory metastatic tumors. The process of building next-generation antibody therapeutics with an improved therapeutic index using site-specific ADCs will be discussed by **Jagath Reddy Junutula** (Genentech, Inc). Finally, **Henry Lowman** (CytomX Therapeutics, Inc) will discuss how Probodies, which are masked antibodies that are activated locally in diseased tissue, can expand the therapeutic index of antibodies and ADCs.