Antibody Engineering & Therapeutics 2015: The Antibody Society’s annual meeting
December 7–10, 2015, San Diego, CA

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Antibody Engineering & Therapeutics, the annual meeting of The Antibody Society, will be held in San Diego, CA in early December 2015. In this meeting preview, the chairs provide their thoughts on the importance of their session topics, which include antibody effector functions, reproducibility of research and diagnostic antibodies, new developments in antibody-drug conjugates (ADCs), preclinical and clinical ADC data, new technologies and applications for bispecific antibodies, antibody therapeutics for non-cancer and orphan indications, antibodies to harness the cellular immune system, overcoming resistance to clinical immunotherapy, and building comprehensive IGVH-gene repertoires through discovering, confirming and cataloging new germline IGVH genes. The Antibody Society’s special session will focus on “Antibodies to watch” in 2016, which are a subset of the nearly 50 antibodies currently in Phase 3 clinical studies. Featuring over 100 speakers in total, the meeting will commence with keynote presentations by Erica Ollmann Saphire (The Scripps Research Institute), Wayne A. Marasco (Dana-Farber Cancer Institute/Harvard Medical School), Joe W. Gray (Oregon Health & Science University), and Anna M. Wu (University of California Los Angeles), and it will conclude with workshops on the promise and challenges of using next-generation sequencing for antibody discovery and engineering from synthetic and in vivo libraries and on computational antibody design.

Monday, December 7, Morning

Keynote presentations

Session chair: James D. Marks (San Francisco General Hospital)

The opening session will feature talks by experts in the field on a broad range of topics relevant to the antibody research and development community. Erica Ollmann Saphire (The Scripps Research Institute) will discuss the Viral Hemorrhagic Fever Immunotherapeutic Consortium, which is a global, field-wide collaboration for antibody therapeutics against Ebola and related viruses. Preliminary results of their work are beginning to reveal which epitopes on the Ebola surface glycoprotein lead to protection, when antibody neutralization in vitro does and does not correlate with protection in vivo, and how antibodies against different sites should be combined to make optimal therapeutic cocktails against Ebola and related viruses.

Wayne A. Marasco (Dana-Farber Cancer Institute/Harvard Medical School) will discuss his group’s past and present contributions to understanding of the basis and bias of human anti-influenza neutralizing antibody responses. Influenza virus remains a serious health threat because of its ability to evade immune surveillance through rapid genetic drift and reassortment. Dr. Marasco’s work, which was initially focused on isolating human antibodies against H5N1 avian influenza, has prompted a paradigm shift in the field, including renewed interest in the quest to develop universal influenza vaccines.

Joe W. Gray (Oregon Health & Science University) will present a spatial systems biological view of cancers. These diseases arise and progress as a result of epigenomic and genomic changes that corrupt normal molecular processes, leading to “cancerous systems” comprising the cancer cells and the environments in which they reside. Much of what we know of these processes has been inferred from assessment of protein levels and associations between proteins using materials extracted from cells and tissues. Now, however, it is possible to directly visualize the multiscale molecular details of aberrant proteins, organelles, cells and tissues using advances in imaging technology, chemistries that enable specific proteins to be made visible during imaging and computational techniques that enable management and interpretation of the resulting information. Dr. Gray’s talk will cover recent advances in analysis of the multiscale “spatial systems” that comprise breast and pancreatic cancers.
Anna M. Wu (University of California Los Angeles) will explain how antibody-based imaging is enabling precision medicine. The development and implementation of targeted therapeutics requires equally powerful and specific diagnostics. Engineered antibodies and fragments provide a broad platform for in vivo imaging based on the cell surface phenotype of cells, tumors, and tissues. ImmunoPET (positron emission tomography) opens the door to specific characterization of disease biology and response to treatment in living organisms and patients, with important implications for selection and monitoring of targeted therapeutics, and whole-body assessment of heterogeneity and immune responses.

Monday, December 7, Afternoon Track A

Antibody effector functions

Session chairs: Dennis R. Burton, The Scripps Research Institute, and Paul W.H.J. Parren, Genmab and Leiden University Medical Center

The antibody Fc fragment provides a critical conduit between the acquired and innate immune system by its role in the activation of a wide range of effector functions. Understanding the full repertoire of Fc-induced effector mechanisms as well as their contribution to antibody-mediated protection against disease therefore remains of high importance for developing novel immunoprophylactic and immunotherapeutic approaches. This session will highlight recent advances and will provide striking examples of how this research may be used to further improve antibody therapy and vaccines.

David G. Brooks (Princess Margaret Cancer Center and University of Toronto) will discuss a novel mechanism of immunosuppression that becomes particularly evident during long-lasting viral infections. Antibodies initially limit viral spread through neutralization and Fc-mediated effector functions. High levels of immune complexes, however, are shown to suppress the activation of cytotoxic effector cells through IgG Fc receptors and through the reduction of antibody-mediated antigen cross-presentation. These findings provide new avenues for improving immune control of persistent infection. George Georgiou (University of Texas) will present a set of novel aglycosylated Fc domains with an exquisite ability to specifically activate individual Fc-mediated effector mechanisms. Significantly, he will demonstrate the importance of the long-disputed significance of in vivo complement activation in the anti-tumor activity of CD20 antibodies. Esther Breij (Genmab) builds her presentation on insights that were obtained from studying complement activation by antibodies at the molecular level. An antibody format was developed that shows an enhanced ability to form hexamers after binding antigen at the cell surface whereas it remains fully monomeric while in solution. A novel approach for antibody therapy of cancer will be presented.

After the networking break, David DiLillo (Rockefeller University) will discuss the long-term effects of immunotherapy. Recent insights show that next to direct Fc-mediated cytotoxic effects, therapeutic antibodies may also induce potent and long-lasting vaccine effects. The potential of improving this aspect, which is mediated via an interaction between antibody Fc and the IgG receptor FcγRIIa, provides new opportunities for marrying vaccine and immunotherapy approaches. Timo van den Berg (Sanquin Research) will provide a dramatic visualization of the killing of tumor cells by neutrophils via a novel mechanism termed trogoptosis. Tumor cells have the ability to escape killing by Fc-mediated cytotoxicity by increasing expression of CD47 which represents a ‘do-not-eat-me’ signal. The possibility to enhance immunotherapy by interrupting interactions with CD47 will be discussed. Marie Kosko-Vilbois (NovImmune) will continue on this theme by presenting a bispecific antibody approach. Bispecific antibodies comprising a CD47 binding arm optimized for affinity, a tumor antigen binding arm and an active IgG1 Fc domain provide a novel means for specific and enhanced tumor cell killing.

The increasing insight into the molecular mechanisms of cross-talk between immune recognition and immune effector as exemplified in this session is leading to new and improved prophylactic and therapeutic approaches in our battle against disease.

Monday, December 7, Afternoon Track B

Getting to reproducibility in research and Dx antibodies

Session chair: Andrew Bradbury, Los Alamos National Laboratory

The issue of reproducibility in biomedical research has become a significant concern, with studies indicating that 50–88% of so-called “landmark” preclinical studies cannot be reproduced, even in close collaboration with the original authors. While there are many reasons for this, antibodies are responsible for many of the problems. In this session, the issue of antibody irreproducibility will be addressed with talks from different stakeholders. Andrew Bradbury (Los Alamos National Laboratory) will provide an overview of the problem, and a solution that involves the broad introduction of recombinant antibodies that can be referred to by their sequences. This will ensure that different researchers are able to use identical antibodies in experiments. David I. Rimm (Yale University) will discuss the use of antibodies in research and clinical settings, and in particular discuss the important role of quantification in antibody staining. He will also provide guidelines for antibody validation to ensure reliable results. Steve Elliot, formerly of Amgen, will discuss a particularly sobering story related to his extensive experience with antibodies against the erythropoietin receptor. Despite reports attesting to their non-specific reactivity, antibodies against EPO-R continued to be sold and used in many publications, leading to widespread misinformation about the receptor. Roberto D Polakiewicz (Cell Signaling Technology) will provide the industrial perspective, and discuss the strategies CST uses to discover and validate high quality reliable monoclonals. Leonard Freedman (Global Biological Standards Institute) will discuss the issues of standards and best practices, as well as the costs, of poor research antibodies. Fridtjof Lund-Johansen (Oslo University...
Hospital) will describe a high throughput flow cytometric approach to validating and characterizing research antibodies, which has the potential to be used at vast scale.

**Tuesday December 8, Morning Track B**

**New developments in antibody-drug conjugates**

*Session chair: James S Huston, The Antibody Society and Huston BioConsulting, LLC*

This Track is both part of the main program and the opening session of 2 days devoted to antibody-drug conjugate (ADC) development. This is our first experiment with a co-located 2-day satellite meeting on a special topic. The first ADC session both stands alone as a singular view into the future of ADCs and also serves as an initial component of the satellite meeting. The centerpiece of this session are the middle 2 presentations by the lead scientists who pioneered creation of the 2 currently approved ADC clinical products that made antibody drug conjugates into a major new focus of therapeutic antibody engineering: **John Lambert** (ImmunoGen, Inc.), who helped develop ado-trastuzumab emtansine, and **Peter Senter** (Seattle Genetics, Inc.), who helped develop brentuximab vedotin. Dr. Lambert will discuss the next wave of innovative ADC technologies, while Dr. Senter will present his views on advancements in drug, linker, and conjugation technologies in ADCs.

The first third of this session is devoted to defining the generalities, challenges, and systems biology of ADCs to facilitate their advanced development. Over the past decade, **Paul Polakis** (Genetech) has carefully analyzed and published on nearly a dozen different ADC combinations. He has deduced a wide range of insights that can be of value in addressing the challenges of ADC development, which he will share with the audience. **Bruce Gomes** (Novartis) developed mathematical models of target cells and ADCs to facilitate quantitative tools for optimal ADC design for different target scenarios. This is expected to stimulate discussion both about its implications and its limitations for experimental design.

The final 2 presentations extend the use of ADCs, **Puja Sapra** (Pfizer) with calicheamicin ADCs, and **Bart de Goeij** (Genmab) with a novel ADC against tissue factor (TF) for treatment of solid tumors. Dr. de Goeij will describe how tisotumab vedotin, a TF antibody coupled to monomethyl auristatin E (MMAE), was highly effective against diverse solid tumors in xenograft models. This novel ADC holds promise as a potent and broadly applicable therapeutic agent for solid tumors.

**Tuesday December 8, Afternoon Track A**

**Bispecific antibodies: new technologies and applications**

*Session chair: Paul J Carter, Genentech*

The concept of bispecific antibodies (BsAb) was first demonstrated in 1960. BsAb are finally emerging as therapeutics, with 2 BsAb now approved for human therapy and over 30 more BsAb in clinical trials. Common clinical applications of BsAb include retargeting of cytotoxic effector cells, such as T cells, to kill tumor cells and dual blockade of 2 different disease mediators. Progress in developing BsAb as drugs has benefited greatly from the clinical and commercial success of monospecific antibodies. Other key factors in the emergence of BsAb include the advent of technologies for efficient production of BsAb and innovation in devising and optimizing BsAb for clinically relevant applications. This session will provide an understanding of diverse technologies for generating BsAb and a broad range of applications from preclinical to clinical stage.

**John Desjarlais** (Xencor) will present a plug-and-play bispecific platform for generating BsAb with long serum half-life. BsAb constructed include anti-CD3 x anti-tumor antigen, such as CD123, CD20 and CD38, for retargeting T cells to acute myeloid leukemia (AML), B cell malignancies and multiple myeloma, respectively. **Eric Krauland** (Adimab) will discuss the efficient identification of BsAb in IgG format. A common light chain strategy is employed together with a chromotographic separation process to derive BsAb in IgG format with all natural sequence. A case study to highlight common light chain Ab discovery and subsequent BsAb generation will be presented. **Yariv Mazor** (MedImmune) will describe an alternative strategy for efficient production of BsAb in a single host cell using an engineering strategy to direct selective assembly of cognate heavy and light chains. Different parameters (e.g., affinity, valence and avidity) affecting dual targeting by BsAb to increase target selectivity will be described.

**Xiaocheng Chen** (Genentech) will present the application of BsAb for enhancing the transport of antibodies across the blood brain barrier. This talk will focus on the identification of new receptor-mediated transcytosis targets to facilitate delivery of BsAb into the brain. **Antonin de Fougerolles** (Ablynx) will demonstrate the use of single domain antibodies known as Nanobodies®, for the facile modular construction of BsAb and multispecific antibodies. Nanobodies are being broadly applied in the fields of inflammation and host defense, including several nanobodies that have reached clinical development. **Bent Jakobsen** (Immunocore) will provide an update on ImmTACs. These bifunctional molecules link an anti-CD3 single-chain variable fragment (scFv) to an affinity-matured T-cell receptor that recognizes target HLA-presented peptides. ImmTACs expand the scope of retargeting of T cells to intracellular targets that are not accessible to conventional BsAb.

**Tuesday December 8, Afternoon Track B**

**Antibody therapeutics for non-cancer indications**

*Session chair: Trudi Veldman, AbbVie*

This session highlights the diversity of approaches to disease modification with antibody therapies, including stable scFv and 2 dual-specific antibody formats. Clinical experience is now emerging with several of these formats, and the studies will inform us whether the preclinical promise of these approaches will translate to the clinical setting. **Patrick Maurer** (ESBATEch) will present the preclinical and clinical experience with
ESBA1008, a highly stable scFv that neutralizes VEGF-A, currently in Phase 3 clinical development for age-related macular degeneration. Jos Raats (Modiquest) will discuss the generation and preclinical data with an anti-citrullinated protein antibody that inhibits NETosis. The process of NETosis recently has gained substantial interest as an important mechanism of action in rheumatoid arthritis (RA). The potential of targeting NETosis in patients with RA will be presented.

Mary Keir (Genentech) has an interesting presentation about etrolizumab, an antibody that blocks $\beta$ integrin. In light of the recent approval of vedolizumab ($\alpha4\beta7$ specific antibody) for the treatment of Crohn’s disease and ulcerative colitis (UC), it will be of interest to learn more about the mechanism of action of etrolizumab, which inhibits both $\alpha4\beta7$ and $\alphaE\beta7$ integrins. The presentation will highlight the experience with predictive biomarkers and patient response in the Phase 2 UC study.

Targeting therapeutics to disease tissue is being explored in a variety of ways to increase the local effectiveness of the drug and, at the same time, to reduce unwanted systemic exposure. Ahuva Nissim (Queen Mary University, UK) has generated a panel of antibodies specific for modified collagen type II in the arthritic joint. She will present preclinical data showing that these antibodies localize to diseased joints, and, when coupled to a disease modifying agent, can provide enhanced efficacy. This approach has great potential for the treatment of osteoarthritis.

The session will conclude with 2 presentations focused on dual-specific approaches to target both tumor necrosis factor (TNF) and interleukin (IL)-17 for the treatment of inflammatory disease. Ullrich Wueellner (Covagen) will discuss the biophysical and pharmacokinetic properties of a bispecific anti-TNF/IL-17 fynomer and the initial experience of the drug in the clinical setting. Lastly, Chung-Ming Hsieh (Abbvie) will present the development of ABT-122, an anti-TNF/IL-17 dual-variable domain Ig (DVD-Ig), that is currently in Phase 2 clinical development for RA. The presentation will include the investigation of translational biomarkers that link the preclinical and clinical mechanism of action.

### Wednesday December 9, Afternoon Track A

**Antibodies to harness the cellular immune system**

*Session chair: Kerry Chester, UCL Cancer Institute*

The session will highlight some of the latest research in development of antibodies to harness the cellular immune system. The session will open with Renier J. Brentjens (Memorial Sloan Kettering Cancer Center) who will talk about cancer therapy with armored CAR T cells, one of the most exiting areas in the field. CARs, which are recombinant antigen receptors generally composed of an extracellular single-chain Fv antibody (scFv) linked to a transmembrane domain and intracellular T-cell signaling domains, can redirect T cells to specifically kill targets in an HLA-independent manner.

John McCafferty (IONTAS Ltd) will then demonstrate the potential to generate new immune-modifying binders utilizing large mammalian display libraries of antibodies and T cell receptors. The talk will explain how the libraries are constructed using site-specific nucleases, and how it is possible to screen millions of clones by flow sorting, providing information on both the level of expression and the extent of binding within individual clones.

The next 4 presentations will focus on different aspects of immune modulatory antibodies, ground breaking and successful new drugs that enhance the anti-tumor T-cell response by interfering with the natural checkpoints that dampen T-cell activation. The first presentation on this topic, given by Sergio A. Quezada (UCL Cancer Institute) will focus on the essential role that the tumor microenvironment and Fc receptors play in the in vivo activity of checkpoint targeting antibodies. The talk will discuss novel developments in this area relating to the mechanism of action and the development of immune modulatory antibodies and combinations that promote intra-tumoral Treg depletion with maximal modulatory activity.

After a networking refreshment break, Jennifer Michaelson (Jounce Therapeutics) will present exciting new work developing an agonistic antibody to the immune checkpoint protein ICOS (Inducible T-cell COStimulator), a CD28-superfamily costimulatory molecule expressed on activated T cells. Preclinical studies demonstrate that anti-ICOS agonistic antibodies are efficacious in syngeneic tumor models, with enhanced efficacy observed in combination with PD-1 inhibition.

Michael Saunders (arGEN-X) will present work on characterization and in vivo evaluation of blocking antibodies against GARP, a novel immune checkpoint target with potential in cancer immunotherapy. arGEN-X have identified anti-GARP antibodies that inhibit active TGF-β production from human Tregs and inhibit the immune suppressive function of human Tregs in vivo. Antibodies against GARP may represent new immunotherapeutic approaches for the treatment of cancer or chronic infections.

The session will conclude with a presentation from Ann White (University of Southampton) in the fascinating area of hinge region biology. Monoclonal antibodies targeting TNF receptors, such as CD40, 4–1BB and OX40, on immune cells generally require Fc receptor-mediated cross-linking for activity. However, human IgG2 is agonistic independent of FcR engagement, due to a unique ability to rearrange hinge region disulfides and adopt a more constrained conformation. The presentation will discuss these different mechanisms of action and how antibody engineering can be used to optimize therapeutic activity.
disease is defined as a condition that affects fewer than 200,000 people nationwide. Numerous pharmaceutical companies are pursuing opportunities to address orphan diseases as a facile route to rapid drug approval. ARGX-113 is an Fc-based FcRn blocker for orphan indications myasthenia gravis and pemphigus vulgaris being developed by arGEN-X, a Belgium-based company. Peter Ulrichs (arGEN-X) will describe preclinical studies and recent work to move ARGX-113 into clinical trials. Next, Vaughn Smider (The Scripps Research Institute and Sevion Therapeutics) will describe antibodies utilizing the long complementarity-determining regions of cows. This technology opens up many difficult therapeutic targets found among G protein-coupled receptors and ion channels. Among these, the Kv1.3 target is particularly interesting for various T cell mediated diseases. Apparently CD40 plays an important role in the pathogenesis of orphan indication primary biliary cirrhosis. Mark de Boer (Fast Forward Pharmaceuticals) will provide preclinical and clinical data on an anti-CD40 antibody, which is a negative allosteric modulator of the CD40 pathway.

Next, Bo Yu (Larix Bioscience LLC) will describe a unique mammalian expression system for the direct identification of anti-receptor antibodies. This technology utilizes so-called antibody membrane switch technology for switchable cell-surface display of antibody libraries. Jim Larrick will describe an antibody that modulates the wnt-pathway for benefit in orphan fibrotic diseases such as idiopathic pulmonary fibrosis. This humanized antibody modulates the activity of co-receptor LRP6 to inhibit multiple wnt ligands and their receptors, the frizzled proteins. The final presentation by Wendy Williams (MedImmune) will focus on antibodies to a ligand-gated ion channel. We anticipate an exciting session of first-rate science in this most important field.

Wednesday December 9, Afternoon Track C

Preclinical and clinical ADC data

Session chair: Mark Alfenito, EnGen Bio, Inc.

Traditionally antibody ‘drug-able’ targets must be expressed on the surface of the cell in order to maximize the potential access and clearing/effector functions conferred by active isotype antibodies. However, once bound to a cell, antibodies are frequently internalized, rendering them mostly useless from a therapeutic standpoint. Further, many cytotoxic drugs are too toxic when delivered to patients, requiring a method to increase local concentration of the cytotoxic at the site of disease. A solution to this is through ADCs. By combining the targeting capabilities of antibodies with the killing capability of cytotoxic drugs, ADCs allow sensitive discrimination between healthy cells and diseased tissue, whether the antibody is internalized or not.

This session covers various pre-clinical and clinical aspects of the broad topic of ADCs. Lioudmila Tchistiakova (Pfizer) will be discussing aspects of selection, engineering and optimization of monoclonal antibodies with an eye toward subsequent ADC development. Eric Feldman (Seattle Genetics, Inc..) is developing an anti-CD33 for AML, in an ADC format. The antibody has engineered cysteines to control for uniform, site-specific conjugation to the cytotoxic pyrrolobenzodiazepine molecule. Phase 1 clinical study data for patients with AML will be presented.

To ensure the success of a drug candidate, patient selection is a critical and often overlooked component. Charles Morris (ImmunoGen, Inc..) will discuss the important contribution of clinical pharmacokinetics and biomarker-based patient selection to establishing an appropriate dosing regimen and target patient population for their Phase 1 candidate, mirvetuximab soravtansine (IMGN853) for platinum-resistant epithelial ovarian cancer. Daniel Maslyar (Genentech) will also be discussing an ADC they have in development for platinum-resistant ovarian cancer, and also for non-small cell lung cancer (NSCLC). Unlike IMGN853, which targets the folate receptor α, lifastuzumab vedotin (DMIB0600A) targets the antigen NaPi2b, which is frequently expressed in ovarian cancer and non-squamous NSCLC. Clinical activity is described in a Phase 1 study population in patients with tumors expressing NaPi2b as assessed by IHC.

David Goldenberg (Immunomedics, Inc..) will show clinical data on sacituzumab govitecan (IMMU-132). IMMU-132 is unique in that the cytotoxic agent, irinotecan, is maybe less toxic, but the target, Trop-2, is well expressed on several cancers at high concentration. Results are most advanced in patients with triple-negative breast cancer, small-cell lung cancer, and NSCLC, where objective responses (CR/PR) have been achieved. IMMU-132 appears to show promising therapeutic activity with an encouraging therapeutic index in patients with advanced and heavily pretreated solid cancers.

ADCs needn’t always be of the simple antibody-cytotoxic conjugate format. Daryl Drummond, (Merrimack Pharmaceuticals, Inc.) will discuss immunoliposomes, which represent a promising novel format for delivering chemotherapeutic agents, allowing for multiple levels of targeting and specificity while providing the flexibility to deliver a wide range of payloads. Clinical development of their lead ErbB2-targeted pegylated immunoliposomal doxorubicin, which is currently in a Phase 2 clinical trial in ErbB2-overexpressing breast cancer patients, will be presented.

Wednesday December 9, Afternoon Special Session of The Antibody Society

Antibodies to watch in 2016

In a remarkable demonstration of the rise of antibodies as therapeutics, the record for numbers of recombinant antibody products that receive first marketing approvals in a calendar year, which was set in 2014, may be met or exceeded in both 2015 and 2016. A high number of first approvals (6 or above) for antibody therapeutics in each of 3 consecutive years would be unprecedented. Janice Reichert (Reichert Biotechnology Consulting LLC), President of The Antibody Society, will give an overview of the newest antibodies entering the market (e.g., secukinumab, dinutuximab, evolocumab, alirocumab), and those anticipated to enter the market in the near future. The
longer-term outlook will be provided through an overview of antibody therapeutics in Phase 3 clinical studies.

**Thursday December 10, Morning Track A**

**Building comprehensive IGVH-gene repertoires: discovering, confirming and cataloging new germline IGVH genes**

*Session chair: Jamie K Scott, Simon Fraser University*

The term, “antibody repertoire,” refers to the collection of antibodies and B-cell receptors produced by plasma cells (which secrete antibodies) and various subsets of B cells (which carry on their surface B-cell receptors, signaling molecules that are the precursors of secreted antibodies), respectively. This repertoire is extremely diverse, with each B-cell clone initially generated in the bone marrow by the recombination of “germline” IGHV, IGHD and IGHJ gene segments in the IGH locus, yielding a VDJ recombinant encoding the VH domain of a complete heavy chain. Following a “productive VDJ rearrangement,” germline IGLV and IGLJ gene segments are recombined at the kappa or lambda locus, yielding a VJ recombinant encoding the VL domain of a complete light chain. Three mechanisms of diversification at the joints between IGHV-IGHD, IGHD-IGHJ and IGLV-IGLJ gene segments lend further diversity to antibody heavy and light chains: imprecise joining, the addition of N and P nucleotides. Thus, the diversity of the initial, “naïve” B-cell repertoire is produced both by combinatorial diversity at the levels of IGHV and IGLV gene-region formation (through recombination of germline V, D and J gene segments and joint diversification), and through the combination of heavy and light chains. After undergoing positive and negative selection, the functional naïve repertoire circulates through the secondary lymphoid tissues, where they encounter antigens. With the proper stimulation, antigen-binding B-cell clones are activated to divide and differentiate into antibody-secreting plasma cells and “memory” B cells that often undergo isotype switching (from IgM and IgD to IgG, IgA and/or IgE), as well as somatic hypermutation of their functional IGHV and IGLV gene regions. Thus, through the process of antigen recognition, memory B cells become “clonally expanded” and from successive rounds of somatic hypermutation, form “lineages” of clonally related sequences. (Note: T-cells undergo similar processes of diversification to form naïve repertoires of CD4+ and CD8+ T cells, and clonal expansion on antigen recognition, though their recombinated TCRV, TCRD and TCRJ genes do not undergo class switching or somatic hypermutation.)

B and T cells form the heart of adaptive immune responses, and are key to our understanding of health and disease, and to the development of therapeutics and vaccines. As such, there is keen interest in methods that can characterize the repertoires of various naïve, memory and effector B- and T-cell subsets. Over the past 5–6 years, high-throughput sequencing (HTS) has been adapted to characterize antibody and T-cell receptor repertoires from the blood and other tissues of people and animals. Briefly, to characterize the IGVH gene regions of B cells, the mRNA is converted to cDNA and then sequenced, using either primers specific for the entire set of germline IGHV genes or by 5’ RACE to cover the 5’ end of the VH region, and isotype-specific primers to cover the 3’ end; this produces tens- to hundreds-of-thousands of IGHV gene-region sequences. While analysis of these sequences can determine clonal expansion (i.e., high-frequency IGHV sequences), the sequences of the germline IGHV genes are required to determine clonal lineages, based on somatic mutation patterns. However, it has become clear that many of the germline IGHV genes are missing in the existing catalog of germline VH gene catalogs, even with the availability of “full” genomic sequences; this is because genome sequencing is typically bad at covering complex loci, such as the IGH locus. Moreover, as will be illustrated in some of the presentations of this session, in some cases, HTS has identified recurrent “somatic mutations” among independent VDJ recombined genes in the IgM populations; these have been inferred to be germline IGHV genes. Thus, this session will cover several approaches to identifying new germline IGHV genes, as well as issues arising that need further clarification by the scientific community.

In the first presentation of the session, Andrew Collins (University of New South Wales) will describe his surprising finding that while C57bl/6 mice and Balb/c mice have large germline IGHV gene repertoires, it appears they have only 5 of the IGHV genes in common. HTS data from the cDNA of splenocytes from both strains were used to infer germline IGHV genes for both strains. Thus cDNA sequences, not genomic ones, underpin this important discovery. Gunilla Karlsson Hedestam (Karolinska Institute) will present data on the germline IGHV gene repertoires of rhesus macaques, an important preclinical model in developing human vaccines. She sequences both genomic DNA and cDNA in these analyses. Thomas Kepler (Boston University) will discuss his work in mapping and assembling the IgH locus of a single rhesus macaque, as well as statistical methods for inferring germline IGHV genes from cDNA-based HTS data.

Another important piece in understanding the humoral immune response involves the question, “Can one’s immune response be predicted based upon a complete knowledge of the naïve B-cell repertoire?” And preceding that, “Can one’s naïve B-cell repertoire be predicted based on knowledge of the IGH locus (i.e., the combination of its germline IGHV gene segments and their locations, cis-acting elements, and epigenetic modifications in developing pro- and pre-B cells)?” Scott Boyd (Stanford University) will present intriguing evidence speaking to the latter question, based on his analysis of identical twins. He has shown that the naïve B cell repertoires of identical twins are very similar, whereas their repertoires of their antigen-experienced counterparts, the memory B cells, are quite different. This indicates that it might indeed be possible to predict the naïve B-cell repertoire were we to understand the “rules” concealed in the depths of the IGH locus. While a complete sequence of one human IgH locus is available, with the diversity among germline IGHV genes, and their alleles and copy numbers having been characterized, the extent of that diversity appears to be enormous. Bronwen Lambson, from South Africa’s National Institute for Communicable Disease, will present 85 novel germline IGHV gene sequences identified through genomic sequences from only 28 individuals.
Thursday December 10, Morning Track B

Overcoming resistance to clinical immunotherapy

Session chair: Louis M Weiner, Georgetown University Medical Center

This session focused on clinical and translational studies related to antibody-engineered therapeutics will be chaired by Louis M Weiner, MD, Director of the Georgetown Lombardi Comprehensive Cancer Center and Chair of the Department of Oncology at Georgetown University in Washington, DC. Antibody engineering has created a vast suite of tools, but their optimal use requires that the products of these tools be matched with disease states where they can be routinely effective. He will speak on targeting cancer’s fragile strengths to overcome immune evasion and promote effective immunotherapy. Most successful malignancies overcome the host anti-tumor immune response through a variety of mechanisms that include immune checkpoints. The fragility of these powerful tumor protective mechanisms is underscored by the exciting successes of antagonist immune checkpoint antibodies in an enlarging list of malignancies. However, these agents are ineffective in many patients, and work only temporarily in others. Hence, it is important to understand immunotherapy resistance mechanisms to identify new agents and strategies that can be used alone, or in combination with immune checkpoint inhibitory antibodies. He has employed an in vivo synthetic lethal screening approach in a murine breast cancer model to identify malignant epithelial cell genes that selectively protect tumors growing in immunocompetent as opposed to SCID syngeneic mice from immune destruction. Inhibition of these new targets by antibodies or small molecules offers promise for immunotherapy.

Kim Margolin (Stanford University Medical Center) will discuss mechanisms of resistance to immunotherapy. Most of the successes using immunotherapy for solid tumors and hematologic malignancy have been in melanoma, using active immunotherapies (cytokines and immune checkpoint inhibitory antibodies) and lymphoproliferative diseases using passive immunotherapies including unmodified and modified antibodies or related structures. The immune response depends on a complex network of tumor cells, various leukocyte populations, stromal and vascular endothelial cells in a tumor immune microenvironment that may change over time, in response to therapy and in different organ sites. The identification of factors that can be modulated to overcome intrinsic resistance, pre-empt acquired resistance, and avoid dangerous toxicities, including immune-based injury, will be critical.

Thomas Davis (Celldex Therapeutics) will discuss progress in the development of Celldex immune checkpoint modulators in a presentation on clinical updates on immunotherapeutic checkpoints. James W. Hodge (National Cancer Institute) will then describe work he has led on radiation-induced immunogenic modulation to enhance T-cell and monoclonal antibody therapy of cancer. Immunogenic modulation describes the mechanism of how antitumor therapies alter the biology of the surviving tumor cells to render them more sensitive to cytotoxic T-lymphocyte (CTL) or natural killer cell-mediated destruction. He has shown in murine models and clinical trials that irradiation of a primary tumor could facilitate systemic anti-tumor activity by engaging the lytic capacity of vaccine-induced antigen-specific CTL. In addition, radiation can significantly increase cell-surface expression of several monoclonal antibody targets, and improve antibody-dependent cell-mediated cytoxicity and antiproliferative effects of selected mAb.

Nizar M. Tannir (The University of Texas MD Anderson Cancer Center) will present work on targeting immune checkpoints in genitourinary cancers, with a focus on rationale and current data. Ipilimumab, an anti-CTLA-4 antibody, was the first immune checkpoint inhibitor to be approved for the treatment of melanoma. This has opened a new field termed “immune checkpoint blockade.” Trials combining nivolumab, an anti-PD-1 antibody, and ipilimumab are ongoing in metastatic renal cell cancer (mRCC) and bladder cancer. In a Phase 1 trial in patients with bladder cancer, MPDL3280A, an anti-PD-L1 antibody, produced 48% ORR in patients whose immune cells expressed PD-L1. Dr. Tannir’s research efforts are focused on the identification of predictive biomarkers of response, mechanisms of innate and acquired resistance, optimal duration of therapy, and best combinatorial strategies.

Finally, progress in antibody-targeted therapies is not limited to checkpoint blockade or immune manipulation. ADCs have had some notable successes in recent years. Alan C. Rigby (Eli Lilly and Company) will provide his perspective on leveraging clinical learnings and patient tailoring to enable next-generation ADC successes.

Thursday December 10, Afternoon Workshop A

The promise and challenges of using next-generation sequencing for antibody discovery and engineering from synthetic and in vivo libraries

Session chair: Sai Reddy, ETH Zürich/Swiss Federal Institute of Technology

Next-generation sequencing (NGS) provides a quantitative approach to measuring the diversity and distribution of antibody libraries. This workshop will introduce and enable researchers on how to design, analyze, and perform antibody NGS studies and how these can be applied for discovery and engineering of
monoclonal antibodies from both synthetic and immune libraries. Furthermore, we will go over practical details of antibody NGS including library construction and quality control, data processing and analysis, and advanced methods for bioinformatic analyses. Finally, we will address important challenges that in the field such as sequencing and PCR errors, multiplex primer bias, and library preparation.

**Thursday December 10, Afternoon Workshop B**

**Computational antibody design workshop**

*Session chair: Gregory P Adams, Viventia Bio, Inc.*

For many years the use of computational design methods in the antibody field was primarily limited to humanizing or improving properties (e.g., increasing their thermal stability or decreasing the propensity to aggregate). Recent advances in the field have significantly broadened our capabilities to design individual antibodies and antibody libraries, and have improved our ability to affinity mature and stabilize existing antibodies. The speakers in this exciting workshop are among the leaders in these efforts and will share their recent advances. Charlotte Deane (Oxford University Kellogg College) will discuss de novo antibody design efforts using the Structural Antibody Database (SABDAB), antibody design platform SABPred and a novel CDR-H3 prediction method (Chimera) and ABodybuilder. Thomas J. Van Blarcom (Pfizer Inc.) will describe a new method to precisely and efficiently map the epitopes of small panels of antibodies in parallel. This rapid method relies on the combination of rational library design, quantitative yeast surface display and next generation DNA sequencing. Matthew K. Robinson (Fox Chase Cancer Center) will describe his work using an understanding of the relationship between amino acid sequence and antibody structure and protein design tools for both the development of de novo antibodies and the improvement of stability and binding affinity. Ralph Adams (UCB) will describe the use of a novel design tool, IOTA, to rationally design mutations to improve protein-protein interactions leading to significant increases in affinity and stability. Johannes Maier (Schrödinger) will address the issue of irreversible self-association of macromolecules to form aggregates, which can be a significant problem in the development of biotherapeutics. He will share their new computational aggregation predicting tool that predicts and visually presents the aggregation propensity regions of a protein based on a combination of the relative arrangements of charged and hydrophobic surface exposed residues, and of the hot-spot analysis from sequence-based aggregation propensity predictions.