MABS
2016, VOL. 8, NO. 3, 617–652
http://dx.doi.org/10.1080/19420862.2016.1153211

Taylor & Francis
Taylor & Francis Group

MEETING REPORT

OPEN ACCESS

Antibody engineering & therapeutics, the annual meeting of the antibody society December 7–10, 2015, San Diego, CA, USA

Matthias Pauthner[a], Jenny Yeung[b], Chris Ullman[c], Joost Bakker[d], Thierry Wurch[e], Janice M. Reichert[f], Fridtjof Lund-Johansen[g], Andrew R.M. Bradbury[h], Paul J. Carter[i], and Joost P.M. Melis[j]

[a]Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, USA; [b]University College London, London, UK; [c]Paratopix Ltd, Cambridge, UK; [d]Scicomvisuals, Amsterdam, The Netherlands; [e]Center de Recherche Servier, France; [f]Reichert Biotechnology Consulting LLC, Framingham, USA; [g]Dept. of Immunology, Oslo University Hospital Rikshospitalet, Oslo, Norway; [h]Los Alamos National Laboratory, Los Alamos, USA; [i]Antibody Engineering Department, Genentech, South San Francisco, USA; [j]Genmab, Utrecht, The Netherlands

ABSTRACT

The 26th Antibody Engineering & Therapeutics meeting, the annual meeting of The Antibody Society united over 800 participants from all over the world in San Diego from 6–10 December 2015. The latest innovations and advances in antibody research and development were discussed, covering a myriad of antibody-related topics by more than 100 speakers, who were carefully selected by The Antibody Society. As a prelude, attendees could join the pre-conference training course focusing, among others, on the engineering and enhancement of antibodies and antibody-like scaffolds, bispecific antibody engineering and adaptation to generate chimeric antigen receptor constructs. The main event covered 4 d of scientific sessions that included 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, building comprehensive IgVH-gene repertoires through discovering, confirming and cataloging new germline IgVH genes, and overcoming resistance to clinical immunotherapy. The Antibody Society's special session focused on "Antibodies to watch" in 2016. Another special session put the spotlight on the limitations of the new definitions for the assignment of antibody international nonproprietary names introduced by the World Health Organization. The convention concluded with workshops on computational antibody design and on the promise and challenges of using next-generation sequencing for antibody discovery and engineering from synthetic and in vivo libraries.

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; ADC, antibody-drug conjugate; AE, adverse events; AML, acute myeloid leukemia; BiTE, bi-specific T cell engagers; bsAb, bispecific antibody; CR, complete response; CDR, complementarity-determining region; DAR, drug-to-antibody ratio; Fc, fragment crystallizable; HIV, human immunodeficiency virus; IgG, immunoglobulin G; IgVH, immunoglobulin variable region heavy chain; IHC, immunohistochemistry; mAb, monoclonal antibody; MDR, multidrug resistance; NSCLC, non-small cell lung cancer; PDX, patient-derived xenograft; PK, pharmacokinetics; RA, rheumatoid arthritis; scFv, single-chain variable fragment; SCLC, small cell lung cancer

ARTICLE HISTORY
Received 5 February 2016
Accepted 8 February 2016

KEYWORDS
Antibody engineering; antibody therapeutics; antibody effector functions; antibody-drug conjugates; bispecific antibodies; clinical; diagnostic antibodies; immunology; immunotherapy; preclinical

Downloaded by [108.49.191.210] at 11:34 25 March 2016

Monday december 7, 2015, keynote presentations

Joost Melis

Antibodies against ebola virus: A global collaboration

Erica Ollmann Saphire (The Scripps Research Institute) opened the conference with an overview of the work of the Viral Hemorrhagic Fever Immunotherapeutic Consortium (VIC). The VIC is a global, field-wide open collaboration for antibody therapeutics against Ebola and related viruses.

Filoviruses, such as the Ebola Virus and the Marburg Virus, have different structural manifestations of their envelope glycoproteins. These different structures can present hurdles to effectively create antibody therapeutics, since target sites are sometimes lost or hidden or serve as potential decoys. Research has only partially elucidated how viruses can best be targeted. The human anti-Ebola monoclonal antibody (mAb) KZ52 from a 1995 survivor has been shown to neutralize the virus, and is able to protect mice and Guinea pigs, but not non-human primates. In 2011, cocktails combining several anti-Ebola mAbs protected against infection in non-human primates, but at least one of these cocktails contained antibodies that were non- or weakly neutralizing. In 2012, many questions regarding cocktails needed answers: which type of cocktails is most suitable, which mAbs are possibly synergistic, which

CONTACT Joost P.M. Melis j.melis@genmab.com

Published with license by Taylor & Francis Group, LLC © Matthias Pauthner, Jenny Yeung, Chris Ullman, Joost Bakker, Thierry Wurch, Janice M. Reichert, Fridtjof Lund-Johansen, Andrew R.M. Bradbury, Paul J. Carter, and Joost P.M. Melis
This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

mAbs are best to be included or which mAbs could possibly compete with one another, and which in vitro assays should be used to select mAbs. These unknowns created a complex problem that needed a significant sample size and input to solve. This initiated the formation of the VIC, which is now aiming to map epitopes, find predictors of what approach works best and how to create the best cocktails.

So far, the VIC has mapped the epitopes of 160 anti-Ebola mAbs and investigated which of these mAbs are able to neutralize the virus and which are able to protect in vivo. Results showed that some epitopes or locations are more likely to cause neutralization than others, however the potency to neutralize does not predict the level of protection.

Additionally, immune effector functions of mAbs might also be of importance for efficacy. The VIC analyzed the effector functions related to the 160 available mAbs. The initial analyses demonstrated a discrepancy between human and mouse results, and indicated different assays or models were necessary to better address the importance of effector functions. The Marburg virus is the next big challenge for the VIC, which is currently mapping epitopes of available mAbs for this virus.

The basis and bias of human anti-influenza - neutralizing antibody responses

The second keynote lecture was given by **Wayne Marasco** (Harvard Medical School). Certain scientific barriers interfere with developing a "universal" influenza vaccine, such as the antigenic drift that can abolish the binding properties of broadly neutralizing antibodies (BnAbs). However, very little changes in the stem domain of the influenza virus occur in respect to the globular head domain. The influenza hemagglutinin (HA) stem is able to elicit a population of antibodies with broad neutralizing activities, which are also recalcitrant to neutralization escape. BnAb F10 is able to bind all subtypes of group 1 HA for example. F10 blocks pH-dependent conformational change and escape from F10 coincides with loss of viral fitness.

Structural analyses of 38 stem-directed IGHV1-69-sBnAbs showed several highly conserved amino acids that are essential for high affinity binding, such as phenylalanine 52 and tyrosine 97–99. These conserved amino acids create promising opportunities for development of BnAbs. IGHV3-30 germline gene-derived BnAbs contain a complementarity-determining region (CDR) 3 HC hydrophobic pocket-stabilized epitope and are effective against group 1 and 2 influenza A strains, which is broader than IGHV1-69 BnAbs.

Part two of the presentation focused on the question if it is possible to vaccinate all individuals of a population, since it is known that there is quite some variation within populations, both pre- and post-vaccination. The research herein focused on the role of genetic V_H segment polymorphisms in anti-influenza antibody responses. In a clinical cohort of 85 H5N1 vaccinated individuals, genotype/phenotype BnAb responses were correlated. For this, the expressed antibody repertoire, the effect of IGHV1-69 genotype on clonal distribution, precursor frequency and copy number were analyzed. In different genotypic groups, HA stem-directed Abs were monitored over time and this analysis uncovered a correlation between IGHV1-69 polymorphism and micro-neutralization titers to H5N1. Further analyses indicated correlations between IGHV1-69 clonal signature frequencies, copy number and utilization of the antibodies as BnAbs. Also, immunoglobulin locus wide associations were assessed and indicated that IGHV1-69 F/L polymorphism, copy number, and gene duplication are different among ethnic groups. Genetic background should therefore be taken into account during vaccine development. Currently, several approaches toward structured-based universal stem-directed influenza vaccines are ongoing. For these current and future developments, not only serological studies should be used, but also genotyping and phenotyping signatures. Moreover, establishing an IGHV haplotype map could prove to be beneficial for development of BnAbs.

A spatial systems biological view of cancer

Joe Gray (Oregon Health & Science University) then discussed a spatial systems biological view of cancer. To generate robust solutions to battle cancer, cancer should be managed as a heterogeneous, adaptive system that includes extrinsic proximal influences, distal influences and intrinsic cell biology. This whole system together dictates cell responses such as proliferation or apoptosis and the eventual clinical outcome. Cancer heterogeneity exists at many scales, from tissue level all the way to molecular structures. Nowadays, imaging technologies allow us to study and integrate biological systems at multiple scales. Electron microscopy, conventional and super-resolution light microscopy, plus anatomical imaging such as positron emission tomography (PET), magnetic resonance imaging (MRI) and computerized tomography (CT) together with bioengineering or computational biology can be integrated to assess and manage tumor heterogeneity. Intrinsic tumor heterogeneity can now be visualized in 3D by using technologies such as light sheet microscopy and tissue clearing; an example of this was shown for triple-negative breast cancer.

To address the question if intrinsic tumor heterogeneity is of functional importance, a cell system using 80 breast cancer cell lines was used to model many aspects of tumor heterogeneity such as genomic aberrations, transcriptional subtypes and differentiation state. Using MEK and PI3 K inhibitors, it was shown that the differentiation state of cancer cells changes in different directions. This is also reflected by dose-dependent changes measured by several "Omics" techniques. Monitoring and directing these changes creates opportunities to possibly steer cancer cells into a more homogeneous state that is more easily treated. This was initially attempted by driving cancer cells into a state of deep CK19+ quiescence. However, once released from selective pressure, the cells started growing again. Another option is to prevent cells from undergoing epigenetic changes, and early results indicate that chromatin modifiers appear to counter resistance-associated epigenomic evolution.

To manage tumor extrinsic heterogeneity, microenvironmental signals that influence tumor cell behavior should be identified. Microenvironment microarrays analysis was used to unravel which signals are important and which ones are not. Both soluble and insoluble proteins were printed on arrays on which each spot hosts space for growth of ~100 cells. Several aspects of tumor biology were measured on these spots, such as

Downloaded by [108.49.191.210] at 11:34 25 March 2016

Downloaded by [108.49.191.210] at 11:34 25 March 2016

morphology, metabolism, cell cycle, nuclear activity and lineage status. Some microenvironment proteins (MEPs) profoundly affected differentiation and proliferation of cancer cells. Selected MEPs can convert gene targeted therapies into stimulatory agents. For example, NRG1 and HGF make neratinib stimulatory in HER2 luminal cells, which is probably caused by trimerization of human epidermal growth factor receptors (HERs). This creates opportunities of receptor multimer formation to manage extrinsic microenvironment.

Results of immuno-targeted siRNA delivery indicated siRNA can be used to overcome resistance of cancer cells to HER2-targeting therapeutics. Anti-HER2-siHER2-NP demonstrated tumor growth inhibition in mice that were tumor resistant to TM1 and paclitaxel, but became sensitive to these drugs again by addition of the siRNA therapeutic.

Multispectral super resolution fluorescence microscopy can be used for direct assessment of receptor complexes on cancer cells. 3D imaging of cancer cells and the interaction with the microenvironment can be visualized and indicates where the receptors are localized on cancer cells. Many of the receptors are present on long protrusions. Functional assessment of protrusions demonstrated a clear role in receptor transport. Receptors were shown to be moving along these protrusions and receptors were visualized being transported between cells. These results initiated the search for protrusion-targeted therapies.

Beyond biopsies – enabling precision medicine through antibody-based imaging

The final keynote lecture was given by **Anna Wu** (David Geffen School of Medicine at University of California Los Angeles). Molecular diagnostics in oncology are mainly still performed by making use of in vitro-based technologies, such as biopsies, blood tests and more novel techniques like circulating tumor cell detection or next-generation sequencing. Molecular imaging using PET can be used to assess the in vivo situation, analyzing living tissue in context and performing spatial and temporal assessments. Since antibody-based therapeutics have improved significantly over the last decade, the use of antibody-based products in molecular imaging can be meaningful for in vivo diagnostics. Several aspects of immune-conjugates for imaging were discussed, such as targeting strategies, conjugation/linker strategies and signal considerations. Improvements in positron-emitting radionuclides have been made for their use in ImmunoPET, e.g., availability of isotopes with longer half-lives. Also, optimized antibodies for imaging are now available; human or humanized antibodies show reduced immunogenicity, antibody engineering can eliminate unwanted effector functions and creates options for site-specific conjugation. Engineering can also influence the pharmacokinetics and targeting capabilities of the antibody-based imaging product. The use of either intact IgG versus diabody or minibody molecules can greatly affect exposure and clearance, and consequently the level of background activity. For instance, an intact antibody takes a week to clear from the body while a diabody is eliminated within 4–7 hours, which effectively means diabodies can be used for same day imaging. Cysteine modification of diabodies allows site-specific conjugation and more uniform products. ImmunoPET can provide info on tumor presence, cell surface phenotype, target expression for therapy selection, target occupancy, internalization/catabolism, response to therapy, and mechanistic or quantification purposes. Data of a ^{124}I PSCA-minibody for quantitative immunoPET imaging of prostate cancer and of an ^{89}Zr-DFO-H2 anti-MET minibody measuring c-MET expression as a marker for resistance to anti-EGFR therapy was presented.

Antibody-based imaging of both cancer cells and immune cells can shed more light on immune responses, immune cells subsets, expansion, trafficking and activation. These imaging techniques can be applied in the field of inflammatory and autoimmune disease and cancer immunotherapy for instance. Since CD8 cytotoxic T cells are very potent immune cells, several examples of CD8 T cell quantitation and biodistribution in tissues were shown: 1) imaging using ^{89}Zr-radiolabeled anti-CD8-169 cys-diabody successfully showed T cell repopulation following HSC transplant, 2) imaging of CD8 T cell infiltration in anti-PD-L1 tumor immunotherapy in syngeneic BALB/c mouse model for CT26 colon carcinoma, and 3) imaging the massive recruitment of T cells into tumor and lymphoid tissues upon 4–1BB antibody treatment. This work is now being extended to CD4 and CD20 imaging.

Track A: Antibody effector functions

Jenny Yeung

The afternoon session on antibody effector functions was chaired by **Paul Parren** (Genmab) and **Dennis Burton** (The Scripps Research Institute).

Suppression of antibody effector activity during persistent virus infection

Persistent viral infections are a global health concern, with over 500 million people infected with HIV and hepatitis B and C viruses. As discussed by **David Brooks** (Princess Margaret Cancer Center and University of Toronto), what they all have in common is a mechanism that evades or circumvents the immune response, creating an immunosuppressive environment. Understanding what governs immunosuppression may help to develop ways in which to restore immune function in persistent infection.

The comparison of the humoral responses elicited by mice infected with 2 variant strains of lymphocytic choriomeningitis virus (LCMV), which induced either an acute infection (Armstrong) or a persistent infection (clone 13), led to the elucidation of a mechanism of immunosuppression mediated by immune complexes. During persistent infection, high total antibody levels and high levels of immune complexes exist in tandem with decreased virus-specific antibody levels, which result in suppression of antibody functions. Using exogenously administered cell-depleting anti-CD4 or anti-CD20 antibodies, it was observed that depletion of target cells was significantly suppressed in persistently LMCV infected wild type mice where antibody levels were high. Depletion of target cells was restored in mice with compromised B cell responses and therefore low antibody levels.

In vitro and in vivo studies showed that the high amounts of immune complexes generated in persistently infected wild type mice led to compromised FcγR-mediated effector functions, such as macrophage phagocytosis and dendritic cell mediated cross-presentation. Immune complex formation blocked activating FcγRs and resulted in suppression of IgG-mediated effector functions. This suppression of the immune system function is reversible, as ex vivo cells from persistently infected mice had decreased levels of antibody on the cell surface after resting, which subsequently restored phagocytosis. These data illustrate a new mechanism through which persistent virus infections can suppress the immune response and have important implication for the design of antibody therapies to control persistent infections.[1]

George Georgiou (University of Texas) next presented a set of novel aglycosylated Fc domains with an exquisite ability to specifically activate individual Fc-mediated effector mechanisms. Notably, he demonstrated the importance of the long-disputed significance of in vivo complement activation in the anti-tumor activity of CD20 antibodies.

Functional aspects and therapeutic application of antigen-dependent formation of IgG hexamers at the cell surface

Esther Breij (Genmab) presented the Genmab Hexabody® platform, which developed from their earlier elegant work showing how monomeric IgGs assemble into an ordered hexameric structure promotes to activation of the complement cascade.[2,3] The understanding of this mechanism can aid or enhance the efficacy of therapeutic antibodies.[4] Formation of the hexamers is determined by Fc:Fc interactions mediated by critical residues. Indeed enhanced formation of hexamers as a result of mutations in key residues can enhance binding to C1q and enhanced complement-dependent cytotoxicity, which creates potential for developing more effective therapeutics. The Hexabody® platform can be applied to a large range of antibodies and to different targets.

Death receptor (DR5) is a receptor for TRAIL, which upon binding to the ligand, initiates an 'outside-in' signaling pathway leading to apoptotic cell death of TRAIL-sensitive cancer cells. While pre-clinical findings showed compelling anti-tumor activity, anti-Death Receptor antibodies have not performed as well in clinical trials. Cross-linking of DR5 is a key factor for the anti-tumor activity. Two anti-DR5 antibodies that do not compete for DR5 binding were developed using the Hexabody® platform and studied in vitro and in vivo: Hx-DR5-03 and Hx-DR5-07. The potency of the Hx-DR5-03 and Hx-DR5-07 was enhanced compared to the wild type IgG1 antibodies IgG1-DR5-03 and IgG1-DR5-07. When used as a combination, the potency of the HexaBody® molecules was enhanced even further, in a number of different DR5-positive cell lines. The potency was relatively ratio independent, whereby a Hx-DR5-03:Hx-DR5-07 ratio of 9:1 or 1:9 was always more potent than the single antibodies, although a 1:3 or 3:1 ratio appeared to be optimal. The key element was that it was a mixture of antibodies. To potently induce cytotoxicity, the Hx-DR5-03/07 combination does not need a secondary cross-linking agent, which is necessary for other anti-DR5 antibodies (conatumumab and tigatuzumab). In seven of 9 murine xenograft models, the Hexabody® combination showed consistently better potency than conatumumab. The maximal therapeutic effect was achieved at 0.5 mg/kg (lowest dose tested) or 2.0 mg/kg. These data demonstrate the therapeutic potential of the Hexabody® platform for potentiating therapeutic antibodies for the treatment of cancer.

After the networking break, **David DiLillo** (Rockefeller University) discussed the long-term effects of immunotherapy. Recent insights showed that next to direct Fc-mediated cytotoxic effects, therapeutic antibodies may also induce potent and long 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.

Neutrophils kill antibody-opsonized cancer cells by trogoptosis

Timo K. van den Berg (Sanquin Research and Academic Medical Center) discussed the process of trogoptosis. Antibody-dependent cell-mediated cytotoxicity (ADCC), mediated by natural killer (NK) cells and phagocytic cells such as macrophages and neutrophils, contribute to the anti-tumor effects of therapeutic antibodies developed for cancer treatment. Each of these effector cell types express Fcγ receptors (FcγRs) and form cell-to-cell synapses with their antibody-coated target cells. NK cells induce target cell apoptosis by release of granzyme B and perforin, and macrophages phagocytose tumor cells and destroy them through intracellular lysosomal degradation. How neutrophils kill antibody-opsonized tumor cells was however not fully understood. As has been previously shown for macrophages, inhibition of the Signal Regulatory Protein Alpha (SIRPα)/CD47 signaling pathway potentiated cell death of opsonized cancer cells by neutrophils.

Formation of a cytotoxic synapse between the neutrophil and opsonized target was a necessary pre-requisite for neutrophil-induced tumor cell death. Blocking SIRPα/CD47 interaction increased synapse formation, a process dependent on CD11b/CD18 integrin. Live imaging studies showed that neutrophils used a trogocytosis-related process, termed trogoptosis (trogo is Greek for gnaw) to kill antibody-coated tumor cells. This novel cell death process did not involve anti-microbial properties of neutrophils, i.e., NAPDH oxidase or cytotoxic granules release. Neutrophils from these patients with genetic defects in these pathways were still capable of killing antibody-coated tumor cells.

During trogoptosis, neutrophils disrupt the plasma membrane of opsonized tumor cells by tearing off pieces at the cytotoxic synapse, leading to leakage of cellular contents and subsequent tumor cell death. Use of pharmacological compounds showed that several signaling pathways were involved in the triggering of trogoptosis, including the calmodulin, Syk, PI3 K and MLCK pathways. There was evidence to show that trogoptosis is a potentially relevant process in vivo as well as in vitro. Using a murine melanoma model, GFP+ neutrophils could be correlated with death of anti-gp75-opsonized B16F10 melanoma cells in the liver of mice. Furthermore, in biopsies taken from HER2+ breast cancer patients after trastuzumab

Downloaded by [108.49.191.210] at 11:34 25 March 2016

treatment, significant infiltration of neutrophils was apparent and these neutrophils contained HER2+ intracellular material.

In summary, trogoptosis is a novel form of neutrophil-mediated cell death of antibody-coated cancer cells, which can be potentiated by disruption of the CD47/SIRPα pathway. Together this could potentially improve the activity of therapeutic antibodies against cancer.

Marie Kosko-Vilbois (NovImmune) continued 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.

Track B: Getting to reproducibility in research and Dx antibodies

Janice Reichert, Andrew Bradbury, Fridtjof Lund-Johansen

As research reagents, commercially available antibodies have been widely criticized for being poorly characterized, and for yielding irreproducible experimental results because they are either not of the correct specificity or not suitable for the type of experiment.[5] In this session chaired by **Andrew Bradbury** (Los Alamos National Laboratory), speakers from the academic, non-profit and commercial sectors reiterated the problem, discussed the approaches currently used to ameliorate the situation, and proposed solutions.

At the crossroads: Getting to reproducible research antibodies

Andrew Bradbury provided an overview of the nature and scale of the problems with research antibodies, and suggested a solution. The research antibody business includes over 300 vendors (as listed on antibodyresource.com) and >2,000,000 antibodies (as listed on CiteAb). Many companies set up their development or manufacturing sites overseas to take advantage of cost advantages, and most producers sell their antibodies to multiple vendors, who add their own labels. The vendors also buy and sell from each other, and then add their own labels. It is thus possible to get the same antibody when purchasing from different vendors. Characterization is expensive (>$200 for a western blot; >$60 per IHC slide), so data from vendors is often minimal and historical (i.e., it often does not apply to the specific lot purchased). The large number of antibodies against popular targets (e.g., receptor-type tyrosine-protein phosphatase C is targeted by 3255 antibodies; 42 proteins have over 1,000 antibodies; 3483 proteins have 100 or more antibodies) makes the selection of effective and reproducible antibodies extremely challenging. Dr. Bradbury noted that substantial time and money (estimated at >$1.1 billion annually worldwide) is wasted on bad antibodies. This represents only the cost of the defective reagents themselves, and takes no account of the money and time wasted on additional secondary reagents, or trying to reproduce irreproducible experiments. In fact, poorly characterized, irreproducible antibodies have been cited as being one of the commonest causes of publications that cannot be repeated.[6-8] Unfortunately, better antibody characterization or ordering from only the best, most reliable manufacturers may not solve the problem, because even good antibodies that are well characterized are not defined or archived forever in a way that allows scientists to unequivocally use the same antibody as that used in a previous study.

In considering solutions, a key point is that research antibodies are sold on the basis of what they (purportedly) recognize, not their physical identity. Dr. Bradbury made it clear that antibodies should be well characterized and validated; this is the solution to poorly functioning antibodies. However, in order to resolve the problem of irreproducibility, antibodies need to be expressed recombinantly and identified by unique "bar codes," i.e., their publicly available sequences. This would address reproducibility issues, as researchers can then repeat/reproduce experiments with the same reagents as those used in previous publications. Good binders will become immortal and never lost, and complete characterization is required only once. In fact, some non-therapeutic antibody companies are selling recombinants, but not revealing sequences. These include AbD-Serotech and Creative Diagnostics (that use in vitro methods), Biosite, REAfinity and ABfinity (that generate recombinant antibodies from immunized animals), Absolute Antibodies (make recombinant antibodies by synthesising genes from patent sequences) and AbCam (~5,000 recombinant rabbit monoclonals, originally derived by Epitomics).

Dr. Bradbury stated that the problem needs to be solved by many different constituencies, including the funding agencies, journals and commercial antibody providers. Funding agencies can contribute by insisting that all antibodies used in research are sequenced and recombinantly expressed, with their sequences published in publically accessible databases. As this is a very ambitious goal, it will require scientists to be informed of these requirements with a long lead time (e.g., 5–10 years). Because of the commercial considerations, it is likely that this would be best implemented if funding agencies established specialized centers for binder selection, sequencing and characterization that would be able to provide well characterized sequenced antibodies to scientists, much like NCI, or Neuromab. Publishers can contribute by Insisting that the sequences of all antibodies used in published papers are disclosed (e.g., within 5–10 years), and that methods descriptions include antibody concentrations and the buffers used. Antibody sequences would be best disclosed using an accession number system that could be cited in publications. Ideally this would be in a separate field, which would allow automatic archiving. He acknowledged that some companies are not keen on the idea of publishing antibody sequences as they are considered intellectual property, and there are no cheap ways to protect antibody sequences, since antibodies used in research do not make enough money to invest in patenting (see the description of Dr. Polakiewicz's seminar below). One way to address company concerns would be the publically funded, specialized centers for binder selection, sequencing and characterization described above. In this scenario, once antibodies are derived and characterized, companies could compete on the production, cost or quality of the publicly available sequence validated antibodies. Dr. Bradbury also raised the possibility that technology may be developed that renders the generation, characterization and production of antibodies so cheap that publication of sequence may not become a concern. However, he thought this could take some time.

Downloaded by [108.49.191.210] at 11:34 25 March 2016

Downloaded by [108.49.191.210] at 11:34 25 March 2016

In conclusion, Dr. Bradbury described progress made by a number of organizations. The Global Biological Standards Institute (GBSI) has established a Research Antibodies and Standards Task Force that will focus on the application of standards that can advance the quality, efficacy, and reproducibility of antibodies used in basic and preclinical research and development. The objectives of the Task Force are to identify specific areas where standards for research antibodies are urgently needed, what types of standards could be designed and implemented to address these needs, and how these recommendations can be effectively implemented by commercial producers and vendors. With The Antibody Society, GBSI is organizing a meeting at Asilomar in the fall of 2016 for all stakeholders on the use of antibodies in rigorous and reproducible research. The Human Proteome Organization (HUPO) has established an Antibody Standards Committee with the goal to define and propose common standards for affinity reagents, and to engage the broader scientific community in dialog around this issue. Ultimately the goal would be to publish guidelines to be endorsed by publishers that offer guidance to both antibody users and providers. The Federation of American Societies for Experimental Biology (FASEB), is organizing workshops on the use of antibodies in rigorous reproducible research, and is also planning to publish guidelines on the use of antibodies to be adopted by researchers.

Use and abuse of antibodies in research and the clinic

David Rimm (Yale University) noted that the problems associated with antibody reagents sold by some vendors pose a barrier to successful translational research. For example, in a study reported in 2008,[9] the success rates for validation of 5436 antibodies from 51 providers for use in Western blotting and tissue microarrays was only 49% on average, and ranged from 0 to 100%. Prof. Rimm indicated that this unpredictability as to whether antibody reagents are suitable for specific applications means that researchers must validate the reagents to ensure that their experiments yield scientifically rigorous and reproducible data. He then discussed the Rimm Lab's algorithm,[10] which is focused on validation of antibodies for immunohistochemistry (ICH) or quantitative immunofluorescence on paraffin-embedded tissues, but could be equally valid or modified for other antibody-based assays. Although antibody validation by the end-user is time-consuming, Prof. Rimm emphasized the need for quantitative analysis, assessment of sensitivity and specificity, as well as reproducibility across lots, platforms, operators and labs. For both ICH and scientific research applications, use of monoclonal and preferably recombinant antibodies was recommended. In concluding Prof. Rimm noted that, although companies should in theory validate antibodies, the end users are ultimately responsible for the accuracy of their experimental data, and therefore the quality of the antibodies they use.

Nonspecific antibodies, what could possibly go wrong? the Epo R story

Steve Elliott (Amgen) who has extensive knowledge of antibodies targeting the erythropoietin receptor (EpoR), discussed long-standing problems with these reagents. He discussed the fact that flawed assumptions and experiments lead to the reporting of flawed data and conclusions, and a cascade of negative effects can occur when researchers then attempt to follow up on the flawed work. As an example of this, Dr. Elliott reviewed flawed data that suggested erythropoietin stimulating agents promoted tumor cell growth through activation of EpoR. Dr. Elliott related that he and colleagues investigated the specificity of the anti-EpoR polyclonal antibodies used for the immunoblotting and immunostaining, and showed that in reality only one was suitable for use in immunoblotting, and none were suitable for immunohistochemistry.[11] Use of these antibodies, however, has continued and led to widespread dissemination of misinformation about the receptor. Dr. Elliott then related a more detailed examination of the problem. Of 219 studies of the "EpoR cancer" hypothesis, only 10% were properly controlled, i.e., used positive and negative control cells or tissue, used validated reagents and reported data that supported the conclusions. A total of 140 of the 219 studies included anti-EpoR antibody data, and only 8 of these were properly controlled, i.e., had EpoR positive and negative tissues or cell lines, and, for westerns, size markers were used and band matched the size of EpoR. Notably, none of the studies that reported results supportive of the hypothesis were controlled. Dr. Elliott highlighted the specific problems with many of the studies, including use of non-specific anti-EpoR antibodies, inappropriate or insufficient use of controls and the reporting of conclusions that were not supported by the data. He then elaborated on the difficulty of validating antibodies. False-positive results are common, even with westerns, and may be difficult to detect. For example, a band on a gel may be the correct size, but the band may result from off-target binding. In addition, false-positive data may be tissue specific. Thus, investigators must be extremely cautious, always use appropriate positive and negative control samples and include additional confirmatory data, e.g., duplicate experiments with a second validated antibody. Dr. Elliott concluded by stating his view on the topic: In scientific endeavors it is rewarding to be right. But it takes courage to admit you are wrong and then correct the record. Failure to do so may be far more damaging than you realize.

Improving antibody quality by combining novel technology and classic concepts for research antibody discovery and development

Roberto Polakiewicz (Cell Signaling Technology) provided the perspective of Cell Signaling Technology (CST), a commercial supplier of research antibodies. He first acknowledged that commercial antibodies are getting bad press. One issue is the economics of the market for research antibodies, which is small compared to the markets for diagnostic and therapeutic antibodies (~$2.5 bn vs $15 bn and $55 bn, respectively) and composed of over 300 providers that offer over 2.5 million antibody reagents. Suppliers may be aggregators or re-sellers of the reagents, and many do not validate their products or provide validation data. The problems are exacerbated by poor or non-existent quality-control (QC) systems or technical support. He then differentiated CST's approach by noting that their mAbs are primarily recombinant mAbs and that CST emphasizes

application validation, QC and technical support. CST does 95% of antibody development and all product-manufacturing in-house.

Dr. Polakiewicz then discussed the process of making a good mAb, including product design, antibody capture/isolation, validation, formulation, and the use of appropriate screening assays at each step. He emphasized that appropriate controls and testing on multiple applications to verify specificity are important, and that each lot must be assessed and compared as part of the QC process. Such a process requires investment of scientific expertise, time and money. Sequenced antibodies have been proposed as a solution to the problems associated with research antibodies, but Dr. Polakiewicz noted that placing the sequences in the public domain jeopardizes the investment in validation and support because the revenue from each product is too small to justify costs of patenting (up to $300 k) and potential litigation. In concluding, Dr. Polakiewicz provided recommendations that, if followed, could alleviate the issues associated with research antibodies. In particular: 1) antibody suppliers should validate properly, share data (excluding sequences) and provide customer support; 2) antibody users should trust companies that validate and share data, follow protocols suggested by the vendor, use antibodies for recommended applications, seek technical support, avoid purchasing from large aggregators offering antibodies of unknown origin, and prefer monoclonals (recombinants, if available); 3) journals should require precise citation of antibody sources (e.g., catalog and lot numbers), validation data, and detailed protocols for experiments involving use of antibodies; 4) independent internet sources should help disseminate data, citations and user feedback; and 5) academic and funding institutions should train young investigators on how to find and use good antibodies.

Closing the reproducibility gap with standards and best practices for antibodies

Global Biological Standards Institute

Leonard Freedman (Global Biological Standard Institute) made the case that standards facilitate the alignment of consensus-based best practices, reduce variance, and improve reproducibility in biomedical research. He discussed the progress made with establishing standards for authentication of human cell lines using short tandem repeat DNA profiling, which is a well-established technique that unambiguously characterizes a number of different loci in the human genome, but noted that there are no universally-accepted guidelines or standardized methods for validation of research antibodies. Dr. Freedman noted that the Global Biological Standards Institute (GBSI) has launched a research antibody validation initiative. The "Research Antibodies: Solutions for Today and Tomorrow" workshop GBSI is organizing with The Antibody Society, has the goals to: 1) identify a set of standards to validate research antibodies, including recommendations for adoption by academia, industry, funders, and journals; 2) develop recommendations for an independent proficiency certification system or open access user ratings service; and 3) develop recommendations, timeline, and follow-up plan for the introduction of sequenced recombinant antibodies as research reagents. In concluding, he discussed steps that could yield substantial improvements in preclinical reproducibility rates, including encouraging vendors to offer only validated reagents (e.g., antibodies, cell lines) and broad utilization of these reagents by principle investigators; ensuring that research funder policies require documented use of validated and non-contaminated reagents and adequate funding to cover these additional costs; ensuring that procedures to document reagent validation are required by publishers.[12]

Reproducibility crisis: Don't blame the antibodies, array them

Fridtjof Lund-Johansen (Oslo University Hospital) argued that researchers may be ignoring a large number of excellent antibodies. Currently, sales are largely limited to a small number of reagents that have been used in many publications. However, there is no evidence that top-cited antibodies are top performers. To find good antibodies with few or no citations, Prof. Lund-Johansen and co-workers have developed array technology that allows parallel validation of thousands of antibodies. In Microsphere Affinity Proteomics (MAP), antibodies bound to color-coded microspheres are used to capture biotinylated proteins from cell lysates. In a multiplexed version of the protein gel blot, the labeled proteins are denatured, separated by gel electrophoresis and eluted into liquid fractions. The fractions are next incubated with microsphere-based antibody arrays, and proteins captured onto the surface of the microspheres are labeled with fluorescent streptavidin and detected by flow cytometry. In a separate assay referred to as Native-MAP, sample proteins are first separated according to subcellular localization and then by size-exclusion chromatography to resolve monomeric proteins and protein complexes. Numerical data from MAP are visualized as line plots that show antibody capture across the fractions. Different targets of the same capture antibody are seen as discrete peaks across the fractions. To discriminate those that correspond to intended antibody targets and cross-reactivity, an aliquot of each fraction is analyzed by mass spectrometry. Thus far, the MAP technique has been used with more than 3000 commercially available antibodies, and the results show that ~10% of antibodies with no or few citations outperform top-cited products. Attendees asked whether MAP can predict performance of antibodies in standard applications such as western blotting and immunohistochemistry (IHC). Prof. Lund-Johansen replied that there is good correspondence between MAP and protein gel blotting, and that studies on IHC are in progress. From a theoretical perspective, antibodies that perform well in capture are likely to be high affinity reagents, and since the assay is used both with native and denatured proteins, one would expect that most relevant epitopes should be covered.

Track A: New aspects of prodrug targeting

Chris Ullman

The second day of the conference opened with a session chaired by **Andreas Plückthun** (University of Zürich) that focused on Prodrug targeting, addressing the challenge of how

Downloaded by [108.49.191.210] at 11:34 25 March 2016

antibodies and antibody drug conjugates can be engineered to be more selective for disease tissue.

Protease mediated activation of tissue/tumor-targeting antibodies and prodrug approaches

Ulrich Brinkmann (Roche) opened this session. The basis of the approach was an adaption of the use of bi/multi-specific antibodies, used to modulate proliferative or inflammatory processes. There are over 50 different bispecific formats described in the literature, which allows the researcher to adjust the size, valency, flexibility, half-life and biodistribution offering an overwhelming choice.[13] Brinkmann stated that he was not a believer in one size and one format fits all diseases and all targets, thus rather than to ask which format is best, it is best to consider which format is optimal for which disease. Modifications to the structures of the bispecific can add additional selectivity so that they become active only at the desired location.

In general, there are 2 groups of bsAbs, those that have an Fc and those that do not. The non-Fc-containing bispecifics function by forming a bridge between T cells and cancerous cell targets. Examples of these formats are Amgen's bi-specific T cell engagers (BiTEs) or Macrogenic's Dual-Affinity Re-Targeting (DART) fragments. A disadvantage of the format is the short half-life, which may require products to be administered via continuous intravenous infusion. Inclusion of an Fc confers advantages of solubility, stability, half-life extension and effector function, each of which can be modified by genetic engineering. Purification is also easier for Fc-containing molecules. The production of such multichain bsAb can be improved by 'knobs-into-holes' technology, or variations of this theme, to ensure correct heavy chain assembly.

Dr. Brinkmann offered examples from the Roche Group. RG7716 (in Phase 1 for wet age-related macular degeneration) and RG7221 (in Phase 2 for colorectal cancer) were built using CrossMab technology, which allows correct heavy-light pairing driven by swapping the CH1 of one heavy chain with the corresponding CL region of the light chain and vice versa. These antibodies simultaneously inactivate 2 ligands that are modulators for angiogenesis - angiopoietin2 and VEGF-A. The bsAb binds each ligand with each arm of an IgG-like molecule containing functional Fc region and prevents activation of their respective receptors, Tie-2 and VEGF receptor kinases. The bispecific format offers advantages as 2 factors are depleted, thereby allowing treatment of tumors that escape angiogenic single anti-VEGF treatment.

RG6013 (developed by Chugai), using a common light chain approach to simplify production, targets FIXa and FX for hemophilia in a full IgG format. The bispecific binding brings together 2 proteins of the same cascade into the same complex that is necessary for sufferers of hemophilia A who lack factor VIIIa, which naturally complexes FIXa and FX. The antibody has advantages over supplementation of FVIII by having a longer half-life in vivo and is less immunogenic in patients. In addition, a dual action Fab (DAF) antibody directed at HER1 and HER3, is in Phase 2 clinical trial for head and neck cancer. This antibody simultaneously blocks HER1 and HER3 through binding the same variable regions, potentially improving cancer treatment by inhibition of MAPK and AKT signaling and growth arrest of pathway-addicted cells. Good affinities against both molecules have been achieved.

These examples served to demonstrate interesting functional formats for bispecifics, but the toxicity associated with some targets can be problematic. A proposed solution is to enable local activation of function through protease cleavage, particularly relevant when binding to death receptors or targets that are also expressed on non-diseased tissue.[14] Overexpression of proteases in tumors can be exploited by activating the binding capacity of half a bispecific. Thus, half of the bispecific tethers the molecule to the diseased cell, thereby bringing a cleavable linker in proximity to the protease to allow activation of the second binding modality. This cleavable disulfide-stabilized Fv format was described for a bispecific targeting HER3 and cMet, in which the binding of cMet is driven by a V_H and V_L domain at the C-terminus of the heavy chain that is activated through cleavage by an enzyme (such as MMP2 or 9, urokinase-type-plasminogen activator or furin). The two anti-cMet variable domains are linked by a disulfide bond (V_H 44- V_L 100) that maintains pairing following the cleavage of the protease sensitive linker ($(G_4S_2)_2$-protease site $(G_4S_2)_2$) connecting V_L to CH3. The cloaking of the binding site affects on-rate of binding, but not the off-rate to cMet, and reduces affinity for receptors where there is an absence of protease.

Probody therapeutics redefine the landscape of antibody modalities in oncology

The theme was continued by **Luc Desnoyers'** (CytomX) presentation "Probody™ Therapeutics Redefine the Landscape of Antibody Modalities in Oncology." CytomX's Probody technology differs by the fact that the antigen binding site of the antibody is masked with a peptide linked to the N-terminus of the light chain through a protease cleavable linker and is applicable to therapeutics and imaging.[15,16] In the case of anti-EGFR Probody PB1, the 21–amino acid masking peptide is recombinantly fused to the light chain through a 26–amino acid linker carrying the 8-residue protease cleavable substrate, flanked by Gly-Ser–rich peptide linkers. Protease activation of the Probody antibody is tightly regulated, producing binding when cleaved by tumor-associated proteases, such as urokinase-type plasminogen activator (uPA), membrane-type serine protease 1 (MT-SP1/matriptase), and legumain, a lysosomal protease found to be released and active in the acidic extracellular tumor microenvironment.[15] This potentially provides a better safety profile and specific examples were described including CX-072, a protease-cleavable anti-PD-L1 Probody therapeutic. CX-072 has been shown to provide equivalent anti-tumor activity in mice to that of its parental antibody, while minimizing binding to peripheral cells through local activation within the tumor, thereby reducing induction of autoimmune diabetes in NOD mice caused by the parental antibody. Further examples included anti-human CD166 Probody drug conjugate (PDC). CD166 is not an obvious target for an antibody-drug conjugate (ADC) because it is expressed on normal tissues, but is highly expressed in many cancers. The anti-CD166 Probody-SPDB-DM4 therapeutic was selected because it binds human and cynomolgus CD166, but doesn't bind to the rodent equivalent. It was well tolerated at 5 mg/kg, with no evidence of on-target

Downloaded by [108.49.191.210] at 11:34 25 March 2016

Downloaded by [108.49.191.210] at 11:34 25 March 2016

toxicity, and off-target toxicity typical of SPDB-DM4 noted at 15 mg/kg. This study demonstrates the potential use of Probody drug conjugates for promising and previously inaccessible cancer antigens through reducing the collateral damage to normal tissues. A Probody T cell-binding bispecific (TCB) platform has also been developed by creating an anti-CD3 Probody bispecific. T cell engagers have shown efficacy in treating hematologic malignancies, but can have toxic side-effects due to binding healthy tissue. An anti-EGFR/anti-CD3 Probody TCB eliminated established $EGFR^+$ HT-29 colorectal tumors in human T cell engrafted NSG mice, having potency within 2- to 3-fold of an antibody-TCB, but gaining a >30-fold increase in the maximum tolerated dose in cynomolgus monkeys relative to the Ab-TCB. Again this demonstrates the potential benefit to safety as CytomX have noted limited leakage of the Probody therapeutic from the sites of cleavage within tumors.

Dr. Desnoyers also commented that the masking of each paratope required a specific peptide mask that can reduce affinity by up to 1000-fold, although, for example, a cetuximab Probody therapeutic demonstrates a ~30-fold reduction. Immunogenicity hasn't been studied widely, but in proof of concept studies with a Probody variant of cetuximab, there was no increase in the anti-Ig response.

Probody therapeutics redefine the landscape of antibody modalities in oncology

Andreas Plückthun (University of Zürich) addressed the theme from an alternative angle in his lecture, presenting data for designed ankyrin repeat protein (DARPin)-toxin fusions whose selectivity was engineered by bioorthogonal veiling.

DARPins are a non-antibody class of small protein affinity reagents engineered from natural ankyrin repeat proteins, one of the most common binding proteins in nature and responsible for diverse functions such as cell signaling and receptor binding. The DARPins have favorable properties of high potency, high stability, high affinity, flexible architecture and ease of production that can be used singularly or in combination in 2 or more to bind multiple targets or epitopes. Examples of clinical phase molecules are Abicipar, an anti-VEGF-A DARPin for wet AMD entering Phase 3 and MP0250, an anti-HGF and VEGF bispecific DARPin in Phase 1 clinical study for solid tumor cancers and hematological malignancies (developed by Molecular Partners AG).

The DARPins are free from Cys and Met and can be made Lys-free, thereby offering choices for coupling drugs, such as monomethylauristatin F toxin and *Pseudomonas aeruginosa* exotoxin A (ETA), through compatible biorthogonal click chemistry. However, reducing the toxicity caused by target-independent uptake of such molecules fused to biological drugs remains a challenge. Therefore, Plückthun and colleagues have developed methods to cloak DARPin-ETA fusions to improve pharmacological properties using site-specific addition of polyethylene glycol (PEG) moieties using azide alkyne cycloaddition. PEG serves as a veil to reduce immunogenicity, improve selectivity and improve the half-life of the molecule. An anti-EpCAM DARPin (Ec1) and a domain I-deleted variant of ETA (ETA″) was developed as a prodrug and both linear and branched PEG moieties were added at specific sites using a linker designed to be susceptible to tumor-specific protease cleavage, unmasking its full activity through loss of PEG. The native REDLK sequence of the toxin was replaced with the canonical eukaryotic ER-retention signal KDEL. Two key positions, one within the catalytic domain and one close to the C-terminal KDEL sequence of Ec1-ETA″ (Ec1-ETA″486Aha-AhaKDEL-3C-PEG) were identified that lowered cytotoxicity 1000-fold in EpCAM-positive tumor cells when both were PEGylated with 20 kDa linear PEG. However, following proteolytic cleavage of the linker-PEG moiety, the molecule was fully potent. The PEGylated Ec1-ETA″ was much better tolerated than Ec1-ETA″, providing a longer circulation half-life (82 min compared to 7.5 min for the unPEGylated format) and an almost 10-fold increased area under the curve (AUC), following systemic intravenous delivery.[17]

Intracellular delivery was also investigated using fusions of EpCAM-targeting DARPins with the full-length pore-forming protein of anthrax toxin, "protective antigen" (N682A/D683A mutant), or the translocation domain of ETA (252–412) and cargo DARPins. Interestingly, for the anthrax toxin, the stability of the cargo had to be less than the threshold thermodynamic stability for anthrax pores due to the requirement for unfolding in retrograde translocation. Therefore, a destabilized DARPin mutant carrying a point mutation was necessary. Assays monitoring delivery were developed that required biotinylation of an AviTag™ sequence (carried on the cargo) by prokaryotic biotin ligase (BirA) expressed in a FlpIn 293 EpCAM/BirA cell line or MCF7 cells that transiently overexpress BirA. Studies using the ETA domain and phosphorylated ERK-binding N2C DARPin demonstrated efficient uptake and high nM concentrations of delivered cargo, even saturating the pathway. Importantly, the modular systems described in the presentation have the potential to overcome critical barriers for the therapeutic use of toxins to modulate targets beyond the reach of current biological drugs in a safe manner.[18]

Pareto optimal biotherapeutic deimmunization: P99 beta-lactamase as a case study for ADEPT fusion partners

Antibody-targeted toxins and prodrug converting enzymes can be powerful anticancer treatments, but therapeutic protein payloads present a risk of undesirable immunogenicity. **Karl Griswold** (Thayer School of Engineering) described experimental validation of integrated protein deimmunization algorithms that deplete immunogenic T cell epitopes while maintaining protein activity. The Pareto optimal methods efficiently and accurately mapped the protein design space, enabling aggressive molecular engineering that balances the tradeoffs between immunogenic potential and therapeutic function.

CRISPR libraries for functional genomics

David E. Root (Broad Institute of Harvard and MIT) discussed CRISPR, which has emerged as a powerful tool for

genetic engineering and functional screens. Work by Prof. Root and others have improved the design of CRISPR libraries to achieve better gene perturbation efficacy and specificity using this technology.

Track B & C: New developments in antibody-drug conjugates

Joost Melis

James S. Huston (Huston BioConsulting LLC) chaired the Track B & C shared morning session, which focused on topics related to ADC development.

Challenges associated with the development of antibody drug conjugates

Paul Polakis (Genentech) discussed challenges associated with the development of antibody drug conjugates. Target-dependent toxicity, heterogenic target expression, target shedding and ADC catabolism were discussed after a short introduction of the multiple components of ADCs. Divergent targeted drug-dependent toxicity of several ADC products were mentioned and showed that drug mechanism of action determines target-dependent killing of normal cells; while NaPi2b demonstrated high levels of expression in normal lung tissue, toxicity was not associated with this tissue in a clinical study of anti-NaPi2b-vc-MMAE. Secondly, MSLN is highly expressed in the pleural lining of lung, yet no adverse pleuritis events were seen in a Phase 1 study of anti-MSLN-vc-MMAE. This was in contrast to previous clinical study in which targeting MSLN with an immunotoxin resulted in florid pleuritis. Data was also presented indicating that toxin-dependent efficacy can vary under different circumstances. Xenograft studies using anti-CD22 ADCs armed with either PNU or MMAE efficacious in a BJAB model, but the PNU ADC remained efficacious in the model made resistant to the MMAE ADC. Targeting of LGR5 in the normal intestine resulted in target-dependent toxicity for PNU, but not for MMAE.

Next, results on the EDNRB target for melanoma treatment were discussed. Separately administrating a MEK inhibitor or ADC demonstrated only moderate efficacy responses, but the combining the 2 treatments showed an enhanced effect in preclinical models. It was shown that induction of EDNRB expression in response to MAKP pathway inhibition (e.g., by a MEK inhibitor) increased the availability of the target for an anti-EDNRB ADC. Melanoma patients in the anti-EDNRB-vc-MMAE clinical study exhibited heterogeneous responses to treatment, suggesting that varying levels of MAPK pathway activity could have contributed to variable target expression, thus influencing the responses to ADC treatment.

Decreased ADC efficacy can be the result of various mechanisms. One possibility is the presence of circulating shed target antigen. This was discussed in the context of the MUC16 ADC clinical study. The MUC16 extracellular domain is shed as CA125, which can potentially interfere with pharmacokinetics (PK) assays and can result in immune complex formation. However, the CA125 expression levels showed no relation to PK in patients. The lack of any PK effect was consistent with analysis showing that clinically high levels of CA125 are still proportionately very low relative to circulating levels of the MUC16 ADC. Finally, 3 examples were provided to illustrate a relationship between rapid pharmacologic clearance of ADCs and increased toxicity. An over-drugged antibody, a Fab fragment and a mutant IgG with compromised binding to the neonatal Fc receptor (FcRn), all cleared more rapidly than wild type IgGs and produced greater toxicity and weaker efficacy when administered at equivalent drug levels. Thus, the rate of ADC catabolism or clearance can be inversely related to efficacy and directly related to toxicity.

Modeling aids design of antibody drug conjugates

Next topic highlighted the development of a mechanistic tumor penetration model to guide ADC design, presented by **Bruce Gomes** (Novartis). The goal of this project was to create a modular mechanistic model for ADCs to aid in parameter design like affinity, half-life, and payload properties. All relevant processes are represented by physical properties, and the aim is to make predictions before reagents are actually available to determine up-front risk and feasibility of ADC projects. The model is ideally flexible enough to be used for different species, can be repurposed for many different targets, and allows rapid answers to research questions. A complete model of ADC takes into account a multitude of parameters, such as ADC distribution, tumor penetration and diffusion, receptor binding, internalization, recycling, linker cleavage, cell sensitivity, peripheral vs. tumor killing, tumor doubling and cell cycle dynamics and killing rate, plus as much info that can be found specific to the target. The presented model identified undesirable tumor properties that can impair ADC tissue homogeneity and compromise ADC success, and explored ADC design optimization scenarios to counteract unfavorable intrinsic tumor tissue attributes. For instance, the model demonstrated the profound impact of cytotoxic payload release mechanisms and the role of bystander killing effects on tumor shrinkage. Cell cycle times, target receptor density and penetration or diffusion from circulation to tumor cells play a role in efficacy. Very high affinity binding or high receptor density can result in the unbalanced distribution of the ADC to tumor cells, mostly targeting the most proximal tumor cells to the capillary and leaving the more distal tumor cells unbound. Tumor mass shrinkage is also a function of target receptor kinetics (e.g., recycling rate) and intrinsic tumor growth properties (e.g., tumor doubling time). Tumor cells with high doubling rates are most effective as targets since this will increase ADC efficacy. A next step in mechanistic modeling involved therapeutic index modeling that has not been that successful yet, since it is troublesome to correctly parameterize and estimate characteristics of both tumor cells and normal tissue. However, the current mechanistic model that was created for ADC treatment allows optimization of properties of all aspects of ADCs and fits with time constraints of drug discovery. It allows rapid hypothesis testing, and the model is suitable to reparametrize to fit different species.

Downloaded by [108.49.191.210] at 11:34 25 March 2016

Downloaded by [108.49.191.210] at 11:34 25 March 2016

Building on the success of kadcyla: The next wave of innovation in ADC technologies

John Lambert (ImmunoGen) then discussed the next innovation wave in ADC technologies, building on the success of Kadcyla®. Ado-trastuzumab emtansine (T-DM1 or Kadcyla®) and the general challenges for a successful ADC (tumor penetration, lysosomal activation, metabolites and safety profiles) were briefly discussed, followed by the maytansinoid ADC platform of ImmunoGen. Linker design was a special focus in this segment, since the nature of the linker determines tumor metabolites in vivo. The release mechanism of a non-cleavable ADC is lysosomal degradation, but some targets recycle to the cell membrane thereby avoiding the lysosome. Disulfide cleavage of linkers in endosomal compartments enables release of neutral toxin molecules within the cell that are also able to kill nearby cells via the so-called bystander effect. Differences between various linkers were highlighted by characteristics of SMCC-DM1 (non-cleavable), SPP-DM1 and SPDB-DM4 (both cleavable). Implementing the hydrophilic sulfo-SPDB linker further enhanced DM4 activity in vitro and in vivo toward MDR+ cells, thereby increasing the potency of the ADC. Clinical efficacy overviews of 3 maytansinoid ADCs were presented, among which was mirvetuximab soravtansine (anti-folate receptor-sulfo-SPDB-DM4). For this drug, bystander killing is one mechanism that likely contributes to clinical profile of the ADC.

The second half of the presentation focused on payload innovation targeting DNA. Some tumor types appear insensitive to tubulin inhibitors, so DNA-acting toxins could create new opportunities for ADCs to addressing these cancers, but the real challenge using these toxins is to create a good therapeutic window. The IGN (indolino-benzodiazepine backbone) payload is an ultra-potent DNA-acting payload that is more potent than PBD SJG-136, exemplified by data showing improved potency on B cell cancer cells and a colon cancer cell line using IGN vs SJG136 head-to-head. The introduction of a phenyl ring further increased potency of the IGN payload, and in vivo a potent dose-responsive anti-tumor activity was established. However, the toxicity profile of this product was less favorable demonstrating delayed or prolonged toxicity, possibly associated with the mechanism of potent DNA crosslinking by the di-imine. As a next step, a DNA alkylating mechanism of action (mono-imine) was designed to prevent crosslinking on DNA strands. Although this decreased the potency by 2-to-3-fold, it resulted in an acceptable toxicity profile and an overall improved therapeutic index. Linker choice can further alter the toxicity profile in vivo in mice. Introducing a disulfide linker for this alkylating format enhanced potency again and further improved the safety profile. IMGN779 (targeting CD33) was mentioned as an example of a promising IGN-based ADC. Adding a cleavable sulfo-SPDB linker resulted in an ADC with average DAR of 3, and picomolar potency was achieved for several acute myeloid leukemia (AML) cell lines. Robust anti-tumor activity was achieved in AML xenograft models at a single 0.5 mg/kg (antibody) dose. Improved activity of IMGN779 against primary patient cells relative to AVE9633 (maytansinoid) was also demonstrated. IMGN779 was well-tolerated in CD-1 mice, with no observed hepatotoxicity or prolonged toxicity (40 mg/kg single dose). An IND for this drug was accepted recently, and a first-in-human trial is expected to begin before the end of Q2 2016.

Innovation continues on the IGN ADC platform, with data presented on 2 novel compounds, "D1" (disulfide linkage) and "P1" (cleavable peptide L-Ala-L-Ala), that demonstrated higher in vitro potency and in vivo efficacy relative to the IGN utilized in IMGN779, plus a maintained or increased therapeutic window in mouse models.

Antibody drug conjugates: Advancements in drug, linker and conjugation technologies

Peter Senter (Seattle Genetics) discussed advancements in drug, linker and conjugation technologies Hodgkin Lymphoma (HL) and systemic anaplastic large cell lymphoma (sALCL) are CD30 positive diseases with unmet needs with~9000 and ~5000 per year in the US, respectively. The chimeric antibody cAC10 targets CD30 and is rapidly internalized upon binding, which makes it a suitable candidate antibody to be used in an ADC format. Brentuximab vedotin consists of the cAC10-directed antibody and the microtubulin inhibitor MMAE linked via cathepsin cleavable valine-citrulline linker. A clinical efficacy comparison of the anti-CD30 ADC brentuximab vedotin and the unconjugated anti-CD30 antibody demonstrated 75% vs 0% OR, respectively, clearly favoring the ADC approach. The toxicity profile of anti-CD30 ADC was discussed briefly, with neutropenia and peripheral neuropathy as the main observed toxicities. This can be linked to the fact that nerve cells are more sensitive to microtubulin inhibition drugs than other cells. Next, data on the stability of the ADC was presented. The vc-MMAE product exhibited a half-life of approximately 1 week in mice and monkeys. It was suggested that MMAE in serum is partly responsible for the demonstrated toxicity profile.

To improve the toxicity profile, efforts were made to avoid the reversible fragmentation reaction of maleimide taking place, which results in detachment of the toxin from the antibody and thereby causing unwanted side effects. Avoiding this reaction resulted in a more stabilized ADC, showing enhanced efficacy and an improved toxicity profile. As an example a decrease in neutropenia in rats was shown. Another improvement was made by reducing the hydrophobicity of the ADC product, which could be an underlying cause of accelerated clearance. The drug-linker was engineered such that unnecessary hydrophobic elements were eliminated, which resulted in an improved PK profile and increased therapeutic window. Implementing a more hydrophilic MMAE product with a DAR of 8 resulted in a higher efficacy than the DAR 4 variant, which was the opposite for the hydrophobic MMAE product. Further improvements were made by reducing the heterogeneity of ADCs. Initial ADC products consisted of a mixture of species with a 0-2-4-6-8 DAR range. The average DAR of 4 is reproducible and high yielding, but more homogeneous products are

desirable for future ADCs. To achieve this, initial attempts to modify the glycan structure for conjugation purposes resulted in active, but unacceptable heterogeneous products. Integrated engineered cysteine residues that are buried in the cavity of the Fc portion resulted in more homogeneous and stable ADCs. This novel technology is now used for the new drug SGN-33A (CD33-PBD ADC) targeting acute lymphoblastic leukemia. SGN-33A demonstrated an improved IC50 over gemtuzumab ozogamicin (Mylotarg®) and ongoing results from the Phase 1 clinical study were presented. Overall, the results presented demonstrated new technological and application advancements for ADC technology.

Calicheamicin antibody-drug conjugates and beyond

Puja Sapra (Pfizer) took the stage to discuss calicheamicin ADCs and other novel payload agents.

Novel ADC against tissue factor for treatment of solid tumors

Next, **Bart de Goeij** (Genmab) presented a novel ADC product for the treatment of solid tumors targeting tissue factor (TF) and highlighting promising results from preclinical and first-in-human studies. TF is present in sub-endothelial cells in the blood vessel wall and upon vascular damage proteins in the blood stream that, upon binding to TF, can initiate the blood coagulation cascade to achieve closure of the ruptured blood vessel. TF expression can also result in PAR-2 activation in a normal situation. Aberrant expression is found in many tumor types (shown for e.g., prostate, cervical, bladder, and ovarian cancer). The critical steps for possible development of TF as an ADC format were investigated. Firstly, expression patterns and distribution of the target were assessed. Confocal microscopy analyses in non-stimulated tumor cells indicated that a large portion of TF is present in intracellular compartments (including lysosomes), indicating a high turnover of TF on tumor cells. This was in contrary to targets such as HER2 and EGFR, which are predominantly present on the plasma membrane. Flow cytometry analyses demonstrated that TF on plasma membrane is able to internalize without an antibody or a ligand being present. Antibody-induced down-modulation of surface-expressing TF was also demonstrated, which was not seen for the targets HER2 and EGFR. Secondly, the internalization rate of TF was assessed for the TF-011 antibody. Compared to HER2 and EGFR antibodies, TF-011 showed very rapid internalization and high levels of lysosomal trafficking.[19]

These results indicated TF is an interesting target for an ADC approach and next, a TF-vc-MMAE ADC was developed and tested for efficacy.[20] Xenograft data on HPAF-II (positive for TF expression) and PANC-1 (negative for TF expression) models were discussed and efficacy was demonstrated for the TF-expressing HPAF-II model. Further anti-tumor activity was shown in PDX models for lung adenocarcinoma (TF expression in 75% of cells) and cervical squamous cell carcinoma (TF expression in 25–50% of cells), also after paclitaxel treatment. Data from the cervical cancer PDX model implied a possible bystander effect of the ADC. The ability of TF-vc-MMAE to induce bystander kill was later confirmed by comparing bystander cell kill effects using MMAE versus MMAF.

Safety profiling in cynomolgus monkeys determined that anti-TF dosed up to 100 mg/kg had no effect on coagulant activities of TF; no differences on functional bleeding time in a Surgicutt assay, prothrombin time or activated partial thromboplastin time were exhibited when compared to the normal situation. IHC analyses of TF expression in healthy human and cynomolgus tissue indicated a wide-spread expression of TF. A toxicity profile was established by administering HuMax-TF-ADC once every 21 d (1, 3, and 5 mg/kg dose groups). Some skin reactions were apparent, which were severe at the highest dose but reversible. A drop in neutrophils was noted at all tested doses, and at 5 mg/kg the red blood cell count was slightly reduced. Overall a narrow toxicity profile was demonstrated with skin, bone marrow and testes as the only target organs. The GEN701 first-in-human dose-escalation study on locally advanced and/or metastatic solid tumors known to express TF demonstrated promising results. 24 patients were dosed up to 2.2 mg/kg with some dose-limiting toxicity apparent. A 1.8 mg/kg dose was well-tolerated, and an intermediate 2 mg/kg dose is now being tested. This study demonstrated encouraging anti-tumor activity in this heavily pre-treated patient population; 10 patients showed a stable disease response and 1 patient a partial response.

Track A: Bispecific antibodies: New technologies and applications

Matthias Pauthner

Paul Carter (Genentech) chaired the afternoon session of Track A, which focused on bispecific antibodies.

Optimization and application of Fc-containing bispecific antibodies

John Desjarlais (Xencor) presented a plug-and-play bispecific platform for generating bsAb with long serum half-life. Xencor has developed a new platform for long-lived and easy to manufacture bispecific antibodies. This platform was applied to rapidly produce CD3 bispecific antibodies targeting CD123 for AML, CD20 for B cell malignancies, and CD38 for multiple myeloma. Each antibody is shown to be potently active in non-human primate studies, and manufacturing at GMP scale is straightforward with yields greater than 2 g/L. Application to multiple formats and target combinations was also discussed.

Facile generation of common light chain bispecific antibodies

Eric Krauland (Adimab) surveyed Adimab's technology for generating common light-chain (cLC) bispecific antibodies (bsAbs). BsAbs based on the IgG format must contend with dimerization events between the heavy chains (HCs) and light chains (LCs). Most technologies used to solve this so-called light-chain pairing problem in a single host cell production setting (e.g., knobs-in-holes, domain swaps, orthogonal Fab interfaces or electrostatic steering) come with the potential disadvantage of introducing mutations into otherwise human

Downloaded by [108.49.191.210] at 11:34 25 March 2016

Downloaded by [108.49.191.210] at 11:34 25 March 2016

sequences. To circumvent this problem, cLCs in both Fab arms present an elegant solution.

Dr. Krauland opened his talk by underlining the theoretical feasibility of cLC discovery for a wide array of targets. Analysis of over 100 antibody-antigen co-crystal structures revealed only 30% LC CDR contributions to binding energy, opposed to 70% contributed by HCs. Further, HC CDRs are more mutated than LC CDRs, in fact CDR L3s often deviate very little from their germline sequences, which is also reflected in the pool of USAN/WHO-INN designated mAbs: as of 2014, there were 14 pairs of mAbs against unrelated targets, but identical CDR L3 sequences.

Dr. Krauland then explained the details of Adimab's cLC discovery platform, which is centered on full IgG libraries with separate genetic elements for HCs and LCs. This feature greatly expedites the library generation for HCs with restricted LC diversity, both if one target mAb is already known (10^4-10^7 HC diversity, 1 week) or for *de novo* identification of 2 HC specificities with a predetermined restricted set of cLCs (10^{10} HC diversity, 1–3 months). Further, known or discovered HC specificities can be shuffled into small (<10) cLC libraries for comparative identification of LCs that work effectively with all desired HCs. A subsequent single round of affinity maturation yielded nanomolar IgG binders in this scenario, which are ready for immediate bsAb formatting.

In the last section of his talk, Dr. Krauland outlined Adimab's in-house purification approach for bsAbs. Consistent with Adimab's overall strategy of keeping mAb constant regions unaltered, the company opted for a purification strategy to enrich correctly paired bsAbs rather than introducing heterodimerization mutations in the Fc region. The purification starts with a Protein A (MAb Select Sure) enrichment, followed by separation of the bsAb species based on intrinsic V_H charge variation using ion-exchange chromatography (Mono S/Mono Q). Starting with a wide linear elution gradient of pH4-11, specificity and yield were strongly increased by closing in on the desired elution pH range. This method was used to purify bsAbs in which the isoelectric point of each V_H varied by less than one-half unit, and with sensitivity down to a single positive to negative charge inversion. Dr. Krauland closed with a case study of an Adimab produced EGFR x HER2 cLC bsAb, which was enriched to >99% purity, retained picomolar affinity to both targets and passed ELISA/FACS based dual-binding characterizations.

Insights into the molecular basis of a bispecific antibody's target selectivity

As discussed by **Yariv Mazor** (MedImmune), dual targeting is thought to enhance biological efficacy, limit escape mechanisms, and increase target selectivity via a strong avidity effect mediated by concurrent binding to both antigens on the surface of the same cell. However, factors that regulate the extent of target selectivity are not well understood. It was shown that dual targeting alone is not sufficient to promote efficient target selectivity. Substantial roles played by the affinity of the individual arms, overall avidity and valence were shown. These findings have important implications on the development of clinically relevant bsAbs.

Efficient production of bispecific IgG in a single host cell

Yiyuan Yin (Genentech) discussed novel protein-engineering based solutions to the light-chain pairing problem, which severely reduces yield in single host cell bsAb production systems. Assuming correct HC pairing, co-expression of light chains that lack intrinsic binding preferences to their cognate HC theoretically only yield 25% correctly paired bsAbs, thereby greatly increasing production cost. Other solutions to this problem include the use of common LCs and domain-swapped Fab arms, while engineering-based approaches utilize knob-in-hole (KIH), electrostatic steering, modified disulfide bonds or complete orthogonal interface design to create specifically interacting HC/LC pairs, thus increasing bsAb production yield.

Dr. Yin started out by introducing the test system for her engineered design, an anti-HER2/CD3 bsAb. To generate HC/LC paring specificity, both mutations in the C_{H1}/C_L interface (1 knob/1 hole, 2 knobs/2 holes - up to 10 residues in both chains altered) as well as engineered charge pairs in the V_H/V_L interface (e.g., Q39 E/Q38 K) were designed and optimized using computation heavy Rosetta modeling. The top hits were than expressed, Protein A or G purified and tested for yield, stability and bsAb functionality using sandwich-ELISAs.

In summary, Dr. Yin's group was able to identify multiple C_{H1}/C_L as well as V_H/V_L interface mutations that were transferable between different bsAb constructs and greatly increased yield, without deteriorating binding characteristics. While wild-type bsAb yield is dependent on the actual specificities - ranging from ~25% (HER2/CD3) to ~90% - the discovered mutations were able to strongly increase yield from 25% to >90 % (HER2/CD3), and for other bsAb specificities up to 100%, thereby providing a great solution for cost-effective single host cell bsAb production. Possible disadvantages to engineering-heavy bsAb designs may include reduced serum half-life due to anti-bsAb immune reactions; however, this remains to be clinically investigated.

Nanobodies as a versatile and clinically validated approach for bispecific development

Antonin de Fougerolles (Ablynx) commenced his talk by introducing Ablynx's proprietary Nanobody® (Nanobody is a registered trademark of Ablynx NV) technology. Nanobodies are derived from the antigen binding V_{HH} domains of heavy chain only antibodies found in llamas and other camelids. These very small 12–15 kDa binding domains offer many advantages, including they 1) can be easily stringed together for increased functionality or binding avidity, 2) are able to bind challenging targets like GPCRs and ion channels, and 3) can be produced robustly and cost effectively at high yield in microbial cells due to absence of canonical N-linked glycosylation sites. Ablynx's discovery process starts by either immunizing llamas with antigens and retrieving the V_{HH} domains or starting from proprietary Nanobody phage libraries. Upon selection and screening, single V_{HH} domain Nanobodies, typically in the low nM and pM binding range, are isolated to the desired target of interest. Subsequently, different Nanobodies can be rapidly formatted into multi-valent or multi-specific

constructs as needed, produced, and evaluated in pre-clinical or clinical tests.

The extraordinary small size and robustness of Nanobodies allow for uniquely modular combinations of identical or heterologous Nanobodies with short GS-linkers. For example, by adding an anti-human serum albumin-binding Nanobody to a string, the serum half-life in humans can be dramatically increased from ~2–18 hrs to 10–20 d. However, the very short serum half-life of unmodified Nanobodies can also be exploited if rapid renal clearance is desired, which is e.g., the case for caplacizumab, Ablynx's in-house Phase 3 clinical candidate designated for the treatment of acquired thrombotic thrombocytopenic purpura.

The modular stringing together of Nanobodies also enables the generation of multi-valent, bi-paratopic and multi-specific conjugates. For example, the tri-valent RSV binding Nanobody, ALX-0171, shows increased potency, with5-fold more clinical isolates neutralized below the lower limit of detection than with palivizumab. Another interesting application is the generation of bi-paratopic conjugates, in which 2 non-competing Nanobodies binding the same antigen are linked together. In one case-study, an anti-IL23 bi-paratopic Nanobody showed a 25-fold increase in in vivo biological activity over bi-valent Nanobodies or benchmark mAbs. Finally, the Nanobody platform also allows the facile generation of bi-, tri- or multi-specific conjugates. In addition to common oncology BsAb applications, Dr. de Fougerolles presented an impressive 160-fold increase in HIV neutralization potency of a CXCR4/CD4 bi-specific Nanobody over an equal mixture of the 2 mono-specific Nanobodies. Other applications for the technology include increased cell targeting specificity for immuno-oncology setting, in which 2 heterologous Nanobodies identify and bind the correct target cell while additional linked Nanobodies can carry out effector functions, like recruiting T cells (anti-CD3).

Bispecific TCR-based reagents for targeted cancer immunotherapy

Bent Jakobsen (Immunocore) discussed ImmTACs, which are bispecific reagents that target tumors via a soluble monoclonal TCR with exceptionally high sensitivity and specificity and redirect host polyclonal T cells via an anti-CD3 antibody fragment. Emerging data from the first ImmTAC to enter Phase 1/2a clinical trials demonstrated durable partial responses in patients with advanced melanoma.

Track B: Antibody therapeutics for non-cancer indications

Joost Bakker

Trudi Veldman (AbbVie Bioresearch Center) chaired the afternoon's Track B session.

ESBA1008 (RTH258, broculizumab), a highly stable scFv for treatment of age-related macular degeneration

ScFv have potential advantages, such as better penetration, durability, and fast clearance, which lead to better safety. However, they are known to be highly unstable and tend to aggregate. **Patrik Maurer** (ESBATech, a Novartis company) and colleagues solved this problem by use of highly stable human frameworks for grafting of CDRs. Broculizumab is an anti-VEGF scFv with rabbit CDRs. The outer loop looks different though no decrease in binding affinity was found. Broculizumab is thermo-stable, and high concentrations can be used for application in the eye. Broculizumab is stable at 4°C for at least one year. Competitors in the field are bevacizumab, ranibizumab and aflibercept.

In in vitro experiments, tissue penetration was higher with broculizumab than with competitors, and no toxicity was observed in animals. Lucentis® (ranibizumab) works well in treatment of age-related macular degeneration, but has to be injected monthly. Treatment with broculizumab might lead to longer duration of efficacy. In a Phase 2 study, efficacy of broculizumab was measured using optical coherence tomography (OCT), which measures leakage and changes in the thickness of the retina. Compared to ranibizumab, broculizumab leads to 30-day prolongation of treatment effect. Compared to aflibercept, the same improvement in sharp sight and reduction of retinal thickness were observed, but with a 3-monthly vs. a monthly injection interval.

A Phase 3 clinical study is currently ongoing and is expected to be completed in 2018. This trial consists of a head-to-head comparison against aflibercept and different dosing schedules. The ESBATech technology for stabilizing scFv molecules appears to work well. Furthermore, no increase in ADA development was observed. This is an important observation since the eye is not completely protected against injection of foreign proteins.

Therapeutic anti-citrullinated protein antibodies reduce inflammation by inhibition of NETosis

Conversion of peptidyL-arginine into peptidyl-citrulline (citrullination) leads to altered protein refolding and loss of function of proteins. Proteins can become autoantigenic after such conversion. A strong link to arthritis in rheumatic arthritis (RA) patients and anti-citrullinated antibodies exists.

Neutrophil extracellular traps (NETs) are associated with a unique form of cell death, distinct from apoptosis or necrosis, whereby invading microbes are trapped and killed. Neutrophil extracellular traps can contribute to autoimmunity by exposing autoantigens, inducing cytokine and chemokine production, and activating the complement system. Therapeutic anti-citrullinated protein antibodies (tACPA) have been identified from RA patients and bind specifically to the conserved citrullinated N-terminus of histones H2A and H4. tACPA binding of these targets in the early stages of NETosis blocks NET formation and thus blocks autoantigen exposure.

Jos Raats (ModiQuest) discussed the company's lead antibody, which is a humanized IgG1 that binds to histones H2A and H4. Different assays for NETosis inhibition have been used, in which MPO/NE activity and immunofluorescence staining showed that NETs formation is strongly reduced after addition of tACPA, but not after adding an isotype control antibody. NETs have been detected in RA joints where they add to the severity of the disease. tACPA treatment was

Downloaded by [108.49.191.210] at 11:34 25 March 2016

Downloaded by [108.49.191.210] at 11:34 25 March 2016

investigated in 2 different RA mouse models. RA inflammation was completely prevented or strongly reduced by tACPA in an early treatment model, whereas, in a therapeutic model, tACPA blocked the increase in inflammation. Moreover, combination therapy with tACPA and dexamethasone demonstrated that inflammation could be reversed completely. With dexamethasone only treatment, severe relapses occurred quickly within a few days after stopping dexamethasone treatment, whereas, in tACPA treated animals, after stopping dexamethasone treatment, relapses occurred much later and inflammation levels were much milder.

Histology data demonstrated that tACPA treatment protected the animals against massive cartilage and bone destruction. NETs are also linked to fibrosis, inflammatory bowel disease, acute lung injury, and cystic fibrosis. Prophylactic tACPA treatment in a pulmonary fibrosis animal model showed protection against fibrosis in the lung. Clinical proof of concept is expected in 2018/2019.

Predictive biomarkers for response to therapy in IBD

A challenge in drug development for irritable bowel diseases has been identifying patients likely to respond to novel therapies. **Mary Keir** (Genentech) discussed results from a Phase 2 study of etrolizumab, a humanized monoclonal antibody that selectively binds the $\beta7$ subunit of the heterodimeric integrins $\alpha4\beta7$ and $\alpha E\beta7$, showed clinical benefit in moderate-to-severely active ulcerative colitis. Post-hoc analysis showed enrichment of clinical remission in αE-high patients, suggesting that baseline αE levels may be predictive of etrolizumab response.

Targeting therapeutic and regenerative biomedicine specifically to arthritic joints

As discussed by **Ahuva Nissim** (Queen Mary University), executed studies have focused on developing a panel of human antibodies that bind specifically to collagen type II post-translationally modified by reactive oxidants (ROS) present in the arthritic joints. ROS are known to play an important role in the disease, and different forms and subforms are present during inflammation. The aim has been to validate their potential targeting for immunotherapy and early diagnosis of arthritis. Importantly, the goal of these new immunotherapeutics is to reduce side effects, increase the response rate, and treat early stage arthritis.

Collagen type II (CII) is a major component of articular cartilage and thus a prominent target of ROS. Antibodies against CII modified by ROS (ROS-CII) were generated and the lead clone has been shown to bind to all forms of ROS-CII (1–11E), human RA cartilage and osteoarthritis (OA) cartilage. Furthermore, the selected clone also binds to cartilage from mice models of arthritis.

Interestingly, fusion of 1–11E clone to etanercept via a matrix metalloproteinase-cleavable peptide linker will result in release of etanercept at the inflamed site, which could locally inhibit TNF-induced inflammation. Indeed, 1–11E fused to etanercept significantly reduced inflammation in arthritic mice, as compared with etanercept alone or etanercept fused to an irrelevant antibody. Also, fusion of 1–11E with viral interleukin (IL)-10 restricted biological activity of viral IL-10 after it has been released from the antibody following matrix metalloproteinase cleavage and thus might reduce inflammation explicitly in the arthritic joints. Indeed, viral IL-10-fused protein induced inhibition of inflammation in mice with inflammatory arthritis; and a reduction in ROS in inflamed knees was observed. In addition, the viral IL-10-fusion protein was able to reduce pro-inflammatory cytokines. The drug can be administered systemically, but the drug is only active locally, at target sites.

There is an unmet need for the development of diagnostic tools for early OA. To investigate whether the selected clone could be used for this purpose, an OA mouse model with destabilization of the medial meniscus (DMM) was used. Histology data demonstrated staining of OA cartilage ahead of evident cartilage damage. The clone can therefore also be used to detect early OA even before evident cartilage damage. Retention of the clone was found to be 4–8 weeks after surgery. Antibody was found only locally in the DMM joints, and not in other areas. This is due to specific binding to arthritic cartilage, as has been observed with histology.

In conclusion, this strategy may potentially have a significant effect on the treatment efficacy and modality for RA. In addition, the selected clones may be developed as an imaging biomarker for early OA diagnosis. Since the antibodies cross-react with small animal tissue, this may facilitate development of disease modifying osteoarthritis drugs.

Bispecific FynomAbs unlock new biology

Ulrich Wuellner (Covagen) discussed the company's development of bispecific FynomAbs by fusing its Fynomer binding proteins to antibodies, resulting in multi-specific therapeutics with novel modes-of-action and enhanced efficacy. FynomAbs have optimal biophysical and pharmacokinetic properties, making them attractive as drug candidates. Here they presented the discovery and development of COVA322, a clinical-stage bispecific TNF/IL-17A inhibitor for the treatment of inflammatory diseases. Dual cytokine inhibition holds great promise to substantially improve current therapies.

ABT-122, an Anti-TNF/IL-17 dual variable domain immunoglobulin (DVD-Ig), mechanisms of translation: Bench to bedside and back again

Chung-Ming Hsieh (AbbVie) discussed ABT-122, an anti-TNF/IL-17 DVD-Ig that is currently in Phase 2 trials for rheumatoid and psoriatic arthritis. To better understand its mechanisms of action and identify potential biomarkers, gene array analysis was performed in a mouse arthritis model followed by evaluation of protein expression in healthy volunteers after a single dose of ABT-122.

In vitro, TNF and IL-17 synergistically upregulate synthesis of chemokines, cytokines, and MMP. Combined treatment of RA mice with anti-TNF and anti-IL-17 results in superior effectivity on RA. We identified multiple novel fully human antibodies to IL-17 by mRNA display technology in vitro. From a dsDNA antibody library, an antibody mRNA library was generated. Using in vitro translation, oligo dT and FLAG

Downloaded by [108.49.191.210] at 11:34 25 March 2016

purifications an mRNA-scFv library was developed. IL-17 antigen was subsequently used for antibody selection. Yeast surface display was used for antibody discovery and engineering. After IL-17 antibodies were affinity-matured, DVD Ig molecules were constructed. Affinity of TNF and IL-17 was measured to be in the 10 to 50 pM range respectively. The half-life of the construct is approximately 14 d in rats.

ABT-122 was investigated for potential use as a biomarker. In an ascending dose study in volunteers, no clinically adverse events were observed, after biweekly subcutaneous injections. Furthermore, ABT-122 fully neutralized TNF and IL-17 in a potency assay, as measured by IL-6 production. Neutralizing activity was present up to 3 weeks after administration to volunteers, and the drug was well tolerated.

Next, it was investigated if ABT-122 affects chemokines, and if these could be used as biomarkers. ABT-122 modulates CXCL10 and CCL23, which are responsible for recruitment of immune cells to the site of inflammation. The effects of ABT-122 on these chemokine receptors were assessed using blood samples from volunteers. Seven days after injection, a reduction in CXCR4 and increase in CXCR 5 was observed. The functional response of immune cells was also investigated and demonstrated a decrease in gm-CSF response.

Track C: Site specific conjugations, novel chemistries and payloads

Joost Melis

John Lambert (ImmunoGen) chaired the afternoon session of Track C, which focused on site-specific conjugations, novel chemistries, and payloads for ADCs.

Site-specific antibody-drug conjugates built on a selenocysteine interface

The first speaker, **Christoph Rader** (The Scripps Research Institute), presented data on site-specific ADCs built on a selenocysteine interface. One of the possibilities to improve an ADC is incorporating site-specific conjugation instead of using random drug conjugation. This can be achieved by making use of natural or unnatural amino acids via chemical or enzymatic conjugation. Selenocysteine (Sec) is the 21st natural amino acid, is analogous to cysteine and serine, but has a selenium-containing selenol group in place of the sulfur-containing thiol group when compared to cysteine. Sec is larger, more nucleophilic and has a lower reduction potential in comparison to cysteine (pKa 5.2 vs 8.3, respectively). Sec is co-translationally incorporated into proteins by recoding the UGA codon. Sec insertion in eukaryotes requires the presence of the Sec insertion machinery and a Sec insertion sequence (SECIS) element. A method was developed for mammalian cell expression of the human IgG1-derived Fc protein with a C-terminal Sec residue by introducing an engineered SECIS element into an expression cassette. This genetically engineered Fc protein displays a unique chemical reactivity that enables selective conjugation to a variable small synthetic molecule at the Sec interface. The platform allows generation of recombinant IgG, Fab, scFv, and IgM with a C-terminal Sec residue, which makes it possible to develop ADCs with a DAR of 1. Sec incorporation is not complete, only 1 in 5 products incorporate a selenocysteine, the other 4 contain stop codons. Still a laboratory-scale production of 4 mg/L is feasible. Selenomabs allow selective conjugation for several formats, although stability issues arose when using maleimide. Using iodoacetamide solved this stability issue. MMAF derivatives can be linked using iodoacetamide, which results in an average DAR of 0.6. This limited DAR still demonstrated efficacy as was shown testing an anti-HER2 ADC in HER2-positive and -negative cell lines. In vivo target-directed killing of the anti-HER2 ADC was also established in a KPL-4 orthotopic xenograft nude mouse model at 1 and 3 mg/kg. Additionally, an anti-CD138 ADC exhibited in vitro efficacy in U266 and H929 cell lines and in vivo target-directed kill in an U266 systemic xenograft mouse model. To improve the low DAR, Sec was moved upstream into a C_H3 position that required Sec incorporation into both heavy chains for functional selenomab assembly. HIC analysis confirmed a DAR of 2 and yield reached 2 mg/L. The technology can also be combined with the Thiomab platform, resulting in a thio-selenomab dual warhead strategy. Firstly, Sec-conjugation occurs under pH 5.2, followed by Cys-conjugation at pH 7.4.

Hapten-directed spontaneous disulfide shuffling: A universal technology for site-directed covalent coupling of payloads to antibodies

Next, **Eike Hoffmann** (Roche) presented a lecture entitled hapten-directed spontaneous disulfide shuffling: a universal technology for site-directed covalent coupling of payloads to antibodies. Individual analyses of known structures of digoxigenin and fluorescein binding antibodies and a new structure of a biotin-binder revealed a "universal" coupling position in proximity to binding pockets, but without contributing to hapten interactions. Hapten-binding antibodies with an accessible cysteine in proximity to the binding pocket were designed to covalently attach payloads to the antibody. Payloads that carry a free thiol are positioned on the antibody and covalently linked to it via disulfides by spontaneous redox shuffling. Attachment at the universal position works with different haptens, antibodies, and payloads and can be achieved by 15 minute incubation of the antibody and the payload in equimolar ratios. Applications include modulation of pharmacokinetics of small compounds as well as payload linkage to targeting vehicles in a reduction-releasable manner.

Antibody pyrrolobenzodiazepine conjugates

Philip Howard (Medimmune/Spirogen) presented work on antibody pyrrolobenzodiazepine conjugates (APCs). Several APCs are currently undergoing clinical investigation. Seattle Genetics is developing an APC targeting CD33 for treatment of AML, plus an APC targeting CD70 in NHL/RCC. StemCentrx is using an APC directed at DLL3 in SCLC and CisR in ovarian cancer. Pyrrolobenzodiazepine (PBD) dimers are sequence-selective DNA minor-groove binding crosslinking agents that block replication and result in cell death, which was verified here by results of an adapted Comet-assay. PBDs are extremely potent (picomolar range) and are not cross resistant with cisplatin, making them clinically interesting payload agents. The

Downloaded by [108.49.191.210] at 11:34 25 March 2016

structure-activity relationships of PBD were discussed. For example, the sugar moiety and electron donators enhance activity and bulky substituents can reduce or abolish activity of a PBD. The PBD payloads tesirine and talirine were highlighted and exemplifying efficacy and safety data was presented. The HER2-SG3249 APC treatment in a NCI N87 gastric carcinoma xenograft model resulted in complete tumor regression at a dose of 1 mg/kg. Promising tolerability data demonstrated no delayed weight loss in mice yet (up to 78 days). The APC was compared to T-DM1 in a BT474 model and APC treatment at 1 mg/kg showed complete tumor regression thereby outperforming T-DM1 at 1 mg/kg. Efficacy was also demonstrated in low HER2-expressing models such as the JIMT-1 model, again showing complete tumor regression with no delayed weight loss in the animals at a 1 mg/kg dose. Antibodies directed at CD25 (component of IL2 receptor - IL2Ra) as a target of hematological malignancies using a PBD payload (also known as ADCT-301) showed good in vitro and in vivo activity. For example, complete tumor regression was established at a 0.5 mg/kg dose in a Karpas 299 Anaplastic large cell lymphoma in vivo model, thereby outperforming brentuximab vedotin (Adcetris®).

Subsequently, the collaboration with StemCentrx in small cell lung cancer (SCLC) and large cell neuro-endocrine carcinoma (LCNEC) was discussed. Data of the APC rovalpituzumab tesirine (SG3249 linker with SG3199 PBD dimer) targeting DLL3 was presented. DLL3 expression in lung tissue and several lung cancers was shown and in vivo activity appeared to correlate with DLL3 expression. In vivo data discussed included complete tumor regression shown up to 150 d in a high expressing model at 1 mg/kg dosing. Also tumor initiating cells are decreased by the APC treatment. In a Phase 1 trial, rovalpituzumab tesirine showed a 44% ORR and 78% clinical benefit rate in DLL3 high expressing patients and 23% ORR and 68% clinical benefit rate in all SCLC patients. A Phase 2 trial is expected to be initiated shortly.

Targeted protein therapeutics for the loco regional and systemic treatment of cancer

After several short oral poster presentations, **Gregory Adams** (Viventia Bio) presented work on targeted protein therapeutics (TPTs) for the loco regional and systemic treatment of cancer. TPTs are fully biologic fusion constructs containing antibody fragments, a serum stable linker, and protein toxin payloads. ScFvs, Fabs or diabodies are herein predominantly used for targeting purposes. The conjugates exotoxin A or deBouganin toxin can be linked to the antibody moiety by either non-cleavable or furin-cleavable linkers. Normal tissues express only low levels of furin, which therefore should make the furin-cleavable TPT predominantly active in cancer cells. The current pipeline includes the locally-administered TPTs vicinium and proxinium, both conjugated to an exotoxin A payload to target EpCAM-expressing cells in high-grade non-muscle invasive bladder cancer (NMIBC) and squamous cell carcinoma of the head and neck (SCCHN), respectively. Systemically-administered TPT VB6-845 d targeting EpCAM in solid tumors using a deBouganin toxin was also discussed in more detail.

NMIBC accounts for 70–80% of all bladder cancers. The initial treatment for this stage of bladder cancer is currently surgical removal of the tumor and possible adjuvant treatment by Bacillus Calmette-Guerin. New drugs for treatment are preferred and since EpCAM overexpressed in >97% of high grade NMIBC, this creates a window of opportunity for vicinium. Phase 1/2 results demonstrated a 41% CR at 3 months, no dose limiting toxicity, and no MTD was reached during this study. PK studies indicated that vicinium is not systemically absorbed, but is contained within the bladder following intravesical instillation. A subsequent Phase 2 study also showed promising efficacy results: 40% CR at 3 months, 17% overall CR at 12 months in the 12 week treatment arm and 13% overall CR at 12 months in the 6 week treatment arm. The median time to recurrence was 408 d and 274 d for the 12 week and 6 week arm, respectively. The Phase 3 status and future plans were discussed, which showed that the treatment regimen was altered without the necessity of a bridging study.

Proxinium is being evaluated for treatment of SCCHN, which is the 7th most common cancer in the world, with 130.000 new cases per year and the current therapy being highly invasive. The Phase 1 trial for this drug resulted in a 13% CR and 40% PR of evaluable patients, with an overall tumor control rate of ~80%. In a Phase 2/3 study with 166 patients, 40% increase of survival was noted by 6 wks, but the trial was terminated prematurely. Proxinium has orphan drug status in both the US and Europe and has been designated as a Fast Track product by the FDA. A new pivotal Phase 3 study is expected to be initiated by the end of Q1 2016.

Besides exotoxin A, deBouganin toxin is used as a payload, which is a highly potent plant toxin in the picomolar range that has been successfully de-immunized, while maintaining its potency. The toxin was compared to DM1 and deBouganin toxin was more potent against 3+ HER2 breast cancer cell lines and demonstrated a superior therapeutic window, thereby limiting the potential for off-target toxicity. Additionally, the deBouganin toxin is unaffected by multidrug resistance (MDR) pumps, cannot bind and enter cells by itself and is only cytotoxic when internalized. VB6-845, directed at EpCAM and using deBouganin toxin as payload, was investigated in Phase 1 trials for treatment of solid tumors. Initially, no immunogenicity was observed for the toxin itself, however the Fab-region of the product caused immunogenicity and the trial was therefore terminated after 4 weeks. Five serious adverse events (AEs) were reported, of which 2 were related to the treatment (both infusion reactions). The VB6-845 Fab has now been de-immunized and a non-human primate study and Phase 1/2 study are expected for the future.

Wednesday december 9, 2015: Track A: Immunotherapeutic antibody mechanisms

Chris Ullman

Combination immunotherapy for synergistic innate and adaptive anti-tumor immunity

Dane Wittrup (Massachusetts Institute of Technology) chaired the session and was the first speaker. He first noted that, in the

Downloaded by [108.49.191.210] at 11:34 25 March 2016

past, understanding of immunotherapy was poor because xenograft models lacked T cells. In these models, innate effectors cells expressing FcγR were shown to be required for the therapeutic effect of monoclonal antibodies, consistent with the mechanism of ADCC. However, these effects diminish when $CD8^+$ cells are depleted in immunocompetent mice with isogenic tumors indicating a major role of the adaptive immune system in addition to the innate. Therefore, Prof. Wittrup and colleagues are investigating methods that bolster both the innate and the adaptive immune response using IL-2. IL-2 administered in combination with mAbs has performed poorly due to the rapid clearance of IL-2, but an extended half-life format of IL-2 fused to Fc (Fc/IL-2) has shown synergistically activity with antibody therapy in 4 different tumor models. The Fc/IL-2 molecule fusion is a single murine IL-2 molecule fused with murine Fc IgG2a containing a D265A mutant, which abrogates complement and Fc gamma receptor binding. Use of Fc/IL-2 demonstrated benefit of monotherapy and led to rapid lymphocyte infiltration at the periphery and within the tumor mass and necrosis, with large increases in T cell populations and NK cell populations. Nearly all cytokines were elevated indicating a cytokine storm, in particular IL-6, IL-1α, IL-1β, and neutrophil-related factors such as G-CSF and MIP-2, causing inflammation and tumor killing through activation of intratumoral NK cells and intratumoral $CD8^+$ T cells. However, T cells alone contributed significantly to the anti-tumor response, producing IFNγ in response to therapy. In order to prove their importance, the numbers of tumor specific CD8+ T cells (Pmel-1) were increased through adoptive transfer in combination with Fc/IL-2 and TA99 a monoclonal against TYRP-1 (a melanocyte marker that becomes surface-expressed on B16F10 melanoma in mouse models). This treatment produced 23/25 cures and immunological memory in a mouse B16F10 melanoma model.[21,22]

The Fc/IL-2 fusion protein has been formulated into a 4 component vaccine (named AIPV) that includes TA99 mAb, Fc/IL-2, anti-PD-1 and a peptide vaccine CD8 T cell epitope derived from a melanocyte differentiation antigen tyrosinase-related protein 2 (TRP2180-188). This immunotherapy seeks to provide an adaptive-centric (lymph node targeting vaccine and anti-PD-1 checkpoint blockade) and innate-centric (anti-tumor antibody and Fc/IL-2) method for cancer treatment. This was demonstrated to successfully treat B16F10 melanoma and TC-1 cervical cancer syngeneic mouse models. Specifically, there was a progressive increase of infiltrating T cells, a large increase in T_{eff}/T_{reg} ratios and stable levels of T cells over 75 d or more. The serum from AIPV-treated mice also displayed responses to many tumor antigens and antigen presentation on dendritic cells was critical for the success of AIPV therapy, as demonstrated in *Batf3−/−* mice. In the melanoma B16F10 melanoma metastasis model, there was a 100% response. Therefore, the conclusion of the study was that the vaccine functions on the tenet that Fc/IL-2 drives an essential T cell response, but the NK response is dispensable; anti-PD-1 sustains the response and peptide vaccine promotes the amplification of T cells. Opsonization of the tumor with antibodies also ultimately helps drive the T cell response.

Unmasking cancer to innate sensing by Anti-CD47 antibody triggers adaptive immune-mediated tumor destruction

The importance of the T cell response was re-iterated by **Yang-Xin Fu** (The University of Chicago), who stated that the adaptive T cell response is necessary for therapy; antibodies help to increasing the stress in tumor tissue through ADCC. In some patients, it is apparent that there is an endogenous anti-tumor lymphocyte repertoire that can be reactivated for therapy by immune modulators such as checkpoint inhibitors. CD47 is cell surface ligand that is highly expressed on stem cells and neoplastic cells providing a "don't eat me signal" to protect against phagocytosis, binding to signal regulatory protein α (SIRPα) on phagocytes. This results in phosphorylation of immunoreceptor tyrosine-based inhibitory motifs (ITIMs) on SIRPα and recruitment of Src homology phosphatases 1 and 2 (SHP-1 and SHP-2), both of which inhibit accumulation of myosin-IIA at the phagocytic synapse. Anti-CD47 antibodies can force the "don't eat" message of the cell into an "eat me" signal through blockade, leading to a potent anti-tumor effect that was initially believed to be mediated by macrophage phagocytosis in xenograft models lacking adaptive responses. However, in syngeneic tumor models in immunocompetent mice, the anti-tumor effects of CD47 blockade have recently been shown to be driven by dendritic cells (DC) that cross-prime T cells within the tumor microenvironment. Therefore, the therapeutic effect of anti-CD47 depends upon type I/II interferons, dendritic cells (DCs) and CD8+ cells, supporting the notion of requiring both the innate and the adaptive immune processes. Responses to these signals generate immunological memory in BALB/c mice inoculated with $CD47^+$ A20 B cell lymphoma cells.[23] In order to determine the underlying mechanism of DC cell activation, it was found that disrupting IFNAR1 receptor function on DCs abrogated the anti-CD47 response, whereas MyD88 and Trif activation were not necessary, suggesting the involvement of a toll-like receptor-independent mechanism. This action is orchestrated through a cytosolic DNA-sensing pathway by the endoplasmic reticulum–resident protein stimulator of interferon genes (STING), a signaling molecule directing the innate response to cytosolic nucleic acids following phagocytosis. Hence phagocytic uptake by DCs ultimately leads to production of type I IFNs causing stimulation of DCs in an autocrine and paracrine manner and cross-presentation of tumor-associated antigens to activate tumor-specific $CD8^+$ T lymphocytes.[23-25]

Another challenge is to understand why only a fraction of patients respond to checkpoint inhibitors and to determine whether tumor infiltrating lymphocytes (TILs) are a pre-requirement for an anti-tumor response. Tumors caused by mouse MC38 colon adenocarcinoma cells respond well to anti-PD-1 treatment and are known to be responsive to T cell infiltration, whereas tumors formed from the murine fibrosarcoma cell line Ag104Ld do not respond. This anecdotally suggests a role for T cell infiltration as Ag104Ld cells secrete high levels of suppressive TGF-β1. Indeed, the

Downloaded by [108.49.191.210] at 11:34 25 March 2016

beneficial anti-PD-1 effects were diminished by action of the drug FTY720 drug (fingolimod) that prevents T cell infiltration. Conversely, a TNF superfamily ligand, TNFSF14 (also known as LIGHT), can cause T cell infiltration and tumor regression by modulating T cell immune responses by signaling through herpes virus entry mediator (HVEM) and lymphotoxin β receptor (LTβR). LIGHT is predominantly expressed on lymphoid tissues, on the surface of immature DCs and activated T cells. Interaction of LIGHT with LTβR on stromal cells results in the upregulation of lymphoid chemokines and adhesion molecules, leading to the increased presence of lymphocytes in peripheral tissues. LIGHT can also serve as a critical ligand for the activation of NK cells. Intratumoral adenoviral delivery and expression of LIGHT can result in rapid rejection of tumors in a NK-dependent fashion through direct activation of tumor-specific cytotoxic T lymphocyte (CTL) proliferation and maturation. In addition, metastases initiated by 4T1 or Ag104Ld cells can also be eradicated by generation of a tumor-specific CTLs that exit the primary tumor site and infiltrate distal tumors.[26] LIGHT can be used in combination with monoclonal antibody therapy and checkpoint inhibition to cause T cell infiltration prior to anti-PD-1 treatment.

Activating and suppressive signals in the head and neck cancer microenvironment affects therapeutic mAb immunotherapy

The immune response in the clinical setting was described by **Nicole Schmitt** (Johns Hopkins University), based on analysis of cetuximab monotherapy and combination therapy administered to head and neck cancer patients. EGFR is overexpressed in over 90% of head and neck squamous cell carcinoma (HNSCC) cancer patients and, therefore, is an attractive target for cetuximab (an anti-EGFR mAb) therapy. However, HNSCC is an immunosuppressive disease, with low lymphocyte levels, impaired NK cell activity, and impaired ADCC. In addition, T_{regs} have been linked to HNSCC tumor progression. Therefore, immunomodulatory therapies that overcome immune suppressive signals in patients with HNSCC have therapeutic promise.[27]

Preliminary analyses indicate that PD-L1 is expressed in 50% to 60% of HNSCCs and that tumor infiltration by PD-1-positive T_{regs} may be more common for HPV-positive than HPV-negative HNSCCs in the tumor microenvironment. In addition, EGFR prevents IFNγ-mediated PD-L1 upregulation suggesting potential synergy through combination. Anti-PD-L1 is being tested in Phase 2 and Phase 3 trials against platinum refractory HNSCC and recurrent/metastatic HNSCC, anti-PD-1 is being evaluated for platinum-cetuximab refractory HNSCC in Phase 2 and both anti-PD-1 and PD-L1 are being separately tested against solid tumors (HNSCC) at Phase 1. Cetuximab therapy can increase the frequency of CTLA-4C Tregs and despite elevated levels of EGFR specific T cells intreated patients compared to na€patients, only 15% to 20% of patients respond. These facts support the use of CTLA-4 blockade in patients treated with cetuximab (Phase 1 trial). A small molecule TLR8 agonist (VTX-2337) and cetuximab, in combination with platinum/fluorouracil are being evaluated in a randomized Phase 2 clinical trial in first-line recurrent/metastatic HNSCC.[27]

Allogeneic IgG tumor therapy

Edgar Engleman (Stanford University School of Medicine) presented research supporting the importance of DCs and T cell responses in cancer immunotherapy, i.e., allogenic IgG tumor therapy. DC therapeutic vaccines have been successful in clinical trials for 5 disease indications, notably causing tumor clearance in a patient with metastatic colon cancer as well as some patients with non-Hodgkin's lymphoma. Sipuleucel-T (Provenge®; APC8015) is the first FDA-approved DC cancer vaccine, specifically for the treatment of prostate cancer. For sipuleucel-T, the vaccine preparatory process involves removing patient DCs and priming these cells with prostatic acid phosphatase (PAP), which is present in 95% of prostate cancer cells, and granulocyte-macrophage colony stimulating factor (GM-CSF) for maturation of the DCs, and then administering the primed cells back to the patient. The treatment can extend the survival of patients with metastatic, asymptomatic hormone refractory prostate cancer by 4.5 months, but has issues that include the cost and the complexity of manufacturing, poorly understood DC biology and susceptibility to immune escape and immunosuppression. New methods are being developed to activate DCs in vivo which take advantage of anti-tumor mechanisms of allo-immunity (an immune rejection of an allogenic tumor) and appear to avoid the immune escape commonly observed when autologous tumors are treated with therapeutic vaccines or monoclonal anti-tumor antibodies. Application of this phenomenon could provide the basis for inducing similar responses against naturally arising tumors. The mechanism in mice demonstrates the importance of anti-tumor IgGs that opsonize disease tissue and bind DCs to allow internalization and presentation of antigens to tumor reactive T cells. This was shown by the induction of potent anti-tumor immunity in vivo from bone marrow derived DCs (BMDCs) loaded with allogenic IgG immune complexes (IC), whereas BMDCs lacking FcγR failed to induce an anti-tumor response. However, additional stimuli were necessary to activate quiescent tumor associated DCs (TADCs) in situ. A combination of CD40L with TNFα enabled stimulation of TADCs and allo-IgG-IC uptake. Indeed, intratumoral injection of allogeneic IgG combined with TNFα + CD40L induced complete elimination of B16 and LL/2 tumors and almost complete eradication of lung metastases from 4T1 breast tumor models following injection into the primary site. [28]

The anti-tumor effect is explained as follows: TADCs are quiescent and not naturally responsive to IgG-IC, requiring stimulation to drive anti-tumor activity against autologous tumors bound by allogenic IgGs; CD40L and TNFα stimulate the DCs to internalize and present antigens, thus stimulating tumor specific T cell responses. In this process, allogenic IgGs enable higher levels of opsonization of tumor antigens. In conclusion, allogenic IgG (either natural or non-natural) administration and DC stimulation offer another mechanism to remove the brakes on the immune response as demonstrated by high-potency in preclinical mouse models of melanoma, breast, colon, pancreas, and lung cancer.

Downloaded by [108.49.191.210] at 11:34 25 March 2016

Therapy-induced antibody responses

Catherine Sabatos-Peyton (Novartis) continued the focus on checkpoint inhibition with a presentation looking beyond the blockade of CTLA-4/PD-1. Multiple strategies are emerging to target the immune system as exemplified through the complementary combination of nivolumab and ipilimumab for treatment of BRAF V600 wild-type unresectable or metastatic melanoma. However, the tumor microenvironment is complex and other checkpoint molecules, such as LAG-3 and TIM-3, are important for offering opportunities for therapeutic intervention, especially as other receptors are upregulated in PD-L1 deficient cells. Indeed, combinations of PD-1 and LAG-3 or PD-1 and TIM-3 blockade have proved to be synergistic and more effective than monotherapy. Both have functions to regulate the immune system: TIM-3 is inversely correlated with IL-12 production from TLR-stimulated macrophages, and LAG-3 is upregulated on natural and induced T_{regs}; LAG-3 T_{regs} are potent suppressors.

Sabatos-Peyton described a Novartis anti-LAG-3 antibody (LAG525) that binds to domain 1 of LAG-3 with picomolar affinity (K_D 109 pM) and to human LAG-3-expressing cells with a K_D of 1.92 nM and cynomolgus monkey LAG-3-expressing cells with a K_D of 2.3 nM. Anti-LAG-3 antibody blocked the interaction between LAG-3 and MHC class II molecules on Daudi cells with an IC50 of 5.5 nM, compared to a human IgG4 isotype control. Blockade of LAG-3 and PD-1 potentiates cytokine secretion. LAG525 is being tested at Phase 1/2b in combination with PDR001, anti-PD1 monoclonal antibody.

Anti-CD8 Immuno-PET detection of anti-tumor immune responses

Due to the critical role tumor-infiltrating cytotoxic CD8 T cells play in determining anti-tumor immune responses, an anti-CD8 antibody fragment was developed for immuno-PET detection of CD8 T cells. As discussed by **Richard Tavaré** (David Geffen School of Medicine at UCLA), anti-CD8 immuno-PET successfully detected alterations in tumor-infiltrating CD8 T cells in response to various methods of immunotherapy.

Track B: Target, target, target … it's all about the target

Thierry Wurch

This session was co-chaired by **Janice Reichert (Reichert Biotechnology Consulting LLC)** and **Janine Schuurman (Genmab)**. Dr. Reichert opened the session by presenting a brief but complete overview of the evolution of targets that are tackled by antibodies in current clinical trials and on the market. The main message was that, although there seems some clustering around a few targets, most of the targets to which these antibodies bind are unique. Some examples in the oncology field: 8 mAbs target the immune checkpoint axis PD-1/PD-L1, same number for HER3. Five mAbs directed against each of the 2 tyrosine kinase receptors EGFR and c-Met are also in the clinic. Nevertheless, about 70 mAbs currently in clinical development in oncology are directed against unique targets. A similar situation is found outside oncology. A complete review of the most exiting mAbs currently in early to late stage clinical development can be found in latest article of the 'Antibodies-to-watch' series by Dr. Reichert.[29] The session covered several of the most studied targets in oncology and other indications, either by conventional antibodies or next-generation protein scaffolds such as DARPINs.

HER2: Trastuzumab - 17 years and still going strong

Gail Lewis Phillips (Genentech/Roche) focused on the tumor-antigen HER2 (or HER2/neu). A comprehensive overview of Roche/Genentech around their product portfolio built around the HER2 target was presented. HER2 (human epidermal growth factor receptor 2, also known as HER2/neu) is a membrane tyrosine kinase receptor that, when activated, affects cell proliferation and survival.[30] HER2 amplification is the primary pathway of HER2 receptor overexpression and is a major driver of tumor development and progression in a subset of breast cancers. HER2 is amplified in about 15% to 20% of breast cancers. The value of HER2 as a therapeutic target encompasses 2 entirely different mechanistic dimensions. The first approach exploits the fact that HER2 is clearly a disease-driving oncogene to deliver HER2 kinase inhibitors, apparently a highly rational approach to the treatment of HER2-amplified cancers.[30] However, the functionally relevant HER2-HER3 complex has proven much more difficult to inhibit than had been anticipated, and because of its modest efficacy, molecules targeting this hetero-dimeric complex such as pertuzumab and lapatinib are currently used predominantly in combinations and in very advanced stages of disease.[30,31] The second approach exploits the massive cell surface expression of HER2 and delivers of a variety of cytotoxic or immunologic effectors with great selectivity to these cancer cells. This approach has proven transformative, essentially thanks to the antibody-drug conjugate ado-trastuzumab-emtansine (Kadcyla®).[32] The clinical development strategy for trastuzumab was described, going from the registration trials in advanced metastatic HER2-positive breast cancer patients to the most recent trials and approval in adjuvant setting.[30] The importance of the development of a diagnostic assay allowing identification of the breast cancer population showing very high HER2 expression status was also highlighted.[30] The existence of such an assay ready for early clinical trials was pivotal for the success of the molecule.

In a second part of her talk, pertuzumab (Perjeta®) was presented. Its main difference compared to trastuzumab resides in the targeted epitope, preventing HER2 from forming heterodimers with other HER receptors.[31] Inhibition of HER2 signaling results in a reduction of tumor cell proliferation, invasiveness and survival. Pertuzumab and trastuzumab bind to different sites on the HER2 receptor and clearly have complementary anti-tumor activities; they act synergistically in inhibiting the growth of HER2-overexpressing breast cancer cell lines in vitro.[31] The efficacy of pertuzumab in combination with trastuzumab plus docetaxel in the first-line treatment of HER2-positive metastatic breast cancer was demonstrated in the randomized, double-

Downloaded by [108.49.191.210] at 11:34 25 March 2016

blind, placebo-controlled, multinational, Phase 3 CLEOPATRA trial.[31] Pertuzumab had an acceptable tolerability profile when added to trastuzumab and docetaxel in the pivotal CLEOPATRA trial.[31]

The last part of the lecture was dedicated to the ADC T-DM1. This molecule is comprised of a potent cytotoxic drug, maytansine, connected via a stable linker to trastuzumab.[32] A Phase 2 randomized trial of T-DM1 in the front-line metastatic breast cancer setting revealed promising activity and improved safety compared with standard chemotherapy plus trastuzumab.[32] Subsequently, EMILIA a Phase 3 trial in patients with trastuzumab-pretreated metastatic breast cancer showed T-DM1 to be associated with prolonged progression-free and overall survival compared with lapatinib plus capecitabine.[32] T-DM1 represents a major shift in the treatment of patients with breast cancer as it replaces traditional non-targeted chemotherapy with a medication that directs the cytotoxic therapy to cancer cells by using a known biomarker.[32]

Targeting the receptors of TNF

The second talk was given by **Allart Stoop** (Rositys Therapeutics) and described the various therapeutic modalities to target tumor necrosis factor α (TNF) for the treatment of chronic inflammatory diseases. TNF is a pleiotropic cytokine associated with both inflammatory and immunoregulatory activities.[33] Its relevance to disease is well established and treatment with TNF antagonists has been highly efficacious in a range of inflammatory disorders, e.g., rheumatoid arthritis.[33] From a biological perspective, TNF mediates its effects by signaling through 2 distinct, specific, high-affinity receptors.[33] TNFR1 is expressed ubiquitously and signals through an intracellular death domain (DD), inducing apoptosis and NF-κB mediated inflammation. In contrast, TNFR2 is expressed on a restricted subset of cells, including endothelial cells and cells of the immune system (T cells), has a TNF receptor-associated factor (TRAF) signaling domain, and has been associated with Akt/PKB-mediated repair and migration.[33] Both TNF receptors signal as membrane-anchored receptors and their numbers are regulated through a combination of receptor synthesis, internalization and shedding, resulting in circulating soluble TNFR1 and TNFR2.[33] As the majority of detrimental effects seem to be mediated by TNFR1 and the more beneficial processes by TNFR2, selective binders to TNFR1 antagonists need to be developed. A small, domain antibody (dAb) with monovalent binding to TNFR1 was screened and characterized. DMS5540 corresponds to a mouse TNFR1 antagonist, constituted by the genetic fusion product of an anti-TNFR1 dAb with an albumin-binding dAb (AlbudAb).[34] It bound mouse TNFR1, but not human TNFR1, and was an antagonist of TNF-mediated cytotoxicity in a L929 cell assay.[34] Surprisingly, the dAb did not compete with TNF for TNFR1-binding. Pharmacokinetic studies of DMS5540 in mice over 3 doses (0.1, 1.0 and 10 mg/kg) confirmed extended in vivo half-life, mediated by the AlbudAb.[34] Target engagement was further confirmed by dose-dependent increases in total soluble TNFR1 levels. Functional in vivo activity was demonstrated in a mouse challenge study, where DMS5540 provided dose-dependent inhibition of serum IL-6 increases in response to bolus mouse TNF injection.[34] Nevertheless, TNFR2 signaling has been shown to promote regulatory T cell function.[35] Blockade of TNFR1 and TNRF2 led to increased effector T cell activity, which was not observed after selective TNFR1 blockade using DMS5540, suggesting an immunoregulatory role of TNFR2.[35] In support of this, TNFR1 blockade, but not TNFR1/2 blockade, expanded and activated T_{reg} cells.[35]

PD-1: Nivolumab - A game changer in immuno-oncology?

Brian Lestini (Bristol-Myers Squibb) presented an overview of the current revolution existing in Immuno-Oncology (IO) with the antibodies modulating the immune checkpoint axis, and especially those targeting cytotoxic T lymphocyte antigen 4 (CTLA-4) or programmed death 1 (PD-1). CTLA-4 blockade was translated to the clinic with a fully human antibody to human CTLA-4 (ipilimumab, Medarex, Bristol-Myers Squibb). Tumor regression was observed in Phase 1/2 trials in patients with a variety of tumor types, including melanoma, renal cell carcinoma, prostate cancer, urothelial carcinoma, and ovarian cancer.[36,37] Ipilimumab (3 mg/kg) was approved in the USA for first-line and second-line treatment of patients with advanced melanoma, and in second-line treatment in Europe.[37] This decision was based on trials showing that ipilimumab alone or combined with a peptide vaccination provided a 3.6 month median survival benefit and a survival benefit of about 33% compared with vaccination alone.[37]

The optimum dose and schedule for ipilimumab is not fully established.[36] Good biomarkers for response are elusive. Immune-related adverse events, an increase in lymphocyte counts, and the presence of NY-ESO-1 antigen seem to be associated with higher response rates.[36] Ipilimumab is a complex drug to give. Adverse events—mostly immune—occur in 40% of patients, including skin rashes, colitis, hepatitis, and hypophysitis. Grade 3–4 adverse events occur in less than 10% of patients, but can be fatal. Adverse events usually resolve spontaneously or after steroid treatment, but endocrine failure frequently needs permanent hormonal supplementation.[36] High-dose steroids are indicated for severe immune-related adverse events, but sometimes anti-TNF (infliximab) might also be needed.[36] Several other late-stage clinical trials are currently ongoing to evaluate to use of ipilimumab in other solid tumor indications such as prostate cancer.

Another T cell intrinsic inhibitory pathway identified after CTLA-4 was the one mediated by PD-1 (programmed death 1) and its ligand PD-L1. PD-1 was initially cloned in 1992 in a study of molecules involved in negative selection of T cells by programed cell death in the thymus.[38] PD-1 is expressed in many tumors in response to inflammation and its engagement on the lymphocyte surface of melanoma cells downregulates T cell function.[38] In preclinical models, PD-1-deficient mice

develop a strain-specific, delayed-onset, organ-specific autoimmunity with incomplete penetrance, supporting a role of the PD-1 axis in self-tolerance. In tumor-bearing immunocompetent mouse models, PD-1 blockade inhibited hematogenous dissemination of B16 melanoma cells and CT26 colon cancer cells to the liver and lung, respectively, through an effector T cell mechanism.[38]

Very large Phase 1 studies were run with 2 anti- PD-1 antibodies (nivolumab and pembrolizumab), including parallel expansion cohorts in various tumor indications (i.e., series of CHECKMATE studies: CHECKpoint Pathway and nivoluMAb Clinical Trial Evaluation).[38-40] Strong clinical responses were rapidly observed with objective response rates of more than 30%, and long-term benefit noted in most responding patients.[39,40] Furthermore, the expression of PD-L1 on the surface of tumor cells seems to be a predictive biomarker. [39,40] Numerous clinical trials are still ongoing either to explore novel potential indications and to study PD-1 blockers within combination therapies.

Targeting VEGF and HGF: First-in-human phase 1 study – interim results of MP0250, the first systemic DARPin

After a refreshment break, **Michael Stumpp** (Molecular Partners) presented a novel, non-Ig-based protein scaffold called designed ankyrin repeat proteins (DARPIN®).[41,42] These are artificial scaffolds based on human ankyrin repeat domain proteins, which are abundant intracellular adaptor molecules that bind to various proteins with different biological consequences, such as inhibition of complex formation. [41] DARPINS are composed of 2 to 4 randomized, genetically-fused repeats that are flanked by N- and C-terminal-capping repeats, which are essential for efficient folding and for avoiding aggregation. DARPIN repeats consist of 33 amino acids and are composed of 2 α-helices and a β-sheet with a molecular mass of between 14 and 21 kDa. Due to their stable structure, DARPIN are very stable proteins. [41]

Abicipar is the most clinically-advanced DARPIN. It is an antagonist of vascular endothelial growth factor A (VEGF-A) that inhibits all relevant subtypes of VEGF-A with very high potency, and currently developed for wet age-related macular degeneration (wet AMD) and diabetic macular edema (DME). The combination of small size, high potency and long intravitreal half-life offers the potential for less frequent injections and higher therapeutic efficacy. In Phase 2 studies, abicipar was shown to provide at least equal or higher vision gains with the potential for fewer injections in wet AMD compared to standard of care treatment as well as less frequent dosing regimen. This product is developed by Pfizer/Allergan.

The next product presented by Dr. Stumpp was MP0250, a multipathway DARPin that inhibits both VEGF and hepatocyte growth factor (HGF) combined to 2 human serum albumin (HAS) binding modules. It simultaneously targets tumor stroma, proliferation, invasion, and metastasis. MP0250 is in Phase 1 clinical studies in solid tumor cancers and hematological malignancies, including 15 patients. MP0250 was well tolerated at doses ranging from 0.5 to 8 mg/kg given every 2 weeks as intravenous infusion and a maximum tolerated dose has not been reached. The most frequent AEs (CTC version 4.03) were transient hypertension (47%), diarrhea (40%), fatigue (40%) and nausea (40%). No anti-drug antibodies were detected within the treated patients. Two patients yielded stable disease for more than 12 months and 8 months, respectively. MP0250 showed a long half-life of around 12 days, giving the potential of dosing every 3 weeks. Repeated dosing led to sustained exposure throughout the treatment periods analyzed, the longest to-date being 12 months.

Several immune-oncology projects have been started based on the favorable physic-chemical and functional properties of DARPINs, they are all in early discovery stage.

Stabilized receptors as antigens for GPCR-antibody discovery

Cath Hutchings (Heptares Therapeutics) described the technology platform developed at Heptares to generate stabilized receptors to increase the efficiency of drug discovery, especially against G protein coupled receptors (GPCRs). The GPCR superfamily is the largest and single most important family of drug targets in the human body. It plays a central role in many biological processes and is linked to a wide range of disease areas. There are over 375 GPCRs encoded in the human genome, of which 225 have known ligands and 150 are orphan targets. GPCRs are the site of action of 25–30% of current drugs.

Drug discovery targeting GPCRs remains challenging however. In contrast to classes of soluble protein drug targets, such as kinases and proteases, the understanding of GPCRs has been severely hampered by the lack of structural and mechanistic knowledge and an understanding of how compounds interact with them. [43] The overriding problem is that GPCRs are very unstable and lose their highly organized structure and activity when taken out of the cell membrane. [43] Through the use of protein engineering methods, mutations could be identified that both increase the thermostability of GPCRs when purified in detergent, as well as biasing the receptor toward a specific physiologically relevant conformational state. The resultant stabilized receptor (known as a StaR) can be purified in multiple-milligram quantities, while retaining correct folding, thus enabling the generation of reagents suitable for a broad range of structural and biophysical studies. [43,44]

Three examples of application of StaRs in drug discovery were presented. The first was an undisclosed target from which a StaR was used to screen Morphosys' Ylanthia® antibody phage library. Strong affinity binders (>10 nM) with good cellular binding properties could be identified.

The second example corresponded to the generation of StaRs derived from the prototypical receptor β-1-adrenergic receptor (β1AR) and the screening for mouse hybridoma using various approaches.[45] The antibodies bind diverse epitopes associated with low nanomolar agonist activity at β1AR. In vitro characterization also verified different antibody receptor interactions reflecting the different epitopes on the extracellular surface of β1AR to which the mAbs bind.[45] The anti-β1AR mAbs only demonstrated agonist activity when in dimeric antibody format, but not as the monomeric Fab format, suggesting that agonist activation may be mediated through promoting

Downloaded by [108.49.191.210] at 11:34 25 March 2016

receptor dimerization.[45] Finally, at least one of these antibodies exhibited in vivo functional activity, producing an increase in heart rate consistent with β1AR agonism.

The third example was the solution of a 3D structure of the human protease-activated receptor (PAR)2 in partnership with AstraZeneca.

Comprehensive discovery of new immuno-oncology targets for antibody therapy

Art Brace (Five Prime Therapeutics) described the drug discovery engine set-up and developed at Five Prime Therapeutics. Their technology includes a large library of secreted, extracellular proteins, derived from more than 100 distinct human tissues, and comprising more than 5,700 human proteins. The library contains proteins that are full-length, structurally complete and biologically active. The second part of the technology concerns complex, cell-based screening assays. Screens were automated, built in-house and analyzed using proprietary software. These platforms are associated with a protein expression system, RIPPS® (Rapid In Vivo Protein Production System). It enables production and testing of the proteins from the secretome library directly in vivo in virtually any rodent model of disease and in high throughput.

Track C: Improving ADC properties, widening therapeutic window or maximizing potency

Joost Melis

Volker Schellenberg (Amunix) introduced the speakers of this special ADC track, which focused on improving ADC properties, widening therapeutic windows or maximizing potencies.

Advancing the dolaflexin platform and other fleximer-based ADCs toward the clinic

Timothy Lowinger (Mersana) discussed advancing the dolaflexin platform and other fleximer-based ADCs toward the clinic. The dolaflexin platform is the most advanced at Mersana establishing a DAR of ~15 of a proprietary auristatin payload and using a fully biodegradable fleximer polymer. The dolaflexin intracellular processing of an auristatin derivative (AF-HPA) with a cleavable linker exerts cell killing potency in the sub-nanomolar range upon internalization and metabolization. The payload is freely cell permeable and therefore capable of inducing bystander cell killing. However, the ADC product is designed as a sort of hybrid and AF-HPA can be further metabolized in the cell to AF, which is charged and therefore a non-cell permeable molecule without bystander capabilities. Tissue accumulation of AF and AF-HPA products at a single time point of 48 h in non-perfused, tumor-bearing mice after 5 mg/kg dosing showed that of the released payload 90 ng/mL was present in the tumor, of which 70% was AF and 20% AF-HPA. Approximately 10 ng/mL was distributed to liver, spleen, and kidney each, while very low concentrations were found in muscle and plasma. The therapeutic index of dolaflexin (calculated by the highest non-severely toxic dose in NHP (mg/m^2) / lowest dose inducing regressions in mouse xenograft (mg/m^2)) compared favorably to other cleavable auristatin ADCs. Currently, XMT-1522 is in late stage preclinical development and expected to move to clinic in the first half of 2016. The ADC targets HER2 and is aimed at treating 1+, 2+, and 3+ HER2 tumors. HER2 1+/2+ positive cancers make up about 60% of metastatic breast cancers in US and EU5. XMT-1522 is built upon the HT-19 antibody that does not compete for binding with either trastuzumab or pertuzumab, but rather binds to a unique epitope (not trastuzumab-based), and a DAR ~15 is enabled via Fleximer polymer conjugation. ADC sensitivity was measured and benchmarked against Kadcyla®. The dolaflexin ADC exhibited single digit nanomolar potency in vitro across a wide panel of cell lines with varying expression levels of HER2. Potency was comparable to ado-trastuzumab emtansine (Kadcyla®) in very high expressing cell lines; in moderate expressing cell lines (>10,000 HER2/cell) potency was significantly greater than Kadcyla®; and in 40% of cell lines tested with 0–10,000 copies of HER2 per cell single digit nanomolar potency was maintained, with none of the cell lines being susceptible to Kadcyla®. In vivo assessment using N87 and JIMT-1 cell line models demonstrated 100% tumor free survivors at lower dosages than Kadcyla. In a SNU5 gastric cancer model with 22.000 HER2/cell, the drug showed also efficacy (tumor growth delay, no complete regression), again outperforming Kadcyla®. Triplet combination of XMT-1522 + trastuzumab + pertuzumab showed improved efficacy over single agent or trastuzumab + pertuzumab treatments in a N87 xenograft model. A resistance N87 model was developed using Kadcyla® treatment, resulting in a model that is 500x less sensitive to Kadcyla® but retains its HER2 expression levels. XMT-1522 treatment exhibited good to full tumor regression in this Kadcyla®-resistant model. In PDX models, like MAXF-1162 for breast cancer, complete tumor regression was also observed for XMT-1522 and similar efficacy was established in other PDX models. Tolerability was assessed in NHP studies and XMT-1522 showed an acceptable safety profile that was comparable to Kadcyla®. Mersana's portfolio further contains an undisclosed ADC targeting solid tumors, XMT-1535, of which IND filing is expected to be in 2017. MERS3-dolaflexin IND filing is expected in 2018.

Probody therapeutics can redefine the target landscape for drug conjugates

Luc Desnoyers (CytomX) talked about the development of Probody™ drug conjugates (PDCs). Probody therapeutics are fully recombinant masked antibodies that are designed to remain inactive in healthy tissue, but are activated specifically in the tumor microenvironment. A masking peptide is tethered recombinantly to the light chain of the antibody portion, which is cleaved in the tumor microenvironment by protease activity upon which antibody binding to the target is possible. This approach is designed to blunt systemic toxicities that can be associated with antibodies. Probody therapeutics can localize activity to the tumor microenvironment, broadening the therapeutic window and enabling development of potent therapeutics against novel targets. The most suitable masking peptide is specific for each Probody therapeutic. The best masking peptide is selected after panel screening, and can, for instance, be selected based on cleavability, e.g., by one or by multiple

Downloaded by [108.49.191.210] at 11:34 25 March 2016

proteases in the tumor microenvironment. The current pipeline includes, among other modalities, PDCs targeted against CD166 and CD71, and Probody therapeutics directed to T cell engaging bispecifics and immune-oncology therapeutics, such as PD-1 and PD-L1. Proof of concept data of an anti-EGFR cetuximab Probody therapeutic were presented. IHZ™ data demonstrated that protease activity in the tumor microenvironment is a lot higher than in healthy tissue in vitro and ex vivo. Preclinical in vivo imaging data in mice demonstrated the same accumulation profile when the EGFR Probody therapeutic with a cleavable linker was compared to cetuximab. A control with a non-cleavable linker showed no accumulation at targeted sites. Additionally, the Probody therapeutic revealed similar efficacy and improved safety profiles over the control antibody.

The CD166-directed Probody therapeutic was discussed in more detail. The role of CD166 in cancer is not known, but the target is highly expressed in prostate, breast, cervical, oropharyngeal and lung cancers, as well as head and neck squamous cell carcinoma. CD166 is, however, also expressed in many normal tissues, which suggests that it is not a good target for a traditional ADC. IHZ data of the CD166 Probody therapeutic showed no binding to normal healthy tissue, but it does bind at tumor sites. In collaboration with ImmunoGen, the CD166-SPDB-DM4 ADC was compared to the PDC. Both the PDC and ADC demonstrated efficacy in a triple-negative breast cancer and an ovarian cancer model, indicated by rapid tumor regression with some regrowth after 1.5 months. Cynomolgus monkey toxicity studies indicated that the PDC was well tolerated and the stability of the PDC is consistent with other SPDB-DM4 conjugated antibodies. Standard DM4 toxicities were apparent at 15 mg/kg dosing, and no off-target toxicity or weight loss was observed. Liver toxicity markers were also not altered by PDC treatment. IND filing for this PDC is anticipated in the first half of 2017. PROBODY and IHZ are trademarks of CytomX Therapeutics, Inc.

Developmental strategy of cancer stromal targeting therapy

Masahiro Yasunaga (National Cancer Center) discussed a developmental strategy of cancer stromal targeting (CAST) strategy. Stroma consists of cellular components (fibroblast, endothelial, blood cells etcetera) and acellular (matrix, collagen) elements and can cause autoimmune reactions. Stroma can also create a barrier for efficient antibody delivery to tumor sites, which was exemplified by staining collagen and identifying barriers to tumor sites in malignant lymphoma and pancreatic cancer. CAST therapy was developed to improve antibody delivery to the actual tumor site. This approach combines administration of a tumor-targeting ADC with a stroma-targeting ADC to basically clear the path to the tumor site. Anti-collagen and anti-fibrin ADCs were developed to target tumor stroma. Two types of linkers were developed for these ADCs, a carbamate-bond (for intracellular release) and an ester-bond (for extracellular release). The safety profile of the anti-collagen 4 ADC was examined and indicated that AST and ALT levels, plus white blood cell counts were acceptable. Also the anti-Coll4 ADC did not induce arthritis in mice in contrary to an anti-Coll2 ADC. In addition, an anti-fibrin ADC was developed. This ADC targets tumor stroma specifically, and not healthy tissue, which is characterized by fibrinogen. The current stroma ADCs have a DAR of 3–4 and are payloaded with MMAE, which is released gradually and distributed throughout the tumor. Tailoring of stroma ADCs to individual characteristics of each tumor stroma is preferred.

XTEN drug linkers with precisely controlled chemical structures for high drug loads and optimized tissue uptake

After a networking break, **Volker Schellenberger** (Amunix) discussed XTEN drug linkers with precisely controlled chemical structures for high drug loads and optimized tissue uptake. A common strategy for increasing therapeutic molecule half-life is the chemical attachment of polyethylene glycol (PEG), which increases the effective size of drugs and thus slows their clearance from the bloodstream. This however results in non-biodegradable, complex product mixtures that are difficult to purify and characterize. XTEN is a homogeneous biodegradable polymer built from 6 amino acid types that are used to form the backbone of precisely-controlled polymers. XTEN polymers can be produced in a wide range of sizes from <10 kDa to 80 kDa. XTEN increases half-lives of therapeutic molecules, is low immunogenic, soluble, stable, and well-tolerated. Recombinant fusion or chemical conjugation to XTEN is possible, creating opportunities for immune activators (ProTIA) and drug conjugates (XDC), for example the XDC FVIIIFc-VWF-XTEN. The biological role of FVIII in the clotting cascade was briefly presented and it was demonstrated that half-life prolongation for this XDC is beneficial for clinic application. Tumor-specific accumulation was demonstrated for an anti-HER2-XTEN of which 40% of the product accumulated at the tumor site. The anti-HER2-XTEN showed similar efficacy results to Kadcyla® in vivo when using a 3, 10, or 30 mg/kg single dose. A long term vision for XDCs is to create molecules that are large within the blood stream, but once trafficked to the tumor sites become smaller by disposing parts of the molecule. This localized size reduction would then facilitate tumor penetration. XTEN polymers facilitate payloading antibodies with different DARs (up to DAR 9 has been tested). Anti-folic acid XDC, FA-XTEN431-DM1, internalized target specifically, plus demonstrated in vivo efficacy after single dosing, while no weight loss was observed after treatment. Next, the ProTIA technology was discussed, in which a XTEN polymer extends the half-life in circulation of bispecific T cell engaging BiTE molecules and prevents premature T cell activation since protease activation near the tumor site is necessary to activate the BiTE. Production of ProTIA is performed in *E. coli* and the characterization of ProTIA was briefly discussed. In vitro efficacy was shown for an EpCAM-ProTIA, which exhibited a half-life of 32 hours compared to 3.5 hours of the targeting component only.

A meditope site-directed conjugation strategy simplifies antibody conjugation and provides a unique way to improve internalization

Elisabeth Gardiner (Meditope Biosciences) presented a site-directed conjugation strategy that simplifies antibody

Downloaded by [108.49.191.210] at 11:34 25 March 2016

Downloaded by [108.49.191.210] at 11:34 25 March 2016

conjugation and provides a unique way to improve internalization. A site-specific novel antibody platform (SnAP) was developed in which a peptide is 'locked' in between heavy and light chains of the Fab arms creating a so-called meditope. The discovery of the SnAP binding site was presented. The peptide was discovered by a peptide binding to cetuximab initially, binding between specific amino acids on the heavy and light chain of the Fab region. These specific sites can now be engineered into all antibodies to tweak peptide binding. Amino acids that can be engineered are in a highly conserved area within the frame work region, which makes SnAP engineering applicable to all antibodies. The meditope site is distinct from antigen binding, so it does not interfere with target binding. Also, when cytotoxins are conjugated to the peptide or fusion proteins are made via the locked peptide, no interference with the normal antibody function is apparent. Meditopes are now available in non-covalent and covalent variants. Covalent binding can be used for cytotoxin conjugation in which the DAR is always conserved to 2. In addition, GFP attachment to the peptide creates opportunities for imaging purposes, radiolabeled attachment can generate new diagnostic tools and the SnAP platform also facilitates possible manufacturing strategies. Antibody to antibody interactions can be promoted at the cell surface facilitated by meditope - antibody binding. Interactions at the cell surface will then enhance internalization and cell death. Two- to 5-fold increases of internalization were achieved compared to the control antibodies.

Track A: Antibodies to harness the cellular immune System

Jenny Yeung

The afternoon session of Track A was chaired by **Kerry A. Chester** (University College London).

Engineering antibodies and T cell receptors by mammalian display

Generation of immune-modifying binders is facilitated by the availability of large libraries of antibodies or T cell receptors expressed on the surface of mammalian cells. As discussed by **John McCafferty** (IONTAS), scientists at IONTAS have demonstrated the construction and use of mammalian display libraries, facilitated by the use of site-specific nucleases. Such libraries allow the screening of millions of clones by flow sorting while providing information on both the level of expression and the extent of binding within individual clones.

Targeting immune regulation at the tumor site

CTLA-4 is a co-inhibitory molecule expressed on T cells that is implicated in the inhibition of T cell-mediated anti-tumor activity and contributes to tumor immune escape. Clinical data has shown that treatment with anti-CTLA-4 antibodies significantly improved survival rates of patients with advanced melanoma, many of whom have durable and long-term responses. As discussed by **Sergio A. Quezada** (University College London), understanding the mechanisms by which immune modulatory antibodies exert their anti-tumor activity can help develop strategies to maximize their activity. The tumor microenvironment and Fc receptors have important roles in modulating the in vivo activity of immunomodulatory antibodies.

The effectiveness of anti-CTLA4 therapy is not solely due to blocking the inhibitory signals to effector T cells (T_{eff}). Studies have shown that CTLA-4 is expressed on T_{eff} cells and on regulatory T cells (T_{reg}). In both murine models and human melanoma patients, there is an altered balance in the ratio of T_{reg} and T_{eff} cells within the tumor; specifically a higher proportion of T_{regs} and a lower level of T_{eff} exists. Although both T_{eff} and T_{reg} cell populations expand in the lymph nodes of mice by treating with anti-CTLA-4 antibodies, specific elimination of T_{regs} within the tumor is promoted. T_{regs} expressed higher levels of CTLA-4 than T_{eff} cells, which leads to preferential depletion through ADCC by tumor macrophages via an FcγR-dependent pathway involving the FcγIV receptor. The isotype of anti-CTLA-4 antibodies administered and therefore their differential binding to Fc receptors was important for anti-CTLA-4 anti-tumor activity.

Dr. Quezada noted that unpublished data using human FcγR transgenic mice helped to determine whether the mechanisms would also apply in the context of human FcγR rather than murine FcγR. This model system showed that tumor-infiltrating macrophages and dendritic cells expressed high levels of the activating human Fc receptors CD16, CD32a and CD64. A mutant anti-CTLA4 hIgG1 antibody that had enhanced ADCC activity prevented accumulation of T_{regs} in the lymph node and enhanced elimination of T_{regs} within tumors, leading to an increase T_{eff}/T_{reg} ratio. Further investigation using this model is required to determine which human FcγRs are required, whether anti-tumor activity is also enhanced, and whether there would be enhanced toxicities.

Analysis of tumor infiltrating cells from human melanoma patients provided evidence that the mechanisms identified in the murine models was also potentially applicable to humans. As in murine models, CTLA-4 was highly expressed by T_{regs} in human melanoma tumors and some of the tumors showed high infiltration of macrophages ($CD11b^+/Fc\gamma R^+$ cells). Freshly isolated human tumor macrophages were capable of mediating anti-CTLA-4 activity in vitro.

Characterization of the expression of immune checkpoints in human cancer can help develop new and more effective immunomodulatory agents and combinations. Analysis of human melanoma tumor infiltrating lymphocytes showed that the activating receptors OX-40 and GITR are more highly expressed on T_{regs} than on T_{eff} cells. In particular, the expression pattern of GITR closely resembled that of CTLA-4 on T_{regs} and T_{effs}. Mouse models showed that agonistic anti-GITR antibodies require ADCC for depletion of intratumoral T_{regs} and maximal activity was dependent on the antibody isotype.

Studying changes that occur in the tumor microenvironment pre- and post- therapy, may help understanding of the resistance to checkpoint inhibition, and facilitate the development of optimal combination therapies to maximize anti-tumor activity.

Downloaded by [108.49.191.210] at 11:34 25 March 2016

An Anti-ICOS agonistic antibody for cancer immunotherapy

Jennifer Michaelson (Jounce Therapeutics) discussed the company's program to develop an agonistic antibody to the co-stimulatory molecule ICOS. Preclinical studies demonstrated that anti-ICOS agonistic antibodies are efficacious in syngeneic tumor models, with enhanced efficacy observed in combination with PD-1 inhibition.

Characterization and in vivo evaluation of blocking antibodies against GARP, a novel immune checkpoint target

Activated regulatory T cells (T_{regs}) specifically express the protein GARP (Glycoprotein A Repetitions Predominant), which is not expressed on other T cell subsets. On activated T_{regs}, GARP forms a complex with latent TGF-β1. GARP is key to the release of TGF-β1, specifically at the T_{reg} level, and results in stimulation of T_{regs} and inhibition of effector T cell functions. Locking of TGF-β1/GARP complex by antibodies can potentially prevent release of active TGF-β1, leading to decreased T_{reg} and can ultimately lead to inhibition of immune escape and increased anti-tumor activity in local microenvironments.

Michael Saunders (argenx) described 2 anti-human GARP antibodies, one murine and one from llama, were identified and engineered with human IgG backbone that recognized the GARP/TGF-β1 complex. These antibodies inhibited TGF-β1 activation on T_{regs} by blocking release. This inhibits SMAD2 phosphorylation, suppressing helper T cell proliferation. A xenogeneic graft-versus-host disease (GVHD) murine model was used to demonstrate the T_{reg} inhibitory effects of anti-GARP antibodies. In this model, the presence of human T_{regs} protects against GVHD whereas treatment with e.g., nivolumab (anti-PD-1) aggravates GVHD. Anti-GARP antibodies were shown to ablate the protective activity of T_{regs} by specifically blocking their function without depleting their numbers. These data showed that GARP function had certain similarities with CTLA-4 functionality.

Exploratory toxicology studies in cynomolgus monkey studies were performed, using the llama anti-GARP antibody clone LHG10.6, upon which ARGX-115 is based. GARP is also expressed on platelets and liver stellate cells and could potentially affect platelet counts and liver function. However, in vitro assays showed no issues with thrombin- or collagen-induced platelet activation. Monkeys being given 3 doses at 14 day intervals at 0.5 mg/kg or 10 mg/kg showed no effect on platelet counts, coagulation parameters, or liver functions after being examined for 26 d after the last dose.

ARGX-115 is a high affinity (40 mM) antibody targeting the GARP-TGFB1 complex and has development potential as a first-in-class immune-oncology product. It has a novel model mode-of-action where TGFB1 is specifically targeting and inhibiting the suppressive effects of T_{reg} function within the tumor microenvironment without depleting T_{reg} systemically. This offers advantages over targeting CTLA-4 for future combination therapy.

Engineering agonistic anti-TNFR antibodies

Ann White (University of Southampton) discussed immunostimulatory monoclonal antibodies that can augment anti-tumor immunity through activation of tumor necrosis factor receptor (TNFR) family members, such as CD40. Binding of CD40 on cells such as dendritic cells and B cells to its ligand CD40L leads to clustering of CD40 and subsequent activation of numerous immune mechanisms, which lead to anti-tumor activity. Multiple mechanisms of TNFR monoclonal antibody agonistic activities discussed are mediated through the Fc region or through the variable domains.

Anti-CD40 antibodies have shown efficacy in preclinical mouse models. The ratio of activating FcγR binding to inhibitory FcγR binding (A/I ratio) is low for mIgG1 and higher for mIgG2a, which contributes to their different effects on anti-tumor activity in BCL1 lymphoma tumor model. Anti-CD40 mIgG1 antibodies induced survival and improved CD8 T cell responses in mice, whereas mIgG2a anti-CD40 antibodies did not. Binding to inhibitory FcγRIIB receptor was shown to be essential for mIgG1 anti-CD40 activity and CD8 T cells responses in FcγRIIB knock out mice. FcγRIIB was also essential for B cell activation induced by anti-CD40 antibodies. The role of FcγRIIB in anti-CD40 therapy in these models was to act as a cross-linking scaffold that increased clustering of CD40 molecules and subsequent activation of T and B cells. These may be general mechanisms that are also relevant to other TNFRs including human 4–1BB, OX40 and CD28.

Choosing the optimal isotype for development of clinical anti-human CD40 is a critical factor. Anti-human CD40 (LOB7/4) was cloned to different human IgG isotypes. Only the IgG2 isotype variant demonstrated strong agonist activity in vitro, but does not bind to FcγRIIB. hIgG2 exists as H2A or H2B isoforms due to rearrangement of disulfide bonds within the hinge and CH1 domains. Mutagenesis can lock them on one form or the other. H2A has a more flexible conformation while H2B has a much more compact and rigid structure. While there is no difference in binding to CD40, H2B anti-CD40 has much better agonistic activity compared to H2A both in vitro and in vivo. The rigid structure of the H2B form resulted in tighter clustering of CD40 molecules upon binding, conferring to better agonistic activity.

Where anti-CD40 antibodies bind on the CD40 molecule also influences their agonistic properties. Comparison of 3 different anti-CD40 antibodies, ChiLOB7/4, SGN40, and CP870893, showed that they bound similar epitopes on the CD40 CRD1 domain and conferred better in vitro B cell proliferation and in vivo CD8 T cell expansion, but antibodies that recognize more proximal domains (CRD2 and CRD3–4) did not.

Antibody engineering can optimize and improve the therapeutic activity of clinical antibodies against TNFR family members. These can act through FcγR-dependent or FcγR-independent mechanisms and can all contribute to the development of better immunomodulatory anti-therapeutic agents.

Track B: Antibodies for orphan indications

Joost Bakker

James Larrick (Panorama Research Institute and Velocity Pharmaceutical Development) chaired the morning Track B session on the development of antibody therapies for indications affecting small numbers of patients, i.e., orphan diseases.

Screening for GPCR antibodies with cell surface displayed antibody libraries constructed by antibody membrane switch technology

Bo Yu (Larix Bioscience) discussed the Antibody Membrane Switch (AMS) technology, which is a unique switchable cell surface antibody display technology that facilitates rapid FACS-mediated identification of high producing cell lines. Cell surface displayed antibody libraries were constructed in CHO cells using AMS technology. Antibodies with superior activities against GPCRs were isolated by direct screening of binding between the cell surface antibody and GPCRs on target cells.

Generating highly potent antibodies to a ligand-gated ion channel

Ion channels are notoriously challenging targets for antibody therapeutics. **Wendy Williams** (MedImmune) presented new data on the generation of potent ion channel modulating antibodies to a ligand-gated ion channel. She discussed a case study on the discovery of ion channel modulating antibodies, including insights into the successful methods of identifying functional antibodies against these targets.

Targeting the Kv1.3 ion channel with an engineered ultralong CDR H3 cowbody

Cows can make unusual ultralong CDR3s that can range to nearly 70 amino acids in length and are comprised of a b-strand "stalk" and disulfide bonded "knob." The knob region is similar in size, shape, and disulfide composition to several naturally occurring venom peptides that inhibit ion channels. **Vaughn Smider** (The Scripps Research Institute) presented data on a humanized cowbody with a Kv1.3 inhibitor venomous peptide that showed potent inhibition of T cells, and could be a unique antibody to treat autoimmune disease.

FFP104: A negative allosteric modulator of CD40 signaling

CD154-blocking mAbs used in human clinical trials resulted in unanticipated vascular complications, leading to heightened interest in the therapeutic potential of antagonist mAbs specific for human CD40. Molecules of the CD40 pathway are (over) expressed in primary biliary cirrhosis target tissue and involved in target cell apoptosis. CD40 plays a central role in immune responses, in B cell activation and macrophage stimulation. Inhibition of the CD40 pathway could thus interfere with the development of autoimmune cholangitis.

Mark de Boer (Fast Forward Pharmaceuticals) discussed the mechanism of action of anti-CD40 mAb FFP104. FFP104 is originally derived from murine 5d12 and antagonizes CD40-CD154 signaling. Interestingly, it does not require physical competition with CD154 to inhibit CD40 signaling and thus acts as a negative allosteric modulator of CD40 signaling. Furthermore, FFP104 inhibits CD154-mediated B cell activation. In addition, binding of FFP104 to CD40 induced degradation of TRAF2 and -3.[46] Validation in primate models has been performed, where a reduction of germinal centers was observed after FFP104 administration, which is consistent with the phenotype in CD40L-KO mice.[47]

FFP104 is currently being tested in a Phase 2a clinical study in primary biliary cirrhosis. In this disease, CD40-L-bearing T cells and CD40-L-bearing macrophages seem to induce apoptosis of biliary epithelial cells.[48] FFP104 can inhibit apoptosis induction by T cells. Also in a clinical Phase 1/2 trial in Crohn's disease, the number of infiltrating lymphocytes decreased after administration of FFP104.

ARGX-113, a novel Fc-based therapeutic approach for antibody-induced pathologies

Peter Ulrichts (argenx) discussed ARGX-113, a proprietary antibody fragment, based on the company's ABDEG technology. ARGX-113 works by preventing pathogenic autoantibodies from being recycled, promoting their degradation and thereby clearing them from circulation. Preclinical data in cynomolgus monkeys proved ARGX-113 to be highly effective in rapidly eliminating pathogenic antibodies, while sparing the broader immune response. The data support further clinical development of this novel therapeutic approach in autoimmune disease management.

Anti-LRP6 antibody attenuates Wnt pathway-mediated pathologies

In general, Wnt signaling orchestrates wound healing, repair and regeneration. Tuning Wnt signaling can enhance wound repair. Elevated Wnt signaling is also observed in human retinas of patients with diabetic retinopathy and various fibrotic diseases.

As described by **James Larrick**, both LRP5 and LRP6 are potential targets, but LRP6 is preferred, because it is required for upstream signaling of VEGF and CTGF. LRP6 also controls profibrotic signaling. Activation of the Wnt pathway via LRP6 cell surface signaling contributes to the severe retinal/choroidal neovascularization observed in VLDLR knockout mice.

Dr. Larrick and colleagues identified a novel murine monoclonal antibody (2F1) that antagonizes LRP6 activity and demonstrated significant inhibitory activity in numerous animal models. The antibody directly inhibits Wnt signaling and downregulates LRP6 cell surface expression.

Wnt pathway is activated in human retina in diabetes. The 2F1 antibody inhibits high glucose-induced endothelial cell migration and tube formation. Inhibition of pathology in multiple forms of eye disease was also observed in a rat model (streptozotocin diabetic rats). In a model of wet AMD, lesion size was found to be reduced. Also in another mouse model for

Downloaded by [108.49.191.210] at 11:34 25 March 2016

wet AMD, 2F1 inhibited cytokines and VEGF production. Benefit was also observed in dry AMD models and in chronic mouse models for eye disease via inhibition of WNT signaling. The 2F1 was humanized using PDL technology. The product, H1L1, attenuated topical alkali burn injury and inhibited inflammation in the eye in preclinical models. These data support clinical development of this novel therapy for a number of conditions including AMD, diabetic retinopathy and fibrotic diseases.

Track C: Preclinical and clinical ADC data

Joost Melis

The afternoon session of this special ADC track was chaired by Mark Alfenito (EnGen Bio, Inc.).

Engineering and optimization of antibodies for ADCs

Lioudmila Tchistiakova (Pfizer) discussed engineering and optimization of antibodies for ADCs, describing several examples. ADCs are complex modalities that require optimization of each component (e.g., antibody vehicle, linker-payload, conjugation strategy), and the bigger picture needs to be taken into account. Candidate selection for a 5T4 ADC antibody for instance resulted in 2 favored ADC candidates: A1 and A3. ADC A3 showed a higher affinity and higher in vitro potency when compared to A1, but A1 demonstrated a greater therapeutic index than A3 and was therefore finally selected as a lead candidate. Selection of the antibody binding epitope for an ADC is also crucial, as was indicated by examples related to 2 anti-Notch3 antibodies with distinct mechanisms of action (inhibitory vs non- inhibitory). Epitope mapping revealed distinct yet overlapping epitopes that were consistent with the effects on signaling activities of both antibodies. Despite antagonizing NOTCH3 signaling, the inhibitory anti-NOTCH3 antibody was unable to induce regression in preclinical tumor xenografts with active NOTCH3 signaling. To enhance their potency, both classes of anti-NOTCH3 antibodies were conjugated to an auristatin-based microtubule inhibitor via a cleavable linker. Unexpectedly, the inhibitory anti-NOTCH3 antibody demonstrated more rapid trafficking to the lysosome than the non-inhibitory antibody, suggesting that the 2 antibodies have distinct internalization routes with important implications for NOTCH3-ADC pharmacology. Furthermore, conjugation efficiency should be taken into account in ADC development. Variable regions should not only be screened for binding properties, but also for efficient conjugation properties for an ADC product. Additionally, ADC recycling via the FcRn could affect the PK of the drug and screening IgG:FcRn complex structures could be beneficial for optimal candidate selection. Biophysical properties of antibodies should also be assessed thoroughly. Setting up a robust characterization toolbox for antibody ranking and selection is recommendable, for example assessing affinity, thermal and pH stability, aggregation propensity, microscale pH buffer scouting, viscosity scouting etcetera. Antibody engineering for site-specific conjugation allows improving ADC homogeneity, in vivo stability, PK and has the potential to increase the therapeutic index.

SGN-CD33A: Preclinical and phase 1 clinical trial results of a CD33-directed PBD dimer antibody-drug conjugate for the treatment of acute myeloid leukemia

Eric Feldman (Seattle Genetics) discussed vadastuximab talirine (SGN-CD33A; 33A), a CD33-directed PBD dimer ADC for treatment of AML. AML treatment has not meaningfully changed in over 30 y. Outcomes in AML remain unsatisfactory, and novel treatments are urgently needed. CD33 may be an optimal target for ADC therapy of AML. ~90% of AML patients express CD33 on leukemic blasts. CD33 is also expressed to a lesser extent on normal myeloid cell. 33A is a cys-engineered site-specifically-conjugated ADC with a cleavable dipeptide linker that is stable in circulation and a DNA-targeting PBD payload. Upon target binding, the ADC-receptor complex is internalized and trafficked to the lysosome where proteolytic cleavage of the linker occurs. The PBD payload is released and diffuses into the nucleus of the cell, where it binds DNA with high intrinsic affinity and induces programmed cell death. Preclinical data demonstrated binding, internalization and rapid lysosomal trafficking by microscopy analyses. Furthermore, apoptotic processes were assessed and increased: gamma-H2AX phosphorylation, increased cleaved PARP and Casp-3 activity were all demonstrated in a dose-dependent manner. Next, cytotoxic activity in a panel of AML cell lines showed activity in the majority of cell lines, also taking MDR status and receptor expression into account and benchmarking the IC50 levels of 33A to gemtuzumab ozogamicin. Cytotoxic activity was also assessed in MDR positive and negative in vivo models and primary patient samples. Subsequently, monotherapy (33A-001) Phase 1 studies were initiated. Pharmacokinetic analysis showed target-mediated disposition and rapid ADC clearance. 91% of the patients had blast reduction at doses of$\geq$40 μg/kg. A dose-dependent blast clearance rate was observed. At doses >40 mcg/kg leukemic clearance occurred, but on-target myelosuppression resulted in a slower count recovery of the patients. The optimal monotherapy dose for further study was therefore determined at 40 μg/kg. Treatment emergent AEs were mostly related to on-target myelosuppression, while limited off-target toxicity was observed. Next, Phase 1 clinical studies with 33A in combination with hypomethylating agents (HMA) were evaluated based on data suggesting that HMAs sensitize cancer cells to cytotoxic agents. Both, azacitidine and decitabine increased CD33 levels on leukemic blasts. Synergism of azacitidine and 33A was demonstrated by increased levels gH2AX and PARP-mediated cell death of AML cells. Also in vivo data supported this synergistic effect. The Phase 1 results indicated that 33A at a dose of 10 μg/kg + HMA may provide a favorable balance of activity and tolerability in older AML patients. Durable remissions were observed during this study: the 65% CR + CRi rate more than doubles what is expected from HMA alone. The median overall survival rate has not been reached yet at the time of this interim analysis (72% of patients is still alive). Future studies include a Phase 1/2 study in front-line higher-risk myelodysplastic syndrome with 33A + azacitidine and a pivotal Phase 3 study for 33A + HMA in older AML patients is scheduled for 2016.

Downloaded by [108.49.191.210] at 11:34 25 March 2016

Downloaded by [108.49.191.210] at 11:34 25 March 2016

Early clinical development of mirvetuximab soravtansine (IMGN853) for platinum-resistant epithelial ovarian cancer

Charles Morris (ImmunoGen) discussed the folate receptor α-directed maytansinoid ADC mirvetuximab soravtansine, which has shown encouraging initial evidence of activity in patients with platinum resistant ovarian cancer in an ongoing Phase 1 clinical trial. Dr. Morris discussed the emerging clinical data, as well as the important contribution of clinical pharmacokinetics and biomarker-based patient selection to establishing an appropriate dosing regimen and target patient population.

Clinical update on sacituzumab govitecan (IMMU-132) in the treatment of advanced triple-negative breast and lung cancers

Cynthia Sullivan (Immunomedics) gave a clinical update on sacituzumab govitecan (IMMU132) in the treatment of advanced triple-negative breast cancer (TNBC) and lung cancers (SCLC and NSCLC). IMMU132 is a RS7-3G11-based, first-in-class ADC, site-specifically conjugated to a moderately toxic SN-38 drug using a pH-sensitive linker and targeting the pan-epithelial cancer antigen Trop2/EGP-1. The antigen shows a broad expression in many different cancers and is a prognostic indicator in certain cancers. The RS7-3G11 antibody binds many human solid tumors, such as breast, lung, colon, renal prostate, and urothelial tumors and is known to internalize upon binding. The antibody and the ADC also have immunotherapy functions (ADCC). IMMU132 has an average drug-to-antibody ratio (DAR) of ~7.6. IMMU132 specificity was determined comparing double strand breaks in Trop2 positive and negative cell lines, in which only the Trop2+ cell lines showed double strand breakage upon selective delivery of SN-38. Also, in vivo efficacy was shown in various human tumor xenografts expressing different Trop2 levels. Activity was achieved in patients that relapsed after multiple prior therapies, accompanied with manageable toxicities (less than the parental payload, irinotecan). Next, IMMU132 clinical Phase 1/2 studies on relapsed/refractory metastatic TNBC, metastatic NSCLC, metastatic SCLC, and metastatic urothelial cancer were discussed. The studies showed promising durable activity for all indications, with some responses exceeding 14 months. Median overall survival has not been reached yet. IMMU132 has an acceptable safety profile in heavily pretreated patients with diverse solid cancers. Dose-limiting neutropenia and rare cases of severe diarrhea were observed. Also, repeated doses can be administered over many months without evoking interfering host anti-IMMU132 antibodies. For the future, among other indications, a Phase 3 trial for mTNBC, and continued Phase 2 trials in lung and urothelial cancers are expected.

Development of immunoliposomes -next generation ADC formats

Daryl Drummond (Merrimack) then discussed the development of immunoliposomes in next generation ADC formats. Firstly the advantages of the Merrimack nanotechnology were presented, such as 1:1 drug-to-lipid ratio by weight, facilitating 30.000–150.000 drug molecules per liposome. In vivo stability and long circulating PK are critical to successful immune-targeted nanoparticle drug delivery, and to reach maximum accumulation at tumor site it needs at least 24 hour stability. MM-398 payload delivery with sustained intratumor levels of payload was presented. Next, data was presented on MM-302, a pegylated immunoliposome payloaded with doxorubicin and targeting ErbB2. When deciding on the type of payload, internalization and processing of the liposome and kinetic rates were all taken into account, since these affect the potency of drug. Doxorubicin could rapidly cross the plasma membrane and demonstrated a favorable PK. The enhanced permeability and retention effect in tumor endothelium provide the initial level of targeting for nanotherapeutics. To further improve targeting, the optimal scFv affinity and number per liposome is determined for a target. For instance, for HER2 increased scFv affinity and number of scFv molecules on the liposome allows to expand treatment to patients with 1+ and 2+ HER2 expression. Currently, the focus of MM-302 in the treatment landscape is 3rd or 4th line of treatment in HER2+ metastatic breast cancer patients. When combining trastuzumab and MM-302 preclinical data supported enhanced activity of the combination over single agents. The MM-302 Phase 1 efficacy study in patients receiving ≥30 mg/m2 MM-302 resulted in 11% ORR, 21% CBR and mPFS of 7.6 months in comparison to the historical 3.3 months. Enhanced efficacy was found in anthracycline naïve patients (24% ORR, 28% CBR, 11 mPFS). MM-302 monotherapy demonstrated an acceptable safety profile with fatigue and nausea being the most prevalent AEs. In total 26% treatment emergent AEs were apparent of which neutropenia was most common and 4.3% of the patients were experiencing severe AEs leading to treatment discontinuation. No treatment-related deaths were observed in this study. Also, no cardiac events with MM-302 monotherapy were noted. The Phase 2 study HERMIONE is now open and enrolling anthracycline-naïve patients that progressed on pertuzumab and TDM-1.

Lifastuzumab vedotin clinical activity in platinum resistant ovarian cancer and non-small cell lung cancer

Final speaker of this special ADC track was **Eric Humke** (Genentech) who talked about clinical activity of Lifastuzumab vedotin in platinum-resistant ovarian cancer and NSCLC. Lifastuzumab Vedotin is an ADC targeting NaPi2b. The discovery of NaPi2b as a possible drug target was discussed and the target appeared highly expressed in lung, ovarian, and thyroid tumors as was indicated by RNA screen and confirmative IHC data. NaPi2b is a multi-transmembrane, sodium-dependent phosphate transporter normally expressed in lungs, testis, salivary gland, thyroid gland, small intestine, mammary gland, and uterus and is involved in transcellular absorption of inorganic phosphate. However, NaPi2b overexpression is believed to be not carcinogenic itself. Lifastuzumab vedotin consists of a mc-vc-PABA-MMAE linker conjugate that showed preclinical target-directed cytotoxicity in lung and ovarian xenograft models, but also causes toxicity indicated by some bone marrow and hematological changes in a dose-dependent manner. Next,

Downloaded by [108.49.191.210] at 11:34 25 March 2016

prevalence, incidence, and division of lung cancer subtypes were discussed. NaPi2b is commonly overexpressed in the non-squamous histologic subtype and anti-NaPi2b is now being explored in those patients without targeted therapeutic options. There is a high unmet need for these patients, of which most undergoing palliative treatment with a PFS of 2–3 months and an OS <1 year. A high unmet need also exists for ovarian cancer patients, who also mostly receive palliative treatment and have PFS of 3–4 months and an OS of approximately 1 y. Companion diagnostics were developed along with the Phase 1 study. This clinical trial indicated that lifastuzumab vedotin was well-tolerated, dose limitations were not due to target expression on normal tissue and clinical toxicities were mainly antigen-independent (e.g., neutropenia, peripheral neuropathy fatigue, nausea, and liver function abnormalities). The study showed significant anti-tumor activity in ovarian (41% PR, 53% CBR) and some activity in NSCLC (0% CR and 20% CBR) IHC 2+/3+ patients. Some CT scan response results of both cancer types were shown. Based on these Phase 1 results, 3 clinical studies have now started; a Phase 2 trial in platinum-resistant ovarian cancer, a Phase 1b trial in platinum-sensitive ovarian cancer and a new expansion trial in NSCLC.

Special session: The antibody society

Janice M. Reichert

Antibodies to watch in 2016

Janice M. Reichert, 2015 President of The Antibody Society, discussed the benefits of participation in The Antibody Society, and gave an update on the Society's numerous activities, which include the organization and promotion of antibody-related conferences, publication of meeting previews and proceedings, and organization of a special issue of *Protein Engineering, Design & Selection* dedicated to antibody-related articles. She encouraged the audience to visit the Society's web page (www.antibodysociety.org) to become a member and to access resources maintained by the Society, including a comprehensive table of therapeutic monoclonal antibodies approved or in review in the European Union or the United States.

Dr. Reichert then discussed the clinical development and approval of antibodies in the biopharmaceutical industry's pipeline. She noted that 2015 was an extraordinary year for first marketing approvals of antibody therapeutics. The number of novel antibody therapeutics that received a first marketing approval in 2015 exceeded expectations, with 8 (alirocumab (Praluent®), elotuzumab (Empliciti®), evolocumab (Repatha®), daratumumab (Darzalex®), dinutuximab (Unituxin®), idarucizumab (Praxbind®), mepolizumab (Nucala®), necitumumab (Portrazza)) granted their first approval as of late December. A total of 9 antibody therapeutics were granted a first US approval in 2015, including all 8 antibodies noted above as well as secukinumab (Cosentyx®), which received a first approval in Japan in 2014. In the European Union, the European Commission also granted marketing approvals to 9 antibody therapeutics in 2015, including 5 antibodies noted above ((alirocumab (Praluent®), evolocumab (Repatha®), dinutuximab (Unituxin®), idarucizumab (Praxbind®), mepolizumab (Nucala®)) and 4 products that had been previously approved in another country. The increase in the number of antibodies gaining first approvals is due at least in part to the larger number of Phase 3 candidates (53 as of late 2015 vs. 39 as of late 2014).

In projecting events that might occur in 2016, Dr. Reichert noted that 7 novel antibody therapeutics (begelomab, bezlotoxumab, brodalumab, ixekizumab, obiltoxaximab, sarilumab, reslizumab) are undergoing regulatory review as of December 2015, and thus may gain their first approvals in 2016.[29] Of the 53 Phase 3 candidates, transitions to regulatory review by the end of 2016 are projected for 8 (atezolizumab, benralizumab, bimagrumab, durvalumab, inotuzumab ozogamicin, lebrikizumab, ocrelizumab, tremelimumab). Other "antibodies to watch" include 15 candidates (bavituximab, bococizumab, dupilumab, fasinumab, fulranumab, gevokizumab, guselkumab, ibalizumab, LY2951742, onartuzumab, REGN2222, roledumab, romosozumab, sirukumab, Xilonix) undergoing evaluation in Phase 3 studies that have estimated primary completion dates in 2016. The increase in the number of mAbs in Phase 3 studies is expected to drive a trend toward first approvals of ~6–8 new mAbs per year. However, the sustainability of this approval trend depends on verification of expected increases in potency of engineered antibodies, antibody-drug conjugates and bispecific antibodies, and the validity of the novel targets.

Town hall forum: Antibody drug nomenclature: What is INN a name?: WHO has been changing them?

Paul Carter

In 2014 the World Health Organization (WHO) introduced new definitions for the assignment of antibody international nonproprietary names (INN). A modification of the existing definitions was required because advances in antibody engineering have made classification into the current 3 main antibody groups (i.e., chimeric, humanized and human) unclear. Unfortunately the new definitions suffer from several major limitations that make them unworkable.[49] The purpose of the town hall forum on antibody drug nomenclature was to update the conference delegates on changes to the INN definitions, discuss their consequences, and solicit input on potential next steps. This session comprised an introductory presentation from **Paul J. Carter** (Genentech) based upon a recent publication by 34 authors from 31 different organizations.[49] The presentation was followed by a panel discussion in which input was solicited from the audience of conference delegates. The panelists were **Matthew P. Baker** (Abzena), **Max Vásquez** (Adimab), **Andreas Plückthun** (University of Zürich) and **Markus Enzelberger** (MorphoSys).

As discussed by Dr. Carter, the WHO established the INN system in 1950 to provide a unique (generic) name to identify each pharmaceutical substance. This system serves the important function of providing clear identification, and safe prescription and dispensing of medicines to patients. Additionally, the INN system supports communication and exchange of information among health professionals and scientists worldwide. The WHO selects INNs based upon the advice of an expert advisory panel.

The "-mab" stem was introduced in 1990 to indicate monoclonal antibody-based therapeutics. Substems were then

Downloaded by [108.49.191.210] at 11:34 25 March 2016

developed in 1997 to describe the antibody origin. The most widely used of these species origin substems are: -xi- for chimeric, -zu- for humanized and -u- for human. The origin substems were developed to classify antibodies based upon their "humanness," and with the assumption that humanness correlates with immunogenicity in patients. Now, nearly 20 y after this system was first introduced, it is appreciated that immunogenicity is a complex multi-factorial problem that is impacted by many parameters beyond amino-acid sequence with no clear sequence-identity threshold for immunogenicity.[5,50] Thus, the antibody origin substem may have outlived its original purpose.

New INN definitions for antibodies were undoubtedly needed as the old definitions had become outdated by rapid progress in antibody technologies. These technological changes include additional methods for humanizing antibodies, an increasing diversity of technologies for discovering human antibodies, and the widespread use of engineering to improve the therapeutic potential of antibodies. The creation of a new and robust naming system for antibody drugs is an exceptionally difficult undertaking, especially as technologies for antibody engineering continue to evolve. This challenge is exacerbated by the ever-expanding repertoire of bispecific and multispecific antibodies that are also entering clinical development.

The most recent (2014) WHO definition of chimeric antibodies was presented[51]:

> "A chimeric antibody is one for which both chain types are chimeric as a result of antibody engineering. A chimeric chain is a chain that contains a foreign variable domain (originating from one species other than human, or synthetic or engineered from any species including human) linked to a constant region of human origin. The variable domain of a chimeric chain has a V region amino acid sequence which, analyzed as a whole, is closer to non-human species than to human."

Similarly, the 2014 WHO definition for humanized antibodies was also described[51]:

> "A humanized antibody is one for which both chain types are humanized as a result of antibody engineering. A humanized chain is typically a chain in which the complementarity determining regions (CDR) of the variable domains are foreign (originating from one species other than human, or synthetic) whereas the remainder of the chain is of human origin. Humanization assessment is based on the resulting amino acid sequence, and not on the methodology per se, which allows protocols other than grafting to be used. The variable domain of a humanized chain has a V region amino acid sequence which, analyzed as a whole, is closer to human than to other species"

Notably, a definition for human antibodies is missing from the 2014 WHO guidance document on INN.[51] As for sequence analysis, comparison to human immunoglobulin heavy or light chain germline V-gene segments (IGHV, IGKV or IGLV) should be done with the Immunogenetics Information System® (IMGT®) DomainGapAlign tool (www.imgt.org). This was clarified during an open session of the WHO Expert Group in April 2015. The WHO INN drug names are used worldwide, with the exception of the USA. In the USA, the American Medical Association (AMA) assigns nonproprietary drug names known as United States Adopted Names (USAN). The drug name application processes are separate for WHO-INN and AMA-USAN.[52, 53] However, the WHO and the AMA work in close collaboration; thus, INN and USAN are commonly, but not invariably identical. The AMA has provided USAN/INN requirements for monoclonal antibodies that includes definitions of chimeric, humanized and human antibodies. [54] These antibody definitions are based upon the sequence identities of their variable regions to human heavy or light chain germline V-gene segments available in the IMGT® reference database: <85% for -ximab with ≥85% being either -zumab or –umab. [54]

Next, the impact of the new INN rules was considered. Most of the approved humanized antibodies are predicted to be "chimeric" or "mixed" under the new rules. Thus, assignment of INN names using the 2014 definitions is often inconsistent with the previous INN names, as well as with decades of scientific literature. Approved human antibodies are likely to retain their "human" classification under the new INN rules. This has created significant inconsistency among antibodies that are, in fact, similarly "human", yet which have been assigned different substem names due to the timing of their approval. In addition, some antibodies cloned from human subjects bear many somatic hypermutations (e.g., anti-HIV antibodies) and have <85% sequence identity with their closest human germline V-gene segments; yet they would fall within the definition of "chimeric". Thus, it is unclear how antibodies should be classified, as human or humanized, since a ≥85% sequence-identity threshold is being used for both. One justification for this classification is that human antibodies are cloned from human subjects whereas humanized antibodies are engineered, regardless of their origin. However, this is inconsistent with the stated goal of the assessment being based upon a resulting sequence and not the methodology that was used to generate it.

Dr. Carter discussed 8 of the major limitations of the 2014 INN antibody naming definitions: 1) The definitions do not allow researchers to determine reliably how an antibody will be classified; 2) The linking of sequence homology definitions to an evolving database of germline V-gene segments, makes the assignment of the origin substems a moving target; 3) It is possible for antibodies to be immunogenic if they are encoded by human germline V-gene sequences that are absent from a given patient's germline. Thus definitions that rely solely on similarity to germline V-gene sequences may provoke a false impression of their propensity for immunogenicity; 4) The definitions are inconsistent with several decades of precedence in naming antibodies in the scientific literature including many previously assigned INN names, i.e., the definitions are incompatible with existing names; 5) There is no WHO definition available for what makes an antibody "human" or how a "human" antibody differs from a "humanized" antibody;[51] 6) The sequence-identity threshold of 85% used in the AMA antibody definitions to determine human and humanized from chimeric antibodies is arbitrary, and the relationship to immunogenicity has not been established; 7) The antibody J region forms a critical part of all V domains, but is not included in the INN process; 8) The extent to which an antibody falls within the definition of human/humanized will be significantly impacted by the identity of the CDRs to the closest human germline V gene segment in the IMGT® database.

Some options for a new INN antibody system were considered, such as developing new substems (e.g., -sy- for synthetic or -e- for engineered). An alternative option of dropping the

origin substem entirely (i.e., use the -mab stem only) was also considered.

The presentation concluded with the message that dialog between the WHO INN Expert Group and key stakeholders is urgently needed to develop a more robust INN system for naming new antibody drugs. The WHO open session with INN stakeholders in April 2016 was identified as an excellent opportunity to initiate such a dialog.

The panel and audience engaged in a lively discussion. In general, participants strongly endorsed the notion that the 2014 antibody INN naming convention has major limitations and urgently needs revision. Audience members recounted a number of examples of nonproprietary names that are illogical in comparison to the prevailing nomenclature. There was very strong support for engaging in a discussion with the WHO to encourage it to address the flaws in the current system. Several participants voiced the opinion that is important that new definitions also govern therapeutic antibody INNs assigned under the 2014 definitions. Several creative suggestions were made for a new naming system. As mentioned, these included dropping the origin substem completely and using only the -mab stem; however, no clear agreement was reached in the time allotted.

Thursday december 10, 2015: Track A: Building comprehensive IgVH-gene epertoires: Discovering, confirming & cataloging new germline IgVH genes

Matthias Pauthner

The session was chaired by **Jamie K. Scott** (Simon Fraser University), who briefly introduced the current research questions pursued in the rather new field of Ig repertoire sequencing or 'antibody-omics'.

A transcriptome-based comparison of the heavy chain germline genes of the C57 BL/6 and BALB/c mouse strains

Andrew Collins (University of New South Wales) started the session by presenting a transcriptome-based comparison of Ig heavy (IgH) germline genes in C57 BL/6 and BALB/c mice. Since mice are both the classic subject of immunological research and the go-to model system for the generation of mAbs, a detailed analysis of murine Ig germline genes and antibody repertoires in commonly used strains is highly relevant.

In the first part of his talk, Dr. Collins presented 2 454 deep sequencing data sets of the C57 BL/6 (~21 k sequences) and BALB/c (~15 k sequences) IgM naive repertoires, sequenced from murine splenocytes. The accurate calculation of mutational frequencies for mature Ab genes from Ab germline genes is heavily reliant on complete germline gene databases for any given species, and still presents a big challenge in the field. When both datasets were queried against the IMGT database (www.imgt.org), the de facto reference database for such applications, C57 BL/6 reads aligned well with deposited IgVH germline alleles and showed narrow mutational distribution patters, as expected for naive IgM sequences. In the BALB/c data set, on the other hand, numerous groups of apparently strongly sequences (e.g., 188 reads calculated to be 18 nt divergent from IgVH1–18*01), revealed both substantial differences between the 2 mouse strains as well as the absence of correct BALB/c IgVH germline genes deposited in IMGT - thus causing falsely high mutational frequencies. In summary, only 50% of detected BALB/c IgVH alleles were annotated in the IMGT database, ~30% were present in the VBASE2/NCBI database and ~20% were entirely novel. Further, in-between the C57 BL/6 and BALB/c IgVH repertoires (99 and 164 detected alleles, respectively), only 5 were shared.

Dr. Collins then dove into the question of how mice build their effective Ig repertoire, given that they have a significantly lower count of total B cells than the theoretical repertoire size arising from combinatorial and junctional diversity (10^8 vs. 10^{12}, respectively). While combinatorial diversity is comparable between human and mice, N-nucleotide addition varies greatly. In humans, on average 7 nt are added to VD and DJ junctions, stochastically introducing a least one fully N-nucleotide encoded amino acid, while mice on average only add 3 nt, 83% of which merely complete codons that are partially germline encoded, and therefore strongly pre-determined by the redundancy of the genetic code. Additionally, mice do not compensate the diminished junctional diversity through somatic hypermutation: ~80% of all murine IgG sequences carry 4 or less mutations while the overwhelming majority of human IgG sequences are mutated from their respective germline genes.

In conclusion, Dr. Collins' findings show that there are strong differences between inbred mouse strain Ig repertoires and suggest that the underlying reason is, that mice compensate for reduced repertoire diversity by relying on a more adapted, germline encoded repertoire, which is likely anticipatory of the commonly encountered pathogens the respective mouse strain co-evolved with. This finding has direct implications on choosing appropriate animal systems for mAb generation, as well as murine immunization studies in general.

Transcriptional control of V(D)J recombination

Ann Feeney (The Scripps Research Institute) discussed the observation that V genes are not equally likely to be utilized, and explained the underlying mechanisms steering V_H gene rearrangement frequencies, which are strongly influenced by the presence of enhancer elements in the IgH locus.

The Ig repertoire of the rhesus macaque

Thomas Kepler (Boston University) discussed his work on mapping and assembling the IgH locus of a single rhesus macaque, as well as statistical methods for inferring the correct germline IgVH genes in antibody repertoire sequencing datasets. Both are considered critical steps toward correctly calculating mutational frequencies and accurately tracking the development of mAb lineages in rhesus macaques, which will be especially useful to HIV vaccine design efforts, once completed.

Somatic and germline genetics of antibody gene rearrangement

Steering away from animal models, **Scott Boyd** (Stanford University) discussed human immune repertoire data obtained

Downloaded by [108.49.191.210] at 11:34 25 March 2016

from a cohort of identical twins. He showed that naive B cell repertoires of identical twins are very similar, whereas their antigen-experienced memory B cell repertoires are quite different. This indicates that it might indeed be possible to predict the naive B cell repertoire, generated by combinatorial and junctional diversity, while the memory repertoire is very much a function of the immunological history of any given person.

Identification of novel germline immunoglobulin alleles in a south african population

In the following talk, **Bronwen Lambson** (National Institute for Communicable Diseases) presented on the identification of novel germline Ig alleles in a South African population. Broadly HIV neutralizing antibodies (bNAbs) differ from strain-specific NAbs in that they can neutralize a wide range of global HIV isolates by targeting functionally conserved epitopes on the HIV envelope. BNAbs are thought to be a key component of a protective HIV vaccine, however, only 15–30% of long-term infected patients develop broadly neutralizing serum responses and known bNAbs show multiple unusual genetic features (e.g., high mutation rates, up to ~30% (nt), very long HCDR3s, up to 39aa), calling for novel immunization strategies.

In the first part of her talk, Dr. Lambson addressed the question of how geographically representative the deposited Ig variable heavy (IgVH) genes in the IMGT reference database (www.IMGT.org) are, by deep sequencing the germline encoded IgVH repertoires of 28 HIV-1 infected donors from the CAPRISA cohort. To that end, DNA was isolated from donor PBMCs, IgVH genes were amplified with general V_H subgroup primers using both 454 and Illumina based sequencing, and the resulting data sets were queried against the IMGT, Ig-BLAST and Ig-PDB databases. Interestingly, this analysis revealed 85 entirely novel alleles and 38 alleles that were deposited in the Ig-PDB database. Strikingly, novel alleles were dispersed among all V_H subgroups, frequently encoded non-synonymous mutations and more than half of newly identified alleles were found in 4 or more donors, suggesting a geographical bias in the reference database.

Dr. Lambson then investigated IgVH copy number variations (CNVs) in the CAPRISA cohort as a possible explanation for the observed increase in IgVH allelic variation. It is known that some V_H genes display CNVs, like the V_H1–69 gene, which is also a commonly used V-gene in HIV bNAbs. To test this hypothesis, Dr. Lambson determined the copy number of V_H1–69 genes using a Droplet Digital PCR assay, which compares the amount of amplicons of a target gene to a known genomic reference. Indeed, most CAPRISA donors had 2–4 copies of V_H1–69, often possessing a mixture of IMGT, non-IMGT and novel V_H1–69 alleles. To address the question whether all novel and non-IMGT alleles are actively used in Ig repertoires, they were queried against antibodies isolated from the CAPRISA cohort, at least 16 of which in fact utilized novel and non-IMGT alleles (7 alleles in total). Additionally, the predicted germline sequences of multiple known bNAb families more accurately matched novel IgVH alleles.

In the final part of her talk, Dr. Lambson addressed the question if germline IgVH repertoires differ between donors that did and did not develop broadly HIV neutralizing serum activity. Interestingly, there was no apparent difference in IgVH repertoire between the 2 groups, suggesting that vaccine design efforts do not need to take into account geographical variations in IgVH germline repertoires, theoretically facilitating the design of globally applicable immunogens. This work was published in the Journal of Immunology in 2015.[55]

Inferring the immune systems dynamics from high-throughput antibody repertoire sequencing data

As discussed by **Gur Yaari** (Yale University), despite the huge promise of high-throughput sequencing of the antibody repertoire, extracting valuable biological information from these large datasets is challenging, and requires continuous development of computational tools. Tools were developed to infer an individual genotype from antibody repertoire sequences, quantify affinity-dependent selection and build a targeting model for the observed mutation spectrum. The applicability of these tools in the context of Multiple Sclerosis and Celiac disease was shown.

Track B: Overcoming resistance to clinical immunotherapy

Jenny Yeung

Chaired by **Louis Weiner** (Georgetown University Medical Center), the last Track B session of the meeting focused on how resistance to immunotherapy might be overcome.

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. As discussed by **Kim Allyson Margolin** (Stanford University Medical Center), 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.

Targeting immune checkpoints in genitourinary cancers: Rationale and current data

Ipilimumab, an anti-CTLA-4 antibody, was the first immune checkpoint inhibitor to be approved for the treatment of melanoma. As discussed by **Nizar M. Tannir** (MD Anderson Cancer Center), 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, a PD-L1 antibody, produced 48% ORR in patients whose immune cells expressed

Downloaded by [108.49.191.210] at 11:34 25 March 2016

PD-L1. 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.

Targeting cancer's fragile strengths to overcome immune evasion and promote effective immunotherapy

Antagonistic immune checkpoint antibodies have been very successful for some malignancies, however their effects are not found in all patients and their effects may not be long lasting in others. As discussed by **Louis Weiner** (Georgetown University Medical Center), understanding how tumor cells develop resistance to these therapeutic agents will enable therapeutic targeting of resistance mechanisms. Identification of new agents and strategies that can be used alone or in combination with immune checkpoint inhibitory antibodies would help to improve response rates.

An in vivo synthetic lethal shRNA screen was carried out using a murine breast cancer model to identify genes that protected tumors grown in wild-type immune-competent mice compared to immune-compromised mice. It was hypothesized that immune selection pressure favored in vivo expansion or contraction of malignant cells upon knock-down of critical genes. A shRNA library was introduced into the murine EO771 breast carcinoma cells that were transplanted into C57 BL/6 mice or C57 BL/6-SCID mice. Analyses of the relative expression of shRNA in tumors, which grew in either wild-type or SCID mice, identified 165 hits that showed adaptive immune selection pressure for cells with specific gene knock-downs. Gene knock-downs leading to under-representation in tumor cells growing in wild-type mice were genes that promoted survival in presence of immune selection pressure. Gene knock-down that led to over-representation in cells growing in wild-type mice represented genes that reduced survival in presence of immune selection pressure.

Validation of this model system was demonstrated by identification of genes with established roles in cancer immunity. CD47 is under-represented in wild-type mice compared to SCID mice where CD47 knock-down cells were rejected in EO771 tumors. CD47 is often overexpressed in tumors cells, which results in inhibition macrophage phagocytosis. The putative Tex9 tumor antigen was over-represented in wild-type mice, where knock-down of Tex9 led to tumors that survived longer in wild-type mice compared to SCID mice. Furthermore, pathway analysis of the data identified TGF-β as a central regulator of the immune response. Knock-down of TGF-β in EO771 tumor cells reduced tumor growth and promoted survival of the wild-type mice compared to immune-compromised mice.

This work demonstrated that a novel functional genomics approach enabled identification of individual genes that regulated the response of the tumor to immune selection pressure. Results validated CD47 and TGF-β as targets for immunomodulation in cancer therapy and identified new immunotherapeutic targets and can aid development of future combinatorial therapies.

Radiation-induced immunogenic modulation to enhance T cell and monoclonal antibody therapy of cancer

James W. Hodge (National Cancer Institute/National Institutes of Health) discussed the various uses of radiation therapy, which is used for treatment of many malignancies and is aimed at tumor destruction by inducing selective cell death of tumor cells. Local and systemic radiation therapy may be combined with immunotherapy to increase anti-tumor activity through immunogenic modulation. Here, the phenotype of the tumor is altered by radiation therapy and renders them more sensitive to killing by immune system.

Cancer vaccine efficacy can be improved by radiation therapy. Radiation therapy changes components of the antigen processing machinery such as proteasome subunits, peptide transporters and protein chaperones. These can result in upregulation of MHC class I as well as translocation of calreticulin, which facilitates T cell-mediated killing. Preclinical data showed that palliative levels of 153-Sm radionuclide increased expression of FAS and ICAM-1, tumor associated antigens such as PSA ad PSMA, as well as increased MHC class I expression, which led to increased antigen-specific cytotoxic T cell killing. In the clinical studies, QUARDAEMT, a 153-Sm containing therapeutic agent for palliation of bone metastasis combined with the PSA-TRICOM vaccine for hormone refractory prostate cancer, demonstrated that the combination therapy increased progression free survival to 3.7 months compared to 1.7 months with QUADRAMET alone.

Finally, radiation therapy was also shown to upregulate therapeutic targets and sensitized them to monoclonal antibody therapy. The targets of cetuximab (EGFR), rituximab (CD20) and trastuzumab (HER2) were all upregulated after radiation treatment. Upregulation of HER2 by doses as low as 2 Gy were maintained over 96 hours, resulted in better trastuzumab-mediated ADCC, and sensitized them to the anti-proliferative effects of trastuzumab. Upregulation of HER2 was also found in triple-negative breast cell lines, which are normally not sensitive to anti-HER2 therapy. These effects of radiation therapy would be particularly useful for tissues that express low amounts of the antigen of interest, could potentially sensitize tumors resistant to monoclonal antibody therapy, plus increase the number of patients eligible for targeted therapy.

Leveraging clinical learnings and patient tailoring to enable next generation ADC success

Alan Rigby (Synta Pharmaceuticals) discussed HSP90-drug conjugates (HDCs). HSP90 is a molecular chaperone that regulates post-translational folding, stability and function of protein substrates or client proteins. These client proteins may have important roles in cell growth, differentiation and survival. HSP90 accounts for 4–6% of total cellular proteins. The conformation and post-translational modification of HSP90 in tumor cells enables higher binding affinity, and thus tumor retention to small molecule HSP90 inhibitors, such as ganetespib. HSP90 inhibitors selectively kill tumor cells through this retention/accumulation in the tumor cell by preventing requisite interactions with oncogenic client proteins, which in turn leads to

Downloaded by [108.49.191.210] at 11:34 25 March 2016

their degradation in the proteasome. Leveraging the learnings of ganetespib, Synta has developed HDCs with an HSP90 inhibitor as the tumor-targeting arm that is conjugated to an anticancer payload through a cleavable linker. HDCs have advantages over ADCs because they are smaller molecules (molecular weight of 550–1500) that target intracellular HSP90 in many tumor types. Their small size enables them to enter cells through passive diffusion or through an activated transporter. This can overcome issues with low antigen density that can be encountered by ADCs, and provides a novel approach to improving the therapeutic index that continues to plague ADC therapies.

STA-12-8666 is a first-in-class HSP90 inhibitor SN-38 drug conjugate developed by the Synta HDC platform. SN-38 is the active metabolite of irinotecan, a widely used potent topoisomerase 1 inhibitor. This first-in-class HDC demonstrated an improved therapeutic window and better efficacy in a number of cancer models, including a cisplatin-resistant small lung carcinoma model, a chemo-resistant pancreatic PDX model and several other cancer PDX models. STA-12-8666 was able to induce durable tumor regression compared to HSP90 inhibitor or irinotecan alone by increased retention times in the tumors. When the tumors in these models relapsed, retreatment with the HDC demonstrated retained sensitivity.

HDCs are able to increase on target exposure of the anticancer payloads, resulting in sustained 'on target' delivery of an active payload into tumors. As demonstrated for STA-12-8666 it appears that HDCs have improved tumor access, tumor penetration and residualization compared to ADCs. To further probe and validate the HDCs mechanism of tumor retention and its contribution to target exposure, which is responsible for the durable responses observed in several PDX animal studies an HSP90-targeted imagining agent is under development. Preclinical toxicology studies also support a superior safety profile of the HDC vs. combination therapy at clinically translatable doses.

Downloaded by [108.49.191.210] at 11:34 25 March 2016

References

1. Yamada DH, Elsaesser H, Lux A, Timmerman JM, Morrison SL, de la Torre JC, Nimmerjahn F, Brooks DG. Suppression of Fcgamma-receptor-mediated antibody effector function during persistent viral infection. Immunity 2015; 42:379-90; PMID:25680277; http://dx.doi.org/10.1016/j.immuni.2015.01.005
2. Diebolder CA, Beurskens FJ, de Jong RN, Koning RI, Strumane K, Lindorfer MA, Voorhorst M, Ugurlar D, Rosati S, Heck AJ, et al. Complement is activated by IgG hexamers assembled at the cell surface. Science 2014; 343:1260-3; PMID:24626930; http://dx.doi.org/10.1126/science.1248943
3. de Jong RN, Beurskens FJ, Verploegen S, Strumane K, van Kampen MD, Voorhorst M, Horstman W, Engelberts PJ, Oostindie SC, Wang G, et al. A novel platform for the potentiation of therapeutic antibodies based on antigen-dependent formation of IgG hexamers at the cell surface. PLoS Biol 2016; 14:e1002344; PMID:26736041; http://dx.doi.org/10.1371/journal.pbio.1002344
4. Melis JP, Strumane K, Ruuls SR, Beurskens FJ, Schuurman J, Parren PW. Complement in therapy and disease: regulating the complement system with antibody-based therapeutics. Mol Immunol 2015; 67:117-30; PMID:25697848; http://dx.doi.org/10.1016/j.molimm.2015.01.028
5. Baker MP, Reynolds HM, Lumicisi B, Bryson CJ. Immunogenicity of protein therapeutics: the key causes, consequences and challenges. Self Nonself 2010; 1:314-22; PMID:21487506; http://dx.doi.org/10.4161/self.1.4.13904
6. Begley CG, Ellis LM. Drug development: raise standards for preclinical cancer research. Nature 2012; 483:531-3; PMID:22460880; http://dx.doi.org/10.1038/483531a
7. Bradbury A, Pluckthun A. Reproducibility: standardize antibodies used in research. Nature 2015; 518:27-9; PMID:25652980; http://dx.doi.org/10.1038/518027a
8. Bradbury AR, Pluckthun A. Getting to reproducible antibodies: the rationale for sequenced recombinant characterized reagents. Protein Eng Des Sel 2015; 28:303-5; PMID:26446960; http://dx.doi.org/10.1093/protein/gzv051
9. Berglund L, Bjorling E, Oksvold P, Fagerberg L, Asplund A, Szigyarto CA, Persson A, Ottosson J, Wernerus H, Nilsson P, et al. A genecentric Human Protein Atlas for expression profiles based on antibodies. Mol Cell Proteomics 2008; 7:2019-27; PMID:18669619; http://dx.doi.org/10.1074/mcp.R800013-MCP200
10. Bordeaux J, Welsh A, Agarwal S, Killiam E, Baquero M, Hanna J, Anagnostou V, Rimm D. Antibody validation. Biotechniques 2010; 48:197-209; PMID:20359301; http://dx.doi.org/10.2144/000113382
11. Elliott S, Busse L, Bass MB, Lu H, Sarosi I, Sinclair AM, Spahr C, Um M, Van G, Begley CG. Anti-Epo receptor antibodies do not predict Epo receptor expression. Blood 2006; 107:1892-5; PMID:16249375; http://dx.doi.org/10.1182/blood-2005-10-4066
12. Freedman LP, Cockburn IM, Simcoe TS. The economics of reproducibility in preclinical research. PLoS Biol 2015; 13:e1002165; PMID:26057340; http://dx.doi.org/10.1371/journal.pbio.1002165
13. Kontermann RE, Brinkmann U. Bispecific antibodies. Drug Discov Today 2015; 20:838-47; PMID:25728220; http://dx.doi.org/10.1016/j.drudis.2015.02.008
14. Metz S, Panke C, Haas AK, Schanzer J, Lau W, Croasdale R, Hoffmann E, Schneider B, Auer J, Gassner C, et al. Bispecific antibody derivatives with restricted binding functionalities that are activated by proteolytic processing. Protein Eng Des Sel 2012; 25:571-80; PMID:22976197; http://dx.doi.org/10.1093/protein/gzs064
15. Desnoyers LR, Vasiljeva O, Richardson JH, Yang A, Menendez EE, Liang TW, Wong C, Bessette PH, Kamath K, Moore SJ, et al. Tumor-specific activation of an EGFR-targeting probody enhances therapeutic index. Sci Transl Med 2013; 5:207ra144; PMID:24132639; http://dx.doi.org/10.1126/scitranslmed.3006682
16. Wong KR, Menendez E, Craik CS, Kavanaugh WM, Vasiljeva O. In vivo imaging of protease activity by Probody therapeutic activation. Biochimie. 2016 Mar;122:62-7; http://dx.doi.org/10.1016/j.biochi.2015.11.003.
17. Stefan N, Zimmermann M, Simon M, Zangemeister-Wittke U, Pluckthun A. Novel prodrug-like fusion toxin with protease-sensitive bioorthogonal PEGylation for tumor targeting. Bioconjug Chem 2014; 25:2144-56; PMID:25350699; http://dx.doi.org/10.1021/bc500468s
18. Verdurmen WP, Luginbuhl M, Honegger A, Pluckthun A. Efficient cell-specific uptake of binding proteins into the cytoplasm through engineered modular transport systems. J Control Release 2015; 200:13-22; PMID:25526701; http://dx.doi.org/10.1016/j.jconrel.2014.12.019
19. de Goeij BE, Satijn D, Freitag CM, Wubbolts R, Bleeker WK, Khasanov A, Zhu T, Chen G, Miao D, van Berkel PH, et al. High turnover of tissue factor enables efficient intracellular delivery of antibody-drug conjugates. Mol Cancer Ther 2015; 14:1130-40; PMID:25724665; http://dx.doi.org/10.1158/1535-7163.MCT-14-0798
20. Breij EC, de Goeij BE, Verploegen S, Schuurhuis DH, Amirkhosravi A, Francis J, Miller VB, Houtkamp M, Bleeker WK, Satijn D, et al. An antibody-drug conjugate that targets tissue factor exhibits potent therapeutic activity against a broad range of solid tumors. Cancer Res 2014; 74:1214-26; PMID:24371232; http://dx.doi.org/10.1158/0008-5472.CAN-13-2440
21. Tzeng A, Kwan BH, Opel CF, Navaratna T, Wittrup KD. Antigen specificity can be irrelevant to immunocytokine efficacy and biodistribution. Proc Natl Acad Sci U S A 2015; 112:3320-5; PMID:25733854; http://dx.doi.org/10.1073/pnas.1416159112

Downloaded by [108.49.191.210] at 11:34 25 March 2016

22. Zhu EF, Gai SA, Opel CF, Kwan BH, Surana R, Mihm MC, Kauke MJ, Moynihan KD, Angelini A, Williams RT, et al. Synergistic innate and adaptive immune response to combination immunotherapy with anti-tumor antigen antibodies and extended serum half-life IL-2. Cancer Cell 2015; 27:489-501; PMID:25873172; http://dx.doi.org/10.1016/j.ccell.2015.03.004
23. Liu X, Pu Y, Cron K, Deng L, Kline J, Frazier WA, Xu H, Peng H, Fu YX, Xu MM. CD47 blockade triggers T cell-mediated destruction of immunogenic tumors. Nat Med 2015; 21:1209-15; PMID:26322579; http://dx.doi.org/10.1038/nm.3931
24. Bronte V. Tumors STING adaptive antitumor immunity. Immunity 2014; 41:679-81; PMID:25517609; http://dx.doi.org/10.1016/j.immuni.2014.11.004
25. Deng L, Liang H, Xu M, Yang X, Burnette B, Arina A, Li XD, Mauceri H, Beckett M, Darga T, et al. STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. Immunity 2014; 41:843-52; PMID:25517616; http://dx.doi.org/10.1016/j.immuni.2014.10.019
26. Fan Z, Yu P, Wang Y, Fu ML, Liu W, Sun Y, Fu YX. NK-cell activation by LIGHT triggers tumor-specific CD8+ T-cell immunity to reject established tumors. Blood 2006; 107:1342-51; PMID:16223768; http://dx.doi.org/10.1182/blood-2005-08-3485
27. Ferris RL. Immunology and immunotherapy of head and neck cancer. J Clin Oncol 2015; 33:3293-304; PMID:26351330; http://dx.doi.org/10.1200/JCO.2015.61.1509
28. Carmi Y, Spitzer MH, Linde IL, Burt BM, Prestwood TR, Perlman N, Davidson MG, Kenkel JA, Segal E, Pusapati GV, et al. Allogeneic IgG combined with dendritic cell stimuli induce antitumour T-cell immunity. Nature 2015; 521:99-104; PMID:25924063; http://dx.doi.org/10.1038/nature14424
29. Reichert JM. Antibodies to watch in 2016. MAbs 2016, Feb-Mar;8(2):197-204; PMID: 26651519; http://dx.doi.org/10.1080/19420862.2015.1125583
30. Moasser MM, Krop IE. The evolving landscape of HER2 targeting in breast cancer. JAMA Oncol 2015; 1:1154-61; PMID:26204261; http://dx.doi.org/10.1001/jamaoncol.2015.2286
31. McCormack PL. Pertuzumab: a review of its use for first-line combination treatment of HER2-positive metastatic breast cancer. Drugs 2013; 73:1491-502; PMID:23982598; http://dx.doi.org/10.1007/s40265-013-0109-0
32. Baron JM, Boster BL, Barnett CM. Ado-trastuzumab emtansine (T-DM1): a novel antibody-drug conjugate for the treatment of HER2-positive metastatic breast cancer. J Oncol Pharm Pract 2015; 21:132-42; PMID:24682654; http://dx.doi.org/10.1177/1078155214527144
33. Feldmann M, Maini RN. Lasker clinical medical research award. TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. Nat Med 2003; 9:1245-50; PMID:14520364; http://dx.doi.org/10.1038/nm939
34. Goodall LJ, Ovecka M, Rycroft D, Friel SL, Sanderson A, Mistry P, Davies ML, Stoop AA. Pharmacokinetic and pharmacodynamic characterisation of an anti-mouse TNF receptor 1 domain antibody formatted for in vivo half-life extension. PLoS One 2015; 10:e0137065; PMID:26352810; http://dx.doi.org/10.1371/journal.pone.0137065
35. McCann FE, Perocheau DP, Ruspi G, Blazek K, Davies ML, Feldmann M, Dean JL, Stoop AA, Williams RO. Selective tumor necrosis factor receptor I blockade is antiinflammatory and reveals immunoregulatory role of tumor necrosis factor receptor II in collagen-induced arthritis. Arthritis Rheumatol 2014; 66:2728-38; PMID:24965881; http://dx.doi.org/10.1002/art.38755
36. Eggermont AM, Spatz A, Robert C. Cutaneous melanoma. Lancet 2014; 383:816-27; PMID:24054424; http://dx.doi.org/10.1016/S0140-6736(13)60802-8
37. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 2015; 27:450-61; PMID:25858804; http://dx.doi.org/10.1016/j.ccell.2015.03.001
38. Raedler LA. Opdivo (Nivolumab): second PD-1 inhibitor receives FDA approval for unresectable or metastatic melanoma. Am Health Drug Benefits 2015; 8:180-3; PMID:26629287
39. Philips GK, Atkins M. Therapeutic uses of anti-PD-1 and anti-PD-L1 antibodies. Int Immunol 2015; 27:39-46; PMID:25323844; http://dx.doi.org/10.1093/intimm/dxu095
40. Rizvi NA, Mazieres J, Planchard D, Stinchcombe TE, Dy GK, Antonia SJ, Horn L, Lena H, Minenza E, Mennecier B, et al. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. Lancet Oncol 2015; 16:257-65; PMID:25704439; http://dx.doi.org/10.1016/S1470-2045(15)70054-9
41. Pluckthun A. Designed ankyrin repeat proteins (DARPins): binding proteins for research, diagnostics, and therapy. Annu Rev Pharmacol Toxicol 2015; 55:489-511; PMID:25562645; http://dx.doi.org/10.1146/annurev-pharmtox-010611-134654
42. Wurch T, Pierre A, Depil S. Novel protein scaffolds as emerging therapeutic proteins: from discovery to clinical proof-of-concept. Trends Biotechnol 2012; 30:575-82; PMID:22944617; http://dx.doi.org/10.1016/j.tibtech.2012.07.006
43. Cooke RM, Brown AJ, Marshall FH, Mason JS. Structures of G protein-coupled receptors reveal new opportunities for drug discovery. Drug Discov Today 2015; 20:1355-64; PMID:26303408; http://dx.doi.org/10.1016/j.drudis.2015.08.003
44. Errey JC, Dore AS, Zhukov A, Marshall FH, Cooke RM. Purification of stabilized GPCRs for structural and biophysical analyses. Methods Mol Biol 2015; 1335:1-15; PMID:26260590; http://dx.doi.org/10.1007/978-1-4939-2914-6_1
45. Hutchings CJ, Cseke G, Osborne G, Woolard J, Zhukov A, Koglin M, Jazayeri A, Pandya-Pathak J, Langmead CJ, Hill SJ, et al. Monoclonal anti-beta1-adrenergic receptor antibodies activate G protein signaling in the absence of beta-arrestin recruitment. MAbs 2014; 6:246-61; PMID:24253107; http://dx.doi.org/10.4161/mabs.27226
46. Bankert KC, Oxley KL, Smith SM, Graham JP, de Boer M, Thewissen M, Simons PJ, Bishop GA. Induction of an altered CD40 signaling complex by an antagonistic human monoclonal antibody to CD40. J Immunol 2015; 194:4319-27; PMID:25795759; http://dx.doi.org/10.4049/jimmunol.1402903
47. de Vos AF, Melief MJ, van Riel D, Boon L, van Eijk M, de Boer M, Laman JD. Antagonist anti-human CD40 antibody inhibits germinal center formation in cynomolgus monkeys. Eur J Immunol 2004; 34:3446-55; PMID:15517614; http://dx.doi.org/10.1002/eji.200424973
48. Alabraba EB, Lai V, Boon L, Wigmore SJ, Adams DH, Afford SC. Coculture of human liver macrophages and cholangiocytes leads to CD40-dependent apoptosis and cytokine secretion. Hepatology 2008; 47:552-62; PMID:17999420; http://dx.doi.org/10.1002/hep.22011
49. Jones TD, Carter PJ, Pluckthun A, Vasquez M, Holgate RG, Hotzel I, Popplewell AG, Parren PW, Enzelberger M, Rademaker HJ, et al. The INNs and outs of antibody nonproprietary names. MAbs 2016; 8:1-9; PMID:26716992; http://dx.doi.org/10.1080/19420862.2015.1114320
50. Wang Y, Zhu M, Yu P, Fu YX. Promoting immune responses by LIGHT in the face of abundant regulatory T cell inhibition. J Immunol 2010; 184:1589-95; PMID:20042587; http://dx.doi.org/10.4049/jimmunol.0901582
51. WHO-INN. International nonproprietary names (INN) for biological and biotechnological substances 2014 (http://www.who.int/medicines/services/inn/BioRev2014.pdf).
52. WHO-INN. International Nonproprietary Names (http://www.who.int/medicines/services/inn/en/).
53. AMA. Application Forms [Internet]. Chicago (IL). [cited 2016 Feb 5]. Available from http://www.ama-assn.org/ama/pub/physician-resources/medical-science/united-states-adopted-names-council/application-forms.page?
54. AMA. Application Forms [Internet]. Chicago (IL). [cited 2016 Feb 5]. Available from http://www.ama-assn.org/ama/pub/physician-resources/medical-science/united-states-adopted-names-council.page?
55. Scheepers C, Shrestha RK, Lambson BE, Jackson KJ, Wright IA, Naicker D, Goosen M, Berrie L, Ismail A, Garrett N, et al. Ability to develop broadly neutralizing HIV-1 antibodies is not restricted by the germline Ig gene repertoire. J Immunol 2015; 194:4371-8; PMID:25825450; http://dx.doi.org/10.4049/jimmunol.1500118