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8th Annual European Antibody Congress 2012
November 27–28, 2012, Geneva, Switzerland

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Abbreviations: ADC, antibody-drug conjugate; ADCC, antibody-dependent cell-mediated cytotoxicity; BBB, blood-brain barrier; CDC, complement-dependent cytotoxicity; CHO, Chinese hamster ovary; DAR, drug-antibody ratio; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; IGF-1R, insulin-like growth factor-1 receptor; mAb, monoclonal antibody; MM, multiple myeloma; MS, mass spectrometry; MTD, maximum tolerated dose; NHL, non-Hodgkin lymphoma; PD, pharmacodynamics; PK, pharmacokinetics; PTM, post-translational modification; TNF, tumor necrosis factor

The 8th European Antibody Congress (EAC), organized by Terrapin Ltd., was again held in Geneva, Switzerland, following on the tradition established with the 4th EAC. The new agenda format for 2012 included three parallel tracks on: (1) naked antibodies; (2) antibody drug conjugates (ADCs); and (3) bispecific antibodies and alternative scaffolds. The meeting started and closed with three plenary lectures to give common background and to share the final panel discussion and conclusions. The two day event included case studies and networking for nearly 250 delegates who learned of the latest advances and trends in the global development of antibody-based therapeutics.

The monoclonal antibody track was focused on understanding the structure-function relationships, optimization of antibody design and developability, and processes that allow better therapeutic candidates to move through the clinic. Discussions on novel target identification and validation were also included. The ADC track was dedicated to evaluation of the ongoing success of the established ADC formats alongside the rise of the next generation drug-conjugates. The bispecific and alternative scaffold track was focused on taking stock of the multitude of bispecific formats being investigated and gaining insight into recent innovations and advancements. Mechanistic understanding, progression into the clinic and the exploration of multispecifics, redirected T cell killing and alternative scaffolds were extensively discussed. In total, nearly 50 speakers provided updates of programs related to antibody research and development ongoing in the academic, government and commercial sectors.

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November 27, 2012: Day 1
Opening Plenary Session

Paul J. Carter and Alain Beck

The 8th Annual European Antibody Congress was opened by the conference Chairman, **Alain Beck** (Centre d'Immunologie Pierre Fabre and Associate Editor of *mAbs*). Since 1986, more than 40 monoclonal antibodies (mAbs) and derivatives, including Fc fusion proteins,[1] have been approved for therapeutic use. In 2012, the first glyco-engineered antibody, mogamulizumab, (humanized anti-CCR4) was approved for marketing in Japan for T cell leukemia-lymphoma.[2] At least 15 additional glyco-engineered antibodies are currently in clinical development.[2] Also in 2012, the anti-human epidermal growth factor receptor (HER)2 antibody, pertuzumab, was approved in combination with another anti-HER2 antibody, trastuzumab, plus docetaxel for first-line treatment of patients with HER2-positive metastatic breast cancer. Currently, there are ~350 antibodies in clinical trials including ~30 antibodies and derivatives in Phase 2/3 or Phase 3 trials.[3]

Paul Carter (Genentech) gave the opening keynote presentation entitled, "Antibody therapeutics: past, present and future." Since the mid-1990s, antibodies have emerged as a clinically and commercially important class of therapeutics. Indeed, 31 antibody therapeutics are currently marketed in the US, with the majority targeting oncology, autoimmunity and chronic inflammatory diseases. In 2011, the worldwide sales of all combined antibody therapeutics were over $45 billion.

Antibodies have several notable strengths and limitations as therapeutics.[4] Strengths of antibodies include that they are often readily generated to targets of interest, their properties are tunable for different therapeutic applications, and they are commonly well-tolerated by patients.[4] Also, antibodies have a relatively high success rate (~17% from first-in-human to approval) compared with other drug classes. Additionally, antibodies are a broadly applicable drug class. Limitations of antibodies as therapeutics

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include lack of access to intracellular targets, inefficient tissue penetration, lack of oral bioavailability, and inefficient delivery across the blood-brain barrier (BBB). Further limitations of antibody therapeutics are high cost and that innate and acquired resistance is sometimes observed. Major drivers to improve antibody therapeutics are to increase benefit to patients and commercial competition between drug developers pursuing antibodies to the same antigens and clinical indications.

Targeting the HER2 proto-oncogene in breast cancer illustrates progress in developing and improving antibody therapeutics, as well as the use of companion diagnostics to identify patients to treat. HER2 is overexpressed in ~20% of breast cancers and is associated with shortened survival. Trastuzumab is a humanized IgG_1 antibody that binds to HER2 and has multiple antitumor activities including growth inhibition, antibody-dependent cell-mediated cytotoxicity (ADCC) and additivity with cytotoxic chemotherapy. Trastuzumab is approved for the treatment of HER2-overexpressing breast cancer and metastatic gastric cancer. Companion diagnostics used to identify HER2-positive patients include immunohistochemistry to detect HER2 protein overexpression and fluorescence in situ hybridization to detect HER2 gene amplification.

Pertuzumab is a humanized IgG_1 antibody that binds to HER2 at a different epitope than trastuzumab. Pertuzumab inhibits ligand-dependent heterodimerization of HER2 with epidermal growth factor receptor (EGFR), HER3 and HER4 and ligand-initiated intracellular signaling. Combining pertuzumab and trastuzumab can result in additive anti-tumor activity in HER2-expressing xenograft models. In June 2012, pertuzumab was approved in combination with trastuzumab and docetaxel for first-line treatment of patients with HER2-positive metastatic breast cancer based on the results of the pivotal Phase 3 CLEOPATRA study.[5] The combination of pertuzumab plus trastuzumab plus docetaxel significantly prolonged progression-free survival compared with placebo plus trastuzumab plus docetaxel, with no increase in cardiotoxicity.[5]

Trastuzumab emtansine is an antibody-drug conjugate in which trastuzumab is conjugated to the potently cytotoxic anti-tubulin agent DM1. Trastuzumab emtansine has robust antitumor activity in tumor xenograft models that are resistant to trastuzumab alone. In the Phase 3 EMILIA trial, patients with HER2-positive advanced breast cancer previously treated with trastuzumab and a taxane, were randomly assigned to receive trastuzumab emtansine or lapatinib plus capecitabine.[6] Trastuzumab emtansine significantly prolonged progression-free and overall survival with less toxicity than lapatinib plus capecitabine in the EMILIA trial.[6] (Post-meeting note, trastuzumab emtansine, with the tradename Kadcyla, was approved in the US in February 2013.)

Following Dr. Carter, **Steve Coats** (MedImmune) gave a talk on how to choose the best antibody targets and technologies, and **Antonio Maschio** (Maschio and Soames LLP) showcased intellectual property strategies to clearing the path to market.

November 27, 2012: Day 1, Track A
Monoclonal Antibodies: Optimizing Structure Function

Paul J. Carter and Alain Beck

Alain Beck (Centre d'Immunologie Pierre Fabre) delivered a presentation entitled, "Antibody structure-guided optimization: OptimAbs." High-resolution mass spectrometry (MS) techniques combined with ultra-performance separation methods allow extensive structural assessment of antibodies. As a result, a continuously increasing number of micro-variants are also identified. The evaluation of these isoforms for stability, antigen and Fc receptor binding and pharmacokinetics (PK)/pharmacodynamics (PD), as well as for safety, is critical for the design of next generation optimized therapeutic antibodies and related products. This structure-function relationship knowledge can also be used to improve homogeneity of antibody lead candidates by genetic engineering to mitigate the chemistry, manufacture and control liabilities (i.e., developability). mAb 6F4 targets the human junctional adhesion molecule A (JAM-A), which is a single transmembrane protein belonging to the immunoglobulin superfamily. JAM-A localizes in tight junctions in epithelial and endothelial cells. Homophilic JAM-A interactions have been shown to be important for regulation of epithelial barrier function. JAM-A was identified by the Centre d'Immunologie Pierre Fabre as a target of interest in oncology and confirmed independently by academic groups. Multiple and complementary MS methods have been used by the OptimAbs network at different stages of optimization and pre-development of a humanized version of 6F4 lead anticancer antibody. Full structural characterization of research lead candidates derived from 6F4 have been performed, as well as identification of hot spots that may be deleterious for stability, PK and pharmacology, resulting in the selection of an optimized antibody candidate for pharmaceutical development.

Paul Carter (Genentech) delivered a presentation entitled, "From knobs-into-holes to onartuzumab (MetMAb)." Bispecific antibodies are one of the major strategies being pursued to develop the next generation antibody therapeutics.[4,7] Over 50 different formats have been described, including many with an Fc region that can provide long serum half-life and optional effector functions.[4,7] Bispecific IgG were initially produced by co-expression of 2 different IgG in a hybrid hybridoma;[8] however, the yield and purity of the bispecific molecules from hybrid hybridomas was low due to unwanted heavy chain homodimerization and light chains pairing with non-cognate heavy chains. Engineering the interface between antibody C_H3 domains with so-called knobs-into-holes mutations provides an efficient way to heterodimerize different antibody heavy chains and minimize unwanted homodimerization.[9,10] Efficient construction of human bispecific IgG was accomplished using knobs-into-holes mutations to direct heavy chain heterodimerization and a common light chain to avoid light chain mispairing.[11]

The first antibody to enter clinical development incorporating knobs-into-holes mutations is the one-armed anti-Met antibody onartuzumab (MetMAb). MetMAb consists of heavy and

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light chains for a humanized anti-Met antibody plus an Fc chain. Knobs-into-holes mutations were use to heterodimerize the anti-Met heavy and Fc chains. MetMAb binds to Met thereby blocking binding of the ligand hepatocyte growth factor/scatter factor (HGF/SF) to Met. MetMAb is monovalent to avoid dimerizing and activating Met. The Fc region in MetMAb allows for long serum half-life by binding to the neonatal receptor, FcRn. MetMAb was produced in *E. coli* in the presence of foldases to promote chain folding and assembly. MetMAb is aglycosylated and does not mediate cytotoxic effector functions against Met positive cells. This was desirable from a safety perspective as Met is expressed on some normal tissues in addition to some tumor cells. MetMAb inhibits ligand-induced activation of Met, as well as cell proliferation and migration in vitro. MetMAb exhibits antitumor activity in vivo, including in paracrine models of non-small cell lung cancer (NSCLC), and is more efficacious in combination with the EGFR small molecule inhibitor erlotinib. In early clinical trials, MetMAb has been well-tolerated and has shown some efficacy in combination with erlotinib in NSCLC tumors with high expression of Met. MetMAb is currently in multiple Phase 2 and 3 clinical trials.

Alexis Rossignol (Clean Cells) gave a talk on standardizing ADCC potency assays for regulatory compliance. ADCC assays for antibodies commonly use peripheral blood mononuclear cell (PBMCs) from human donors as a source of effector cells. The ability of PMBCs from different donors to support ADCC is highly variable for multiple reasons, including polymorphisms in FcγRIIIA that affect ADCC. Standardized ADCC assays were developed using T lymphocyte cell lines engineered to express FcγRIIIA as effector cells. ADCC assays with the engineered T lymphocytes were much more reproducible than ADCC assays with PBMCs.

Steffen Hartmann (Novartis) delivered a presentation on assessing antibody developability in the selection of optimal therapeutic antibody candidates. Antibody developability was evaluated based upon multiple parameters, including amino sequence liabilities, expression titer and purification yield, aggregation, stability, physicochemical profile, off-target binding, PK half-life and immunogenicity.

The starting point for antibody candidate selection was a large panel of antibodies with favorable biologic characteristics such as target antigen binding, in vitro potency and in vivo efficacy. Initial developability profiling was used to triage the antibody panel to ~4 candidates. More extensive developability profiling was then used to select a lead antibody for development.

Antibodies are susceptible to many different post-translational modifications (PTMs), including pyroglutamate formation, asparagine deamidation, aspartate isomerization, tryptophan and methionine oxidation, proline amidation and lysine glycation. The potential risk of PTMs on antibody developability varies from minimal to high, behooving case-by-case assessment. Significant potential problems encountered include loss of potency, reduced safety, increased immunogenicity and altered PK. Other potential liabilities from antibody PTMs include reduced stability, problems in manufacturing, formulation and storage, plus the necessity of additional analytical methods.

PTM profiling during antibody developability assessment included sequence-based prediction of potential PTMs and experimental evaluation, often under conditions chosen to accelerate their occurrence. It is sometimes possible to engineer the antibody sequence to remove the PTM site without perturbing binding affinity or biologic potency.

Developability assessment also considered critical parameters such as aggregation by size exclusion chromatography, expression titer and purification yield, as well as other risk factors such as melting temperature, hydrophobicity and isoelectric point (pI). A traffic light ranking system was developed where high, moderate and low risks were represented by red, yellow and green colors, respectively.

High throughput formulation assessment was also included during candidate profiling. A case study was provided in which 4 Fab candidates were evaluated for an application requiring formulation at high concentration. The Fab with the best developability profile was selected based upon consideration of multiple parameters, e.g., pI, hydrophilicity, protein self-interaction, solubility, viscosity, purification experience.

In addition to binding their cognate antigen, some antibodies show significant binding to other antigens. This so-called off-target binding poses multiple potential risks to antibody drug development, such as accelerated PK clearance, reduced efficacy and safety. Off-target binding was assessed by binding of the antibody candidates to chips with 384-arrayed proteins (Protagen).

Binding of IgG to the neonatal receptor, FcRn, is important for maintaining the long serum half-life of antibodies.[12] Binding of antibody candidates to FcRn from multiple species was evaluated by surface plasmon resonance. PK experiments in rats were included for in vivo fitness assessment of antibodies.

Antibodies are potentially immunogenic in patients.[13] Immunogenicity risk was assessed by proteomic identification of peptide sequences from antibody candidates that are processed and presented by MHC class II on antigen-presenting cells. In silico prediction was used to modify the antibody sequence to avoid MHC class II binding. Antibody variants were evaluated for biologic function, developability and proteomics to verify reduced presentation of antibody drug-derived peptides.

Dietmar Reusch (Hoffmann-La Roche) gave a presentation entitled, "State of the art analytical methods for the characterization of therapeutic antibodies." Fc glycosylation is important in the development of antibody drugs since it is almost always necessary for the antibody to support effector functions such as ADCC and complement-dependent cytotoxicity (CDC).[14,15] Moreover, Fc glycosylation can be tailored to enhance antibody ADCC and CDC activities.[14,15]

A major focus of this talk was on high throughput analysis of IgG Fc glycosylation.[16]

IgGs were captured from Chinese hamster ovary (CHO) cell culture harvest fluids using immobilized protein A and then digested with trypsin. Released glycopeptides were purified by hydrophilic interaction liquid chromatography and identified and quantified by MS.[16] The method developed is automated and can be used for selecting CHO clones.

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Liquid chromatography-MS (LC-MS) methods were also developed to quantify other PTMs including asparagine deamidation and aspartate isomerization in the antigen-binding loops of antibodies.[17] Antibodies were incubated at elevated temperatures (≤ 40°C) to accelerate degradation, proteolyzed and peptides identified by LC-MS. This analytical method was used to determine the influence of process conditions on asparagine deamidation and aspartate isomerization. Additionally, the extent of side chain modification was sufficient to evaluate the functional impact upon antigen binding by the antibody. Another LC-MS method was developed to quantify the extent of oxidation of critical methionine and tryptophan residues.[18] Accelerated oxidation was achieved using *tert*-butylhydroperoxide as an oxidizing reagent.

Patrick Haddad (LFB Biomanufacturing) gave a talk on the production of recombinant antibodies with enhanced ADCC activity. It was previously known that IgG_1 antibodies expressed in the rat hybridoma cell line YB2/0 typically have Fc glycosylation with lower fucose content than corresponding antibodies produced in CHO cells, leading to more efficient ADCC activity.[19] The YB2/0 cell line was adapted for growth in suspension in a chemically-defined media (YB2/0-E). The EMABling® platform includes the use of optimized expression vectors, the YB2/0-E cell line and a cell line development process to identify clones producing antibodies in gram per liter quantities.

Hervé Broly (Merck Serono) discussed the topic of Quality by Design (QbD) in the context of optimizing biosimilar and next generation antibodies. The concept of QbD for pharmaceutical development has been described in several guidelines from the *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use* (ICH) (www.ich.org). The goal of QbD is to build quality into the drug in a proactive way, rather than just testing for quality at the end of the process. One important facet of QbD is the identification of critical quality attributes (CQA) that may affect clinical efficacy or safety.[20,21] Identification of CQA includes careful consideration of clinical and nonclinical data with the drug, as well as data from related drugs, and the scientific literature. Quality attributes associated with antibody therapeutics and other protein drugs include product-related impurities and substances, process-related impurities, product attributes and contaminants. Ideally these quality attributes are individually tested for their influence on the drug's biologic activity, PK, PD, immunogenicity and safety.[20]

Protein aggregates are a product-related impurity that may be found at low levels in protein drugs. Aggregates can either attenuate or augment the activity of the drug. Moreover, aggregates can induce an immune response that in some cases significantly affects the efficacy or safety of the protein drug, e.g., induction of a neutralizing antibody response to the protein drug may limit efficacy. Removal of aggregates from protein drugs is desirable, with upper limits set for individual protein drugs. **Christof Finkler** (Hoffmann-La Roche) also presented on quality by design, focusing on the control strategy for products developed.

Achim Knappik (AbD Serotec) discussed the generation of anti-idiotypic antibodies that are highly specific for individual antibody drugs as tools for assay development. Applications of these anti-idiotypic antibodies include immune response assays for the detection of anti-drug antibodies in serum of patients. Anti-idiotypic antibodies are also commonly used for the quantification of human antibody drugs in serum for PK and PD studies. Special requirements for anti-idiotypic antibodies are sensitivity down to the ng/ml range and the need to detect the antibody drug in the presence of up to ~10^6-fold excess of very closely related molecules, namely human IgG.

Three different types of anti-idiotypic antibodies were identified. Type 1 anti-idiotypic antibodies are specific for the paratope of the antibody drug, i.e., the region involved in antigen binding. Type 1 anti-idiotypic antibodies inhibit binding of the antibody drug to its target antigen and can detect antibody drug only when it is free and not complexed to antigen. Type 2 anti-idiotypic antibodies are not paratope-specific, non-inhibitory and detect total antibody drug, i.e., free or bound to the target antigen. Type 3 anti-idiotype antibodies bind to the antibody drug-target antigen complex, are non-inhibitory and only detect antibody drug-target antigen complexes.

Anti-idiotypic antibodies were obtained in ~8 weeks by panning a large (45 billion members) human antibody phage display library, HuCAL PLATINUM.[22] Selection conditions were varied according to the type of anti-idiotype antibody sought. Affinity maturation of the anti-idiotypic antibodies if needed or desired was performed by trinucleotide cassette mutagenesis.

Anti-idiotype antibodies to several different marketed antibody products are commercially available, including ones recognizing adalimumab, alemtuzumab, bevacizumab, infliximab, rituximab, trastuzumab and ustekinumab. Assay data for anti-idiotypic antibodies from several different types of assays were presented, including epitope binding, detection of immune responses and analysis of PK samples.

János Szebeni (Semmelweis University) gave the last presentation of the session: "Adverse immune reactivities of monoclonal antibodies and testing for their prediction." Acute infusion (hypersensitivity) reactions are common side effects of intravenous administration of antibody and other protein therapeutics, as well as nanomedicines such as liposomal drugs and micellar systems.[23,24] These infusion reactions are usually mild and well-tolerated but on rare occasion can be severe or fatal.

Evidence presented suggests that these infusion reactions are mediated, at least in part, by complement-activation, rather than by IgE associated with allergy. This complement activation-induced pseudo-allergy (CARPA)[23] shares some common symptoms with IgE-mediated type I allergy. Symptoms unique to CARPA include that the adverse reaction arises at first exposure, the reaction is milder or absent on repeated doses with prolonged latency, premedication with anti-inflammatory drugs can be efficacious and acute pulmonary infiltration may occur.

Complement activation by nanomedicines can lead to anaphylatoxin release that may result in hypersensitivity reactions and enhancement of specific immune response. Additionally, complement activation can lead to opsonization of particles, resulting in rapid clearance by the reticulo-endothelial system and toxicity.

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Several different assays are being evaluated as potential predictive tests for CARPA. The long-term goal is to help in the prediction, prevention and treatment of drug-induced infusion reactions and thereby improve drug safety.

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November 27, 2012: Day 1, Track B
Antibody Drug Conjugates: Middle and Late-Stage ADC Progresses

Hans-Peter Gerber

Hans-Peter Gerber (Pfizer Oncology Research) pointed out that ADC research and development (R&D) is undergoing a substantial transformation based on novel insights into biological mechanism controlling safety and efficacy of ADCs. He started his presentation with a review of focus areas with the greatest potential to improve ADCs' efficacy and safety. He concluded his talk by reviewing the clinical and preclinical data generated with conjugates employing DNA damaging payloads like calicheamicin (inotuzumab ozogamicin) and tubulin inhibitor payloads such as auristatins (5T4-ADC).

Dr. Gerber emphasized the lack of clean ADC targets for solid tumor indications, i.e., most if not all of the solid tumor antigens are also present at various levels in normal tissues. One way to address these limitations is the identification of novel linker and payloads with limited activity in normal tissues. Dr. Gerber reviewed the results from high throughput screening efforts to identify novel cytotoxic compounds, in particular natural products. He also discussed the results from a phage display-based screen, which led to the identification of targets expressed on chemo-resistant, but not chemo-sensitive, parental tumor cells. Dr. Gerber pointed out that there are limitations in the numbers of linkers that are currently employed for ADCs and suggested the use of molecular and cell biology-based approaches to identify linkers that are selectively cleaved in tumors, but not in normal cells.

Safety improvements are critical for the advancement of the ADC platform because off-target toxicity is frequently limiting the dose escalation in humans. The majority of ADCs are cleared via hepatic and renal clearance, and toxicities in these organs are observed across different ADC platforms. Therefore, understanding the basic biological concepts leading to off-target toxicity will be the foundation for the development of novel technology to reduce the off-target toxicity of ADCs.[1]

Dr. Gerber finished his presentation by reviewing the clinical data generated with CMC-544 (inotuzumab ozogamicin),[2] a calicheamicin conjugate targeting CD22, in non-Hodgkin lymphoma (NHL). Calicheamicin has a fundamentally different mechanism of action compared with tubulin inhibitors because it introduces DNA double strand breaks in cells irrespective of their proliferation rates. This may be advantageous for tumors with low proliferation rates. CMC-544 is currently being tested in Phase 3 trials in NHL in combination with rituximab. Finally, Dr. Gerber reviewed the preclinical data generated with a novel, tubulin inhibitor-based ADC (5T4-ADC, A1-mcMMAF), targeting the most malignant cell population within tumors, the

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tumor initiating cells (TICs[3]). The preclinical efficacy and safety data established a promising therapeutic index that supports clinical development of A1-mcMMAF.

John Lambert (ImmunoGen) discussed the key limitations of IgG-based biotherapeutics, in particular their distribution rates to tumors and normal tissues, which result in a median tumor uptake of 0.01% of the injected dose per gram of tumor. Highly potent linker payloads that are stable in circulation and able to selectively release the payloads to tumors are required to overcome the limitations in biodistribution of IgG-based biotherapeutics. Dr. Lambert then reviewed the progress made with maytansine conjugates (DM1 and DM4 derivatives) in the clinic, with a focus on the biological differences between the various linkers currently employed (cleavable vs. non-cleavable). Ten maytansine conjugates being tested in the clinic. He emphasized the lessons learned when developing the most advanced ADC program in solid tumors (T-DM1[4]). Marketing applications for trastuzumab emtansine submitted in the US and Europe were based on strong Phase 3 data from breast cancer trials, where T-DM1 was combined with standard of care in HER2 positive, locally advanced or metastatic breast cancer patients.[5]

Dr. Lambert discussed the importance of ADC target selection, antibody optimization, linker design and the mechanism of action of the cytotoxic payloads employed. He emphasized on the critical contribution of both linker and payload to safety and efficacy. In addition, Dr. Lambert reminded the audience that the vast majority of ADCs (> 50%) is eliminated via catabolism in normal tissue, including liver and kidney. Improvement in the off-target toxicities of ADC in these organs is an area for future improvement for ADCs. Over 30 ADCs are currently being tested in the clinic, and the information gained will help the entire field of ADC research to design future ADC platforms.

George Badescu (PolyTherics) discussed the ThioBridge™ next generation conjugation technology. He presented a novel method to achieve site-specific conjugation of ADCs by introducing interchain disulfides between existing cysteines of light and heavy chain. Thiobridges are non-maleimide-based compounds used to conjugate a variety of mAbs to various payloads. The loading of mAb can be carefully controlled, and homogenous mixtures were obtained of conjugates with a drug-antibody ratio (DAR) of 1–2 or 3–4.

Bertrand Coiffier (Hospices Civils de Lyon, Université Lyon) presented a talk ADCs in hematology. He reviewed the compounds currently developed in liquid tumor indications and emphasized the difficulties of designing meaningful clinical trials with the appropriate control groups, given the large variety of combination regimens in this space, including various combination regimens of chemotherapies and rituximab. The total number of antibody conjugates currently developed for liquid tumors is greater than 12, including two approved radioimmunoconjugates (Bexxar®, Zevalin®), and CMC-544, which is in Phase 3 trials, with most of the remaining compounds in Phase 1 studies. Dr. Coiffier suggested that the critical element for the clinical success of these compounds will be the durability of the response because the conventional chemotherapeutic compounds are very efficacious already.

Charles Dumontet (INSERM) outlined the clinician perspectives on ADCs, with a focus on the question of which are the most promising disease indications for ADCs in oncology. He emphasized that the tumor types with the lowest survival rates among all cancer patients are pancreatic, lung, metastatic carcinomas and sarcoma. The indications where mAb therapeutics have been most successful in the past, however, are NHL, breast and colorectal cancer. Therefore, a combination of liquid and solid tumor indications may provide the optimal risk mitigation strategy for ADC development in the clinic. Dr. Dumontet then reviewed the underlying causes for withdrawal of Mylotarg® from the US market in 2010. Major factors that may have contributed to this decision include certain aspects of the target biology, with CD33 being expressed on hematopoietic progenitor cells, and the very high potency of the payload.

Dr. Dumontet then used the clinical data generated with T-DM1[6] to illustrate the status of clinical development of ADCs. Clinical benefit was observed in both efficacy and safety endpoints compared with relevant standard of care regimens in breast cancer. The off-target toxicities of tubulin inhibitor-based conjugates, however, should be additionally addressed to improve this therapeutic modality. For example, peripheral neuropathy and thrombocytopenia were dose- limiting for SGN-35,[7] and these side effects are believed to be non-antibody target-related. Peripheral neuropathy represents a well-described side effect of tubulin inhibitors, which are known to interfere with peripheral neuronal functions when administered systemically. Dr. Dumontet suggested that the ideal ADC should have non-overlapping toxicities when combined with standard of care to provide opportunities for combination treatment with front-line therapies. When developed as single agents, development strategies that include consolidation or maintenance treatments for ADCs should also be considered. Dr. Dumontet identified liver toxicity to be intrinsic to all ADC platforms, and improving liver toxicity should be a critical focus area of future ADC development. Similarly, the peripheral neuropathy observed with certain tubulin inhibitor conjugates will make it difficult to combine these ADCs with taxanes and platinum drugs, vincas or proteasome inhibitors. There should also be an increased focus on ADC resistance mechanisms, such as target downregulation, induction of alternative intracellular trafficking pathways and alterations in apoptotic pathways in response to ADC treatment. Dr. Dumontet suggested the development of novel classes of payloads with non-redundant mechanism of action to complement the tubulin inhibitor platform most widely used in the clinic.

Sarah Fredriksson (Genovis) discussed antibody peptides and fragments in drug discovery as key molecules in antibody characterization. LC-MS analysis of ADCs is becoming an increasingly important tool to characterize the changes in DARs of ADCs in patients. To quantify the proteolytic degradation of ADCs via LC-MS, fragmentation of large molecules into smaller pieces is required. Dr. Fredriksson provided an overview of two unique enzymes isolated from the pathogen *Streptococcus pyrogenes* that cleave mAb and ADC at selected sites. EndoS (termed IgG ZERO) specifically and rapidly cleaves N-linked glycans from antibodies, leaving one N-acetyl-D-glucosamine and one fucose.

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IdeS (termed FABRICATOR) is a unique cysteine protease that cleaves GG amino acid motif separating F(ab')2 and Fc antibody fragments. Dr. Fredriksson recommended the broader use of these enzymes to streamline MS and structural analysis of ADCs.

Thorsten Fritz (SAFC) presented the talk "Manufacturing of ADCs to enable preclinical and clinical testing." He pointed out that his organization generated 41 GMP batches and 71 GMP stability studies, underlining the increased interest of organizations engaged in oncology R&D in this therapeutic modality.

Christoph Uherek (Biotest) discussed preclinical and clinical data generated with a tubulin-based ADC targeting CD138 (nBT-062-SPDB-DM4, BT-062). CD138 represents a reliable target for multiple myeloma (MM) because it is expressed at lower levels on normal myeloid cells and is present on the surface of almost all MM cells regardless of whether the patients were previously treated. Among a series of different linker payload combinations tested, the SPDB-DM4 conjugate was the most potent. The bystander effect elicited by this drug-linker combination was an important factor to its selection as lead compound. A single injection of 25 mg/kg on day 11 post-tumor cells implantation resulted in cures or significant regressions until about day 45. Dr. Uherek then reviewed the clinical data from Phase 1 dose escalation and Phase 2a studies in MM patients. A maximum tolerated dose (MTD) of 160 mg/m^2 was identified for the every 3 weeks regimen, and 140 mg/m^2 for the weekly dose regimen. It will be important to understand the reason for the comparable MTDs between weekly and every 3 week dosing regimens. Based on Immunohistochemistry analysis of a variety of solid tumor indications, CD138 was identified in 40 to 70% of solid tumors; among them, triple negative breast cancer was identified as a potential area for clinical development for BT-062.

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November 27, 2012: Day 1, Track C
Bispecific and Alternatives: Exploring Bispecific Formats

Alexey A. Lugovskoy

The first session in the bispecific and alternative proteins track of the 2012 European Antibody Congress was dedicated to case studies of bispecific antibody formats. **David Szymkowski**, Senior Director of Biotherapeutics at Xencor, chaired the session. He opened the session by reviewing the history of bispecific antibodies, which goes back almost 50 y. Dr. Szymkowski expressed his confidence that the field has matured as evidenced by multiple bispecific formats advancing toward the clinic or progressing in clinical trials.

Roland Kontermann (University of Stuttgart) discussed recombinant bispecific and bifunctional antibody fusion proteins for tumor therapy. He suggested that, regardless of the architecture of a bispecific molecule, it invariably contains a targeting unit and an effector unit that are the critical moieties for optimization. Professor Kontermann's work focused on single-chain diabodies, which are recombinant bispecific antibodies devoid of constant regions. These molecules can be used for retargeting of T cells and delivery of co-stimulatory molecules for the immune system to the aberrant cells. He gave an example of a single-chain diabody that cross-linked fibroblast activation protein (FAP) with CD3 on the surface of T cells. This molecule triggered immune system-mediated lysis of FAP-expressing cells. Professor Kontermann also described fusion molecules that contained either 4–1BBL cytokine or a fragment of CD86 co-stimulatory protein. Co-administration of these fusion proteins increased the potency of single-chain diabody cross-linker of FAP and CD3. Not surprisingly, all of these molecules cleared quickly from the systemic circulation and required application of half-life extension strategies. Professor Kontermann's group tested multiple approaches, including pegylation, albumin fusion, and the introduction of an N-linked glycosylation site or albumin binding domain. They concluded that introduction of albumin binding domain was the best strategy to increase circulation half-life of single-chain diabodies. Next, Professor Kontermann presented his work on interleukin 15—fusion proteins targeted to FAP. These molecules activated CD4$^+$ and CD8$^+$ T cells and natural killer cells and significantly reduced the number of metastasis in a xenograft model of lung cancer. Professor Kontermann concluded his talk by describing engineering of epidermal growth factor receptor (EFGR) targeted TNF-related apoptosis-inducing ligand (TRAIL). These molecules were active in cancer xenograft models where they also increased the activity of proteasome inhibitor bortezomib.

Christian Klein (Roche Pharma Research and Early Development Roche Glycart AG), gave a detailed overview of the CrossMAb antibody platform. In the beginning of his talk Dr. Klein summarized his recent review on bispecific heterodimeric IgG-like antibodies[1] and highlighted the remaining challenges in the development of bispecific antibodies. He discussed engineering steps needed to overcome these challenges within the

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CrossMAb format and illustrated its properties by presenting a case study of an angiopoietin-2 and vascular endothelial growth factor (VEGF)-targeting molecule. This CrossMAb binds simultaneously to both targets, displays the PK profile of a typical IgG in rodents and monkeys and is more potent that a mixture of monospecific antibodies targeting angiopoietin-2 and VEGF in xenograft models. Dr. Klein's team confirmed the structural integrity of their clinical lead by X-ray crystallography, showed that Fc receptor binding and effector functions were unaffected, and generated a CHO manufacturing cell line with productivity of 4 g per liter. Evaluation of this molecule in a Phase 1 clinical study is currently on-going. Dr. Klein concluded by discussing additional opportunities within the CrossMAb platform that include monovalent molecules, dual Fc antibodies, and tetravalent antibodies. These formats are robust and have differentiated properties that could enable new clinical applications.

Tariq Ghayur (AbbVie) discussed the selection of dual-variable-domain–immunoglobulin (DVD-IgG) development candidates with good drug-like properties. He remarked that, for each new technology platform, time and diligent effort are required to understand its properties. His team has developed such an understanding of DVD-IgGs and advanced two molecules into clinical development. Along the way, they have built hundreds of molecules, including ones that co-target tumor necrosis factor (TNF) and prostaglandin E (PGE), CD20 and CD22, EGFR and receptor for macrophage stimulating protein (MSPR or RON), HER2 and insulin-like growth factor 1 receptor (IGF-1R), interleukins 12 and 18, and DVD-IgGs that targeted multiple epitopes on EGFR and HER2. Most commonly, Dr. Ghayur's team observed additive effects from DVD-IgG modules directed at soluble cytokines and synergistic effects from DVD-IgG modules directed at cell surface targets. Additional opportunities for this platform include monovalent and T cell recruiter DVD-IgGs. Next, Dr. Ghayur discussed the influence of linkers and module orientation on the properties of DVD-IgGs and concluded that optimal module orientation was more important to achieve desired activity. He concluded his talk by describing a streamlined triage scheme for assessment of DVD-IgGs manufacturability. For every project in AbbVie, 50–90 DVD-IgGs are built. Once these molecules are shown to be potent in functional assays and to have high target affinity, they are evaluated using two sets of "drug-likeness" filters. These data, combined with information on their expression in CHO cells and their PK profile in rodents and cynomolgus monkeys, are used to select the clinical lead and 3–4 back-up molecules.

Alexey Lugovskoy (Merrimack Pharmaceuticals) presented a case study on engineering of MM-141, a human tetravalent antibody for the treatment of cancer. MM-141 targets the phosphoinositide 3-kinase/v-AKT murine thymoma viral oncogene homolog 1/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway that is activated through IGF-1R and HER3 (also known as ErbB3) and their heterodimerization partners. The PI3K/AKT/mTOR pathway promotes tumor cell survival and is often activated in cancers in response to cytotoxic and targeted therapies. To date, antibody blockers of IGF-1R have proven to be clinically ineffective, and Merrimack's data suggests that this is because HER3 receptor and its ligand heregulin provide strong compensation for IGF-1R blockade. Therefore, only the dual IGF-1R/ErbB3 antibody co-inhibitor can completely block the IGF-driven activation of PI3K/AKT/mTOR. Merrimack's team used a network biology approach to come up with the blueprint of an "optimal" bispecific antibody. They used focused yeast antibody module display and rapid prototyping approach to construct therapeutic candidates from modules with desired affinities and stabilities. The selected clinical molecule, MM-141, binds to IGF-1R and ErbB3 with subnanomolar affinities, blocks IGF-and heregulin-induced PI3K/AKT/mTOR signaling, and induces strong downregulation of receptor complexes containing IGF-1R and ErbB3. MM-141 is stable in solution and manufacturable. While MM-141 showed monotherapy activity in xenograft models of Ewing sarcoma and pancreatic, prostate, and breast cancers, it is likely to be most effective in combination with chemotherapeutics and targeted therapeutics. In fact, everolimus, gemcitabine and docetaxel all activated PI3K/AKT/mTOR and MM-141 inhibited this acquired resistance mechanism in xenograft models. These results suggest that MM-141 has the potential to become an effective therapeutic for treatment of advanced solid tumors that depend on PI3K/AKT/mTOR.

The topic of targeted payload delivery with bispecific antibodies was discussed by **Michael Grote** (Roche). He described a flexible platform based on the antibody fusion of a single-chain variable domain that recognizes digoxigenin with high affinity. Digoxigenin can be linked to various payloads, e.g., cytotoxic compounds, small interfering RNAs, and then loaded on the bispecific antibody. Dr. Grote also discussed a multispecific antibody that co-targets HER3 and hepatocyte growth factor receptor (c-Met). This molecule can be modified to bind digoxigenin and then can be used to increase specificity of targeted payload delivery.

Jochen Kruip (Sanofi) reviewed results from Sanofi's bispecific antibodies in clinical trials. He focused on SAR156597, a bispecific molecule for the treatment of idiopathic pulmonary fibrosis (IPF) that targets interleukins 4 and 13. This molecule contains a tandem pair of variable domains fused to an Fc module giving it dual functionality. Dr. Kruip remarked that, similar to DVD-IgGs, placement of variable domains is influenced by strong positional effects. The SAR156597 drug product is lyophilized and can be formulated at 100 mg/ml for subcutaneous delivery. The investigational new drug (IND) application was filed in the first quarter of 2011; a Phase 1/2 clinical study (NCT01529853) in IPF is on-going. Dr. Kruip also presented a newer bispecific format, the crossover dual variable domain antibody, which has VH_1-VH_2-CH_1-Fc: VL_1-VL_2-CL topology, and again pointed to the importance of linker optimization to achieve the desired biological activity.

Gabriele Schaefer (Genentech) presented a case study of MEHD7945A, a "two in one" mAb targeting HER3 and EGFR. The molecule has 0.4 nM affinity to HER3 and "dialed-down" 19 nM affinity to EGFR. In clinical studies, MEHD7945A showed nonlinear PK that reached saturation at 10 mg/kg. A flat dose of 1100 mg every other week (Q2W) or 1650 mg every third

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week (Q3W) was selected for Phase 2 studies in colorectal cancer, squamous cell carcinoma of head and neck and lung cancer. Dr. Schaefer highlighted the importance of biomarkers for clinical development of biotherapeutics and stated that Genentech intends to focus on evaluating levels of phosphorylated HER3 and its ligand heregulin as predictors of patients' response. In Phase 2 study, a retrospective analysis of heregulin expression levels in archival biopsies will be conducted. In addition, patients that carry mutations in V-K_i-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) gene will be excluded from the colorectal cancer study.

The final talk of the session was delivered by **Aran Labrijn** (Genmab). Dr. Labrijn gave an update on the Duobody platform for bispecific antibody generation. It is well known that IgG4 antibodies undergo rapid arm exchange and Genmab scientists have explored this property to create F405L/K409R mutant of IgG1 as a scaffold for production of bispecific Duobodies. Dr. Labrijn illustrated this approach by describing three bispecific molecules targeting CD20, CD3 and HER2. These molecules had excellent manufacturability with production yields as high as 20 g per liter at 25 L scale. Dr. Labrijn remarked that production of Duobodies is easily conducted at standard bench and commercial manufacturing scale.

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November 28, 2012: Day 2, Track A
Monoclonal Antibodies

Thierry Wurch

The second day of the 'Monoclonal Antibody' track, chaired by **Steve Coats** (MedImmune/AstraZeneca), addressed three major topics: (1) the influence of collaborations on the R&D process; (2) three case studies of clinical success stories; and (3) the identification and validation of novel targets with a particular emphasis on antibodies targeting immune check-point modulators.

The topic of collaborations was covered by two speakers who presented both sides of an efficient and fruitful partnership between two companies, Nascent Biologics, represented by **Mark Glassy**, and Catalent Pharma Solutions, represented by **Greg Bleck**. Nascent Biologics' lead product, pritumumab, a natural human antibody, has been used to treat 250 brain cancer patients. After 5 y, patients treated with pritumumab have an overall survival rate of 25–30% compared with 3% for standard therapies. Pritumumab is a human IgG1 kappa antibody derived from a B cell isolated from a regional draining lymph node of a patient with cervical carcinoma.[1] It binds to a pan-carcinoma neo-epitope present in the coil 2 of the central rod domain of vimentin present in brain, lung, thyroid, pancreas, breast and colon cancers, making the pritumumab epitope highly tumor-specific and restricted to various cancers and not normal cells and tissues. In several clinical trials in Japan spanning over the past 20 y, 249 patients with brain cancer were treated with pritumumab.[1] The overall response rate was between 25–30%, with several survivors beyond 5 y post-treatment. The patients were on a low-dose regimen of 1 mg given twice a week for a course of 24 weeks, for a total dose of 48 mgs per course.[1] Pritumumab appears to be a safe and effective therapy in patients with malignant gliomas.[1] The original owner of pritumumab, the Higawara family, sold the entire IP and ownership to Nascent Biologics Inc. in 2009. A partnership was then established with Catalent Pharma Solutions for GMP grade manufacturing of the antibody and joint clinical development in brain cancer. The exact nature of the partnership was not disclosed; nevertheless, the established contract was without cash funding and Catalent took stock options on the future financial profit on the molecule. It is a win-win situation, as explained by Dr. Glassy.

Three case studies on the R&D process of successful mAbs were presented, two in the field of infectious diseases and one in oncology. **Robert Friesen** (Crucell) discussed the identification of neutralizing antibodies showing a broad spectrum of activity against several influenza A and B serotypes. It is currently a big challenge to treat influenza with a therapeutic antibody approach and even a bigger challenge to prevent the disease using a unique vaccine.[2] Dr. Friesen explained that influenza hemagglutinin (HA) is an antigenic glycoprotein found on the surface of the influenza viruses and it is the main antigenic determinant; neutralizing antibodies can affect HA binding to the cell receptor and subsequent fusion. In 2008, Crucell discovered CR6261, a mAb that is able to prevent and cure influenza by binding to a highly conserved epitope in the HA stem with heavy chain only.[3] Dr. Friesen highlighted that CR6261 is able to neutralize a broad range of group 1 influenza viruses, including H1N1, which is a common seasonal influenza strain, and the highly pathogenic H5N1 ('bird flu') virus.[3] Group 2 HAs such as H3, H7 and H10 strains, however, are not recognized by CR6261 because they have a glycan structure in the hydrophobic pocket that prevents recognition by the VH1–69 germline gene of CR6261. Crystal structures of CR6261 Fab were obtained in complex with HAs from the human 1918 H1N1 pandemic virus and from H5N1 virus; the epitope corresponds to a highly conserved helical region in the membrane-proximal stem of HA1/HA2. CR6261 neutralizes the virus by blocking conformational rearrangements associated with membrane fusion.[3] Another series of mAbs, especially CR8020 and CR8043, neutralized multiple group 2 influenza subtypes, including H3, H4, H7, H10, H14 and H15 and protected mice from lethal challenge with H3N2 and H7N7 viruses.[4,5] These mAbs seem to block the pH-induced conformational change of HA, thereby preventing the proteolytic cleavage necessary for virus replication.[5] Using competition binding experiments and computational modeling, a second overlapping conserved epitope has been identified in group 2 influenza viruses.[5] These mAbs prevent infection through two different mechanisms. They either block pH-induced conformational change or prevent proteolytic cleavage.[4] Dr. Friesen concluded by reiterating that group 2 mAbs have defined a second conserved epitope on HA that is critical to

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virus replication and that these H1/H3 mAbs are an important addition to the arsenal against influenza.

The second case study was in oncology. **Liliane Goetsch** (Centre d'Immunologie Pierre Fabre) presented the discovery and non-clinical evaluation of mouse 7C10, an antibody directed against human IGF-1R.[6] The antibody was generated by conventional mouse hybridoma technology and was selected for its potent and efficient inhibition of IGF-1R phosphorylation and inhibition of tumor growth.[6] A particular emphasis was given to the importance of blocking not only IGF-1R signaling, but also the function of the related hybrid receptor engaging an IGF-1R and an insulin receptor (IR) moiety.[6,7] This particular receptor type occurs in tumor cells overexpressing IGF-1R and IR isoform A.[7]

Dr. Goetsch showed that the 7C10 mAb and its humanized form h7C10 potently and efficiently inhibited binding of IGF-1 and insulin to these hybrid receptors and were able to downregulate them.[6,7] As many as 12 different anti-IGF-1R antibodies were discovered by various companies and reached the clinical development stage with a total of 124 clinical trials started or in progress.[8] Nevertheless, only limited signs of therapeutic benefit were obtained despite a positive expression of IGF-1R on the treated tumors. This demonstrates the crucial need for biomarkers to select a population of patients that might better benefit from anti-IGF-1R therapies.[8,9] Following in vitro preclinical studies and an in-depth evaluation of the complex network between IGF-1R, IR and HRs and their physiological regulation, a putative association with a growth hormone signature was suggested. Its current evaluation in in vivo models may reinforce its use as a clinical biomarker for better patient stratification.

The third example was another anti-infectious disease antibody directed against the pathogenic bacterium *Staphylococcus aureus*, currently developed by Kenta Biotech and presented by **Michael Rudolf**. This program deals with the treatment of nosocomial infections acquired in hospitals and nursing homes, which affect an estimated 10% of all hospitalized patients and are an important cause of morbidity and mortality. Patients with compromised immune functions are especially susceptible. It has been observed that patients in the intensive care units (ICUs) get hospital-acquired infections more frequently than patients who are on the standard wards of the hospital. A high proportion (28–50%) of mortalities linked to nosocomial infections in ICUs was attributed to *S. aureus*. One of its critical infection determinants is an extracellular, highly conserved virulence factor termed α-toxin or α-hemolysin, which attacks various human cell types causing cell death by inducing apoptosis/necrosis. KBSA301 is a human IgG1 mAb specifically targeting *S. aureus* α-toxin. Upon binding, KBSA301 represses functional toxin pore formation, leading to protection of susceptible cells from α-toxin dependent destruction. Hence, its mode of action is independent of the antibiotic resistance profile of *S. aureus*, and therefore covers infections caused by bacteria that are antibiotic-resistant and sensitive. When testing the therapeutic activity of KBSA301 in preclinical animal studies of localized and systemic infection, administration of the mAb resulted in reduced bacterial loads and significantly improved the survival rates of infected animals. Based on the in vivo efficacy results and favorable safety profile, a Phase 1/2 trial could be conducted directly in ICU patients with severe *S. aureus* pneumonia.

Werner Meier (Biogen Idec) presented an overview of the current antibody discovery engine set up at Biogen Idec by capitalizing on decades of knowledge, both internal and from the scientific community, accumulated around antibodies and antibody-associated technologies. As an introduction, the global antibody market over the last decade was summarized based on the recent analysis by Arrowsmith.[10] Next, the current selection criteria for antibody discovery were presented. The predominant novel mAb source is hybridoma technology at Biogen Idec (about 60%), then classical parameters on binding characteristics (affinity, selectivity, and species cross-reactivity), a set of CMC-related criteria such as aggregation, post-translational modifications, stability and finally a 'production platform fit assessment' is performed. All these parameters are evaluated on a high throughput basis not to slow down the program, but to maximize the chances of success of the potential lead candidates. Therefore, biological/pharmacological activity and drug-like properties are evaluated in parallel for selection of optimal preclinical candidates. An example was further developed regarding antibodies for central nervous system applications. Since crossing of the BBB is a crucial issue in the field, single domain VHH fragments were screened for their selective binding to brain endothelial cells and for their capacity to migrate through the BBB.[11] One candidate, FC5, demonstrated efficient BBB crossing after infection in mice.[11]

Nicolas Beltraminelli (Vivalis) presented the strengths of the VIVAScreen™ technology platform dedicated to the identification and isolation of rare, therapeutic mAbs directly from human B cells. The technology is founded on the principle that humans naturally develop humoral immune responses, particularly the expression of antibodies, not only against exogenous but also against self-antigens. Nowadays, even with all the advances made in antibody engineering, human-derived antibodies are still considered the best candidates for developing mAb therapeutics. Therefore, the VIVAScreen™ platform enables the isolation of human B lymphocytes from peripheral blood using a high throughput, single-cell screening system based on a micro-array chip technology called ISAAC (ImmunoSpot Array Assay on a Chip). Access to this ISAAC technology was made possible through the acquisition in 2011 by Vivalis of the Japanese biotechnology company Single Cell World Inc. The ISAAC technology allows rapid and precise mass screening of millions of B lymphocytes.[12] The VIVAScreen™ technology was made possible thanks to a privileged and quick access to thousands of samples of blood from healthy and diseased volunteers through an agreement with the French blood collection sites or 'Etablissements Francais du Sang'. Following the screening of pools of B lymphocytes for biological function, B lymphocytes are captured individually in single wells using a micro-array system, one at a time. Candidate B lymphocytes are identified using a known antigen of interest. Using a micro-pipetting technology, these individual cells can be isolated, individually, off the micro-array chip. After two weeks of culture, RNA of selected cells is retrieved by PCR and antibody genes cloned and produced as recombinant mAbs.

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The method also allows isolation of antigen-specific B cells present at low frequency (< 2×10^{-8}) in peripheral blood of human donors. Finally, Dr. Beltraminelli described the use of the EB66 cell line, derived from duck embryonic stem cells, to produce low-fucosylated mAbs with enhanced ADCC. These cells can proliferate in suspension in stirred tank bioreactors to reach high cell densities in serum-free media, with a yield of mAb production of about 1 g/L.

William Finlay (Pfizer) presented a novel antibody generation platform developed at Pfizer that is based on chicken immunization and downstream generation of immune chicken antibody libraries using phage display.[13,14] Major advantages of this approach are the large phylogenic distance allowing the selection of human/mouse/cyno cross-reactive antibodies and single V germline gene for heavy and for light chain allowing easy cloning of V-gene repertoire. They are moreover closely homologous to human germline V-genes, allowing easy humanization.[13,14] A rapid phage selection method based on time-resolved energy transfer was set up. Dr. Finlay presented a case study of the generation of highly selective antibodies directed against three phosphoepitopes of tau protein, with mouse/primate/human species cross-reactivity.[15] Each antibody showed full specificity for one single phosphopeptide and sub-nanomolar affinities. He presented data on crystallographic and 3D modeling of the structure of pT231/pS235 Fab in complex with its cognate phosphopeptide at 1.9 Å resolution, underlying binding mechanisms to explain such remarkable specificity.[15] The Fab fragment exhibits novel complementarity-determining region (CDR) structures with a "bowl-like" conformation in CDR-H2 that tightly and specifically interacts with the phospho-Thr-231 phosphate group, as well as a long, disulfide-constrained CDR-H3 that mediates peptide recognition. This binding mechanism differs distinctly from either peptide- or hapten-specific antibodies classically described.[15]

After the lunch break, **Jérôme Tiollier** (Innate Pharma) highlighted the strategy of his company to capitalize on their knowledge on innate immunity to develop novel antibody therapeutics targeting immune checkpoint modulators. One of these druggable proteins is the killer cell Ig-like receptor (KIR) protein family. Natural killer (NK) cell activity against tumor cells is regulated by a complex balance of inhibitory and activating signals, which are mediated by the binding of NK cell receptors to activating and inhibitory ligands expressed on tumor cells. KIR2DL1/2 and 3 are inhibitory receptors of the immune response of NK cells and all three are targeted by IPH-2101 (1–7F9) antibody.[16] It is a fully human IgG4 for the treatment of hematological malignancies, such as acute myeloid leukemia (AML) and MM.[16] In preclinical studies, IPH-2101 selectively bound to its cognate receptors and exposure of KIR-transfected target cell lines to IPH-2101 led to an augmented NK-cell-mediated lysis.[16] A Phase 1 study of IPH2101 was conducted in elderly patients with acute myeloid leukemia (AML) in first complete remission.[17] Patients were enrolled and received escalating doses (0.0003–3 mg/kg) of IPH2101 following a 3 + 3 design. Adverse events were mild and transient, consisting mainly of infusion syndrome and erythema.[17] The MTD was not reached, although full KIR saturation (> 90%) was sustained for more than 2 weeks at 1 and 3 mg/kg. There was a clear correlation between mAb exposure and KIR occupancy. Neither hematologic toxicity nor significant changes in the numbers and distribution of lymphocyte subsets, NK cell receptor expression, or in vitro cytotoxicity were seen.[17] At the highest dose levels (0.3, 1, and 3 mg/kg), transient increases in TNF and MIP-1β serum concentrations and NK cell CD69 expression were observed.[17] A second Phase 1 trial was conducted with IPH2101 in patients with relapsed/refractory MM.18 IPH2101 was administered intravenously every 28 d in 7 dose-escalated cohorts (0.0003–3 mg/kg) for up to 4 cycles. A total of 32 patients were enrolled. The biologic endpoint of full KIR2D occupancy across the dosing cycle was achieved without dose-limiting toxicity or reaching the MTD.[18] IPH2101 enhanced ex vivo patient-derived NK cell cytotoxicity against MM. No objective responses were seen. No evidence of autoimmunity was observed.[18]

The penultimate talk of the session was about the pipeline of immune-regulatory antibodies discovered and developed by the biotechnology company 4-Antibodies and presented by its chief executive officer, **Robert Burns**. After providing a brief overview of the company, Dr. Burns described the company's core technology platform, 'retrocyte display'. In brief, multiple human antibody libraries are constructed based on various blood sources (cord blood, healthy donors and patients) as well as mutated and semi-synthetic (CDR-3) antibody libraries. All sequences are cloned into a retroviral expression system, by splitting the genetic information for heavy and light chains onto two different virus particles, allowing easy chain scrambling to increase library diversity. Then, an immortalized mouse pre-B cell line deficient in endogenous B cell receptor (BCR) expression -1624–5 cells- are transduced in a two-step sequential process. The human antibody libraries are expressed as membrane-bound full-length IgGs in these cells. Expression into engineered B cells allows optimal folding and expression of the antibodies as a natural Ig-BCR complex. Cell surface expression also allows library screening by flow cytometry using a fluorescently labeled antigen (purified protein or living cell). The selected B cell clones expressing the best binders are converted into a CHO-expressing platform called Retro-CHO using the same retroviral system to reach productivities up to 50 μg/ml scale; up to 10,000 clones can be produced at this scale. The Retrocyte display technology was exemplified with targets belonging to three major families of interest in oncology: (1) immune checkpoint regulators, (2) tumor metabolism and (3) tumor stroma. Examples essentially taken from the scientific literature were described, such as PD-1, LAG-3, TIM3 and CA9.

The last presentation of this session was delivered by **Thierry Wurch** (Servier Research Institute) who discussed new ways of developing biologics by engaging the immune system. The topic was illustrated by three case studies, two of them involving recent partnerships between the French pharmaceutical company 'Les Laboratoires Servier' and the US biotechnology company MacroGenics. The first example was MGA27, an antibody targeting the immune checkpoint regulator B7-H3 currently being evaluated in a clinical Phase 1 study. This antibody was selected from MacroGenics' platform because of its high selectivity for tumor cells compared with healthy tissues.[19] Optimization of

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the Fc portion was performed by mutagenesis to increase binding affinity to the human FcγRIIIa receptor, and particularly its low affinity allele Phe158 and to decrease binding to the inhibitory receptor FcγRIIb.[20] Therefore, this antibody is likely to exert enhanced ADCC activity on NK cells, as clearly demonstrated both on in vitro cell cytotoxicity models and in vivo on tumor xenograft models.[19] Complete inhibition of tumor growth was obtained with A498 (renal), AGS (gastric) and HT-1197 cell lines at doses as low as 1 mg/kg.[19] A second antibody targeting B7-H3 was also identified which is suitable for immuno-histochemistry evaluation and potential use as a companion diagnostic reagent in the clinic.[19]

The second case study presented a novel bispecific, antibody-based protein scaffold called DART, for 'Dual Affinity Re-Targeting'. It corresponds to a Fab-like association via a disulfide-bridge of two distinct antibody variable domains; nevertheless the heavy chain VH of one Fv is associated with the light chain VL of the second Fv specificity to create the two functional binding domains.[21,22] The design and pharmacological characterization of an anti-CD19×CD3 DART was presented, and compared with the prototypical blinatumomab bispecific T cell engager (BiTE) molecule from Micromet/Amgen. Although comparable binding affinities for each target (CD19 and CD3) was maintained between the DART and BiTE structures, superior T cell activation and cytotoxic efficacy and potency was obtained with the DART[23] compared with the equivalent BiTE. This enhanced efficacy was explained by a more optimal structure of the disulfide-bridge of the DART compared with the linker of the BiTE, allowing better cell-cell contact between T cell and target cell.[23] Several DART molecules are currently at the preclinical development stage, and first-in-human studies are expected in a near future. The final part highlighted the novel concept of putative implication of antibody treatment in triggering an adaptive immune response as shown for rituximab and trastuzumab.[24,25] In these examples, adaptive immune responses initiated by antibody treatment could protect treated animals from subsequent tumor challenge even in the absence of novel treatment.[24,25] Altogether, these different examples strongly suggest that activation of both innate and adaptive immune responses via different pathways may be the ultimate path to eradicate residual cancer disease and in refractory, multi-relapsed disease situations.

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November 28, 2012: Day 2, Track B
Antibody Drug Conjugates

Jagath R. Junutula

The second day of the ADC track, chaired by Alain Beck (Centre d'Immunologie Pierre Fabre), addressed R&D on next generation ADCs. The first speaker, **Patrick van Berkel** (ADC Therapeutics Sarl), discussed a rational approach to developing a portfolio of ADCs armed with a novel potent class of pyrrolobenzodiazepines (PBD) warhead. Dr. van Berkel highlighted that there are over 50 ADCs in development and the majority of them are associated with one class of cytotoxic drug, tubulin inhibitors. Thus, there is an urgent need for ADCs with a PBD-type linker drug that complements existing cytotoxic drugs in the ADC portfolio. PBDs are originally natural products isolated from Streptomyces species. PBDs form a covalent aminal linkage with N2 of guanine in the minor groove of DNA and their preferred target DNA sequence is in the following order: Pu-G-Pu > Pu-G-Py or Py-G-Pu > Py–G-Py. The rationale presented for using the PBD class of cytotoxic drugs for ADC development was that their in vitro potency (1–20 picomolar) is superior to existing cytotoxic drugs (auristatins, calicheamicins and maytansines, with potencies of 50–200 picomolar) that are in the clinical development. Dimerization of PBD is shown to increase the cellular potency. He summarized the mechanism of action of PBD dimers as they cross-link DNA in a sequence-selective fashion, thereby blocking DNA replication. Cells treated with PBD dimers undergo cell cycle arrest and cells enter apoptosis at G2/M interface. PBD dimers do not distort the DNA helix, thereby avoiding DNA repair.

Dr. van Berkel summarized by stating that many forms of PBD dimers with robust scalable synthetic routes, varying potency and lipophilicity are being developed. These PBD warheads are shown to display 0.3–16 picomolar IC_{50} values on A2780 ovarian cancer cell line. Unlike tubulin inhibitors, the potency of PBDs is not compromised in multi-drug resistant cell lines. He concluded in his presentation that the PBD class of drugs can complement existing tubulin inhibitor-based ADCs by targeting tubulin-binding refractory, resistant diseases, slowly proliferating cancers, and low copy number targets and they also provide a unique opportunity to perform treatment at lower doses.

Paul Parren (Genmab) gave an overview to building a novel portfolio of ADCs by exemplifying CD74 and tissue factor (TF) ADCs as case studies. Dr. Parren highlighted in his presentation that ideal auristatin-based ADCs should have the following critical properties: binding to cell-surface expressed target antigen; ADC internalization and trafficking to the lysosome driven by target antigen-antibody binding; and enzymatic degradation of ADC that releases auristatin. Both CD74- and TF-targeting ADCs seem to display these critical properties and are considered excellent ADC targets by Genmab. TF is expressed in many solid tumors including lung, pancreas, and bladder cancers. HuMax-TF-MC-vc-PAB-MMAE ADC was selected as a lead molecule for development based on a payload linker license obtained from Seattle Genetics. Dr. Parren discussed features of the HuMax-TF antibody ADC: it is a human antibody that interferes with TF-mediated signaling but does not interfere with coagulation; it is also a diagnostic reagent that binds well for a wide range of solid tumor biopsies; and it displays potent in vivo tumor regressions in a wide-range of xenograft models. He also noted that HuMax-TF-MC-vc-PAB-MMAE ADC displayed acceptable safety findings in preclinical models and is poised for IND filing in 2013.

Dario Neri (ETH Zurich) discussed comparisons of various payloads of armed antibodies. He started his presentation by refreshing the audience on random tissue distribution of chemotherapeutic drug due to more efficient penetration into normal tissues compared with tumor tissues using data on doxorubicin imaging as an example.[1] In contrast, antibodies directed to tumor antigens showed selective localization to tumors. He then described strategies for ligand-based targeting of tumor neo-vasculature. He showcased two examples, oncofetal fibronectin and oncofetal tenascin, as excellent classes of markers for angiogenesis. Oncofetal fibronectins are overexpressed in many different cancers, including breast, lung, and pancreatic cancers, as well as lymphoma. Its expression in normal tissues was observed only in placenta and in the endometrium during proliferative phase.[2]

L19 and F8 antibodies specific to the EDB and EDA domains of oncofetal fibronectin, respectively, and an F16 antibody specific to oncofetal tenascin were used to describe various arming modalities (cytotoxic drugs, cytokines, radionuclides) in his presentation. Professor Neri also used Phase 1 results to discuss radioimmunotherapy with radretumab in patients with relapsed hematologic malignancies. The Phase 1 results showed that selective tumor uptake was found in 14 of 18 patients; a favorable benefit and risk profile in advanced relapsed lymphoma patients; and an induced complete response in two heavily pretreated, relapsed Hodgkin lymphoma patients and in one diffuse large B cell lymphoma patient.[3] He went on to describe selective tumor targeting of L19-scFv-TNF fusion proteins and described exploratory trial results that evaluated safety and clinical activity of L19-TNF plus melphalan-containing isolated limb perfusion (ILP) in extremity melanoma patients.[4] He presented the data on F8-scFv-IL10 fusion protein in targeting inflammatory diseases arthritis, endometriosis and atherosclerotic plaques. Professor Neri ended his presentation by summarizing the applications and therapeutic utility of antibodies against vascular targets by arming with various payloads.

Daryl Drummond (Merrimack Pharmaceuticals) presented a talk on antibody-targeted nanotherapeutics for solid tumors. The use of anthacyclins in combination with anti-HER2 targeted therapies is generally restricted because this class of chemotherapy drugs has become associated with cardiotoxicities. However, this combination was possible with HER2-targeted liposomal doxorubicin because it restricts the uptake of doxorubicin into normal tissues and promotes selective delivery into HER2-positive tumor cells through target antigen mediated endocytosis. Dr. Drummond highlighted engineering, functional characterization of immunoliposomes and their applications in treating cancer. He pointed out that targeted delivery of chemotherapeutic drugs through immunoliposomes is advantageous, as each liposome has

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the ability to carry up to 10^4–10^5 drugs. In contrast, ADCs or immunotoxins could only deliver 1–8 drugs or 1 toxin per target molecule, respectively. Recently, Merrimack Pharmaceuticals presented Phase 1 data at the 2012 CTRC-AACR San Antonio Breast Cancer Symposium, held Dec. 4–8, 2012 in San Antonio, TX (Abstract #: P5-18-09) on MM-302, a HER2-targeted liposomal doxorubicin.

Timothy Lowinger (Mersana Therapeutics) discussed a Fleximer technology to create next generation ADCs that can enable utilization of diverse payloads, higher drug loading and alternative targeting agents. Fleximer is a biodegradable, clinically-validated polymer molecule that can be exploited for covalent attachment of multiple, diverse payloads. Upon breakdown, Fleximer results in the safe metabolic products, glycerol and glycoloate. Solubility of several small molecule payloads was improved upon conjugation to Fleximer, e.g., camptothecin by 5000-fold, paclitaxel by > 1000-fold and a PI3-kinase inhibitor by > 500-fold. In addition, Fleximer also improves the PK and biodistribution properties of diverse payloads upon conjugation. The circulating half-life of IFNα is improved by > 70-fold and a small molecule fumagillol derivative by > 280-fold.

Fleximers appropriate for various antibody formats (scFv, diabody, minibody and IgG), different payloads, and lysine-based, cysteine-based, or site-specific conjugation methods were developed and can be used to build a diverse ADC portfolio. Dr. Lowinger presented the data on a trastuzumab-Fleximer-vinca ADC, which had 16 vinca drugs per antibody. Vinca alkaloids are mitotic inhibitors that cause cell death by inhibiting microtubule formation. The trastuzumab-Fleximer-vinca ADC and other similar auristatin-based Fleximer ADCs showed excellent in vivo efficacy in multiple HER2-positive breast cancer xenograft models at 2–10 mg/kg doses. A trastuzumab-Fab Fleximer ADC also showed complete regressions at three repeat dosing of 7 mg/kg. Finally, Dr. Lowinger reminded the audience that Mersana's Fleximer technology can aid in building novel next generation ADCs with payload diversity, increased payload per antibody and target/linker diversity.

Jagath Reddy Junutula (Genentech) delivered a talk on engineered THIOMABs for designing next generation ADCs. Dr. Junutula started his presentation by giving an overview to ADCs and he described that an ADC is a three component molecule and all three components (antibody, linker, cytotoxic drug) are equally important in building a successful ADC therapeutic for a given tumor specific antigen. He reviewed three conjugation methods used in current ADC development for preclinical and clinical studies: (1) linker-drug conjugation using lysine side chain amine groups; (2) conjugation with cysteine sulfhydryl groups activated upon reduction of inter-chain disulfide bonds; and (3) site-specific conjugation through engineered cysteine residues. The former two conjugation methods produce heterogeneous products containing a mixture of different molar ratios of drug (0–8 drugs) to antibody linked at different conjugation sites.[5,6] In contrast, ADCs derived from engineered cysteines (THIOMAB platform) result in homogenous preparation with defined stoichiometry (2 drugs per antibody).[7] He showcased several examples of in vivo efficacy and safety studies on conventional ADCs and their corresponding engineered ADCs in his presentation. Despite having a lower drug load, engineered ADCs with 2 drugs per antibody showed similar in vivo efficacy compared with conventional ADCs with an average of 3.5 drugs per antibody. This was reasoned to be due to improved PK properties of engineered ADCs compared with conventional ADCs.7 Engineered ADCs in the context of a non-cleavable maytansine (MCC-DM1) or a cleavable auristatin-based linker drug (MC-vc-PAB-MMAE) displayed reduced liver and bone marrow toxicity compared with conventional ADCs.[7,8]

Dr. Junutula highlighted the role of the conjugation site in the in vivo stability and therapeutic activity of ADCs. He summarized the data on engineered ADCs using three trastuzumab THIOMAB variants that differed in solvent accessibility and local charge. These three variants had similar in vitro properties (binding to target antigen and in vitro potency), but differed in their in vivo efficacy and PK properties. A detailed biochemical mechanism on the stability of cysteine-maleimide-based antibody conjugates in plasma in vitro and in vivo was dissected and discussed. The maleimide exchange from an ADC to reactive thiols in plasma constituents such as albumin, cysteine or glutathione resulted in a decrease in ADC stability and therapeutic activity. In contrast, hydrolysis of a succinamide ring in the ADC enhanced the stability and therapeutic activity. ADCs with different conjugation sites seem to vary in the maleimide exchange and succinamide ring hydrolysis characteristics depending on their solvent accessibility and local charge properties.[9]

In the final part of his talk, Dr. Junutula reviewed the in vitro potency, in vivo efficacy, and safety data of 4-drug load engineered ADCs and 4-drug load conventional ADCs. Both these conjugates were studied by using the same antibody and linker drug (MC-vc-PAB-MMAE) and they only differed with respect to method of conjugation as described above. ADCs derived from both methods showed similar in vitro potency, while engineered ADCs displayed a two-fold improvement in in vivo efficacy over conventional ADCs. In conclusion, Dr. Junutula highlighted that engineering site-specific ADCs will improve ADC manufacturing, define DAR, and improve safety. He also concluded that our ability to analyze and understand the in vivo metabolism of ADCs in preclinical and clinical studies will help us immensely in developing next generation ADC therapeutics.

Giulio Casi (Philochem) discussed the use of non-internalizing antibodies and traceless linkers for ADC development. Oncofetal fibronectin and oncofetal tenascin represent well-characterized and validated targets of angiogenesis for vascular targeting. Antibodies against these proteins selectively accumulate around tumor neovasculature structures.[10,11] Dr. Casi used L19, an anti-oncofetal fibronectin antibody, to address utilization of non-internalizing antibodies in developing ADCs for vascular targeting. Early findings come from SIP-L19-PS, a scFv-CH4 dimer (~80 kDa) of antibody-photosensitizer conjugate. Following intravenous administration of SIP-L19-PS, it localizes to the sub-endothelial extracellular matrix around the tumor and blood vessels. Reactive oxygen species are released upon irradiation, which diffuse into the immediate surroundings to cause damage to tumor vasculature and cause tumor cell death.[12]

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Dr. Casi highlighted that the same principle could be applied for ADCs where a photosensitizer can be replaced with a potent cytotoxic drug that can freely diffuse into tumor cells upon release from the target antibody. To demonstrate this point, cemadotin, a dolastatin analog with a thiol reactive group, was conjugated to thiol groups of cysteines derived from a disulfide bond of a SIP-F8 antibody (anti-oncofetal fibronectin SIP format antibody, specific to EDA domain). The resulting SIP-F8-cemadotin ADC was analyzed to have 2 drugs per antibody and shown to display target-dependent tumor efficacy at very high doses of ADC (> 40 mg/kg).[13] Dr. Casi also highlighted similar traceless linker technology, where a cemadotin-CHO reactive group was coupled to antibodies or antibody fragments (SIP, diabody) that contain an N-terminal cysteine residue. The introduction of a cysteine residue at the N-terminus of the heavy chain of an antibody provides a 1,2 –aminothiol moiety, which is suitable for traceless coupling to aldehyde groups. In summary, Dr. Casi described applications of traceless linkers and utilization of non-internalizing antibodies for vascular targeting of ADCs.

Chris Lloyd (MedImmune) presented engineering strategies to generate site-specific ADCs. He highlighted advances to ADC technical development with respect to rational design and selection of target antibody, linker and cytotoxic drugs that lead to successful transition of over 20 ADCs into various stages of clinical development. Dr. Lloyd summarized limitations to generating conventional ADCs that are a mixture of heterogeneous ADCs with 0–8 drug load species.[6,7] Due to the increased hydrophobicity of high drug load species, these ADCs are shown to undergo fast clearance and decreased stability,[14] which contribute to a decreased in vivo efficacy and lower tolerability. Engineered site-specific ADCs can overcome these challenges, as was discussed in the previous THIOMAB platform presentation by Dr. Junutula, and are advantageous.

Dr. Lloyd described production and characterization of engineered cysteines (S131C, S132C, S134C, T135C, S136C, T139C) in the CH1 domain of an antibody and also the combination of double/triple mutant variants. Conjugation with maleimide-PEG2-biotin resulted in up to 50–60% conjugation efficiency. It was found that incomplete conjugation was due to formation of mixed disulfide bonds with existing cysteines in the inter-chain disulfide bonds. Dr. Lloyd showed results with new THIOMAB variants, which lack inter-chain disulfide bonds, yielding over 90% conjugation with maleimide-PEG2-biotin. He also described additional THIOMAB variants with cysteine engineering in the Fc domain that did not seem to have an issue of mis-paired disulfide bonds. Also presented was data with single (T289C), double (T289C-A339C) and triple (T289C-A339C-S442C) mutants, where resulting ADCs had DARs of 1.8, 3.7 and 5.7 drugs/antibody, respectively. Dr. Lloyd described analytical and functional characterization of these site-specific ADCs and their plans to proceed for further testing in preclinical efficacy and safety models with their lead ADC therapeutics.

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November 28, 2012: Day 2, Track C
Bispecific and Alternatives: Morning session

Roland Kontermann

Robert Mabry (Adimab) described the company platform technology for rapid identification of bispecific antibodies utilizing the IgG-scFv format, especially regarding stability influenced by linker length, orientation of variable domains and the formation of disulfide bonds.[1] Examples were shown of how error-prone PCR in combination with a yeast presentation and expression system can be used to generate bispecific antibodies with improved properties.

Horst Lindhofer (TRION Pharma) gave an overview of the Triomab® technology, trifunctional full-length bispecific antibodies generated from hybrid hybridomas. Besides removab

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(catumaxomab), an anti-EpCAM × anti-CD3 Triomab® approved for the treatment of malignant ascites, data of various other Triomabs®, e.g., directed against HER2 (rexomun), CD20 (FBTA05), and GD2 (extomun) were presented. Data from clinical trials of catumaxomab established a prolonged mean overall survival. Results with surrogate Triomab® antibodies for use in mouse tumor models further revealed the importance of a secondary T cell-driven immune response, demonstrating that Triomab® antibodies can result in a therapeutic cancer vaccination.[2]

Roman Kischel (Amgen) provided data from clinical trials of blinatumomab (AMG103) a bispecific BiTE directed against CD19 and CD3 developed for the treatment of B cell malignancies. New results from a Phase 2 study of blinatumomab in B-lineage ALL patients with persistent or relapsed minimal residual disease were presented, showing the induction of a long-lasting complete remission.[3] These results further established the importance of effector memory T cells stimulated by the bispecific antibodies.

Bent Jakobsen (Immunocore) presented data on T cell receptor-based bispecifics (ImmTACs) targeting HLA peptides. These molecules are based on soluble, disulfide-stabilized T cell receptors further engineered for high-affinity binding of HLA-displayed peptides, fused to an anti-CD3 scFv for T cell recognition.[4] Data from in vitro and preclinical studies were shown demonstrating T cell retargeting and killing of tumor cells. First results from a Phase 1/2 clinical trial of IMCgp100, recognizing a $gp100_{280–288}$ peptide with picomolar affinity, were presented.

Carrie Enever (GlaxoSmithKline) gave a talk on liver-specific biopharmaceuticals for the treatment of chronic hepatitis C infections. Data for a molecule composed of interferon-α fused to a liver-specific domain antibody (dAb) recognizing the asialoglycoprotein receptor were presented, including imaging studies with radiolabeled dAbs and fusion proteins, as well as data on efficacy and an improved safety profile in HBV transgenic mice.

Christine Rothe (Pieris) summarized data for PRS-190, a bispecific antagonistic duocalin targeting the cytokines IL-17 and IL-23, which are proinflammatory and involved in autoimmunity and hyperinflammation. The duocalin was generated by genetically fusing two anticalins.[5] Data on the functionality of the individual anticalins and the duocalin were presented.

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November 28, 2012: Day 2, Track C
Bispecific and Alternatives: Afternoon session

Robert Mabry

The afternoon session of the second day, chaired by **Tariq Ghayur** (AbbVie), comprised three presentations on therapeutic leads in different bispecific formats.

David Szymkowski (Xencor) unveiled recent Fc-engineering advances in his talk entitled "Novel formats for full-length bispecifics." The presented strategy for generated bispecific antibodies focused on the isolation of Fc heterodimers during purification. This approach deviates from traditional efforts employing Fc mutations to increase heterodimer percentages. Dr. Szymkowski reported the integration of amino acid substitutions derived from other IgG isotypes into one heavy chain within the human Fc region. These substitutions result in altered binding to protein A and permit the isolation of the heterodimeric from homodimeric Fc during protein A elution. The proposed strategy may mitigate risk for immunogenicity compared with non-IgG residue substitutions. Using this approach, Xencor generated a CD19 × CD32b bispecific antibody (Xmab5871) in which each of the antibody arms are single-chain variable fragments (scFvs) fused to one chain of the human Fc region. The anti-CD19 arm of the molecule is fused to the modified heavy chain for the purification of the heterodimer. Xencor has also combined this Fc-based purification strategy with the common light chain approach in which two variable heavy regions (V_{Hs}) with different specificities have the ability to pair with the same variable light chain (V_L). This obviates the need for conversion to single-chain fragments which can typically introduce stability issues that influence antibody development.

Dr. Szymkowski also presented on the recent progress of a CD19 × CD3 bispecific scFv-Fc fusion (XENP1138). Xencor conducted a head-to-head comparative study of the bispecific Fc fusion with the same antibody variable regions in the BiTE format (Micromet/Amgen, tandem scFv). Interestingly, the variable regions in the BiTE format were approximately 2 orders of magnitude more potent in two in vitro assays compared with the Fc-fusion format. A significant advantage of XENP1138 is the cynomolgus cross-reactivity of the anti-CD3 arm, which provides more suitable in vivo options for preclinical modeling. As expected, the Fc-fusion exhibited greater serum persistence than the BiTE format in cynomolgus monkeys, which translated to more potent reduction in $CD20^+$ B cells with single intravenous bolus dosing. The work shared by Dr. Szymkowski highlights the next generation of CD3 engaging molecules and potential improvements in administration, dosing, and efficacy attributed to FcRn-mediated serum persistence.

Ezio Bonvini (MacroGenics) presented on the continuing work surrounding the Dual-Affinity Retargeting Platform (DART). In this talk, he highlighted the modularity of the DART molecules and varying formats to accommodate valencies for tailoring therapeutic strategies against different targets. The anti-CD3 antibody arm of the DART molecules also cross-reacts with cynomolgus CD3 and has been paired with multiple specificities for targeting both liquid tumors (CD19) and solid tumors (B7H3). In addition,

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Dr. Bonvini presented on the culmination of targets derived from the cancer stem cell technology as part of the Raven acquisition in 2008. MacroGenics has generated innovative cancer stem cell lines to yield more than 70 targets representing validated and novel targets for antibody discovery. Antibodies raised against these targets have been paired with CD3 targeting to generate bispecifics for therapeutic investigation.

Dr. Bonvini also presented a head-to-head comparison of the DART format with the BiTE format. In this study, the DART diabody format with a *C*-terminal disulfide exhibited higher potency than the tandem scFv BiTE format with the same variable regions. These results suggest superior conjugation of T and B cells for the DART format, yet the underlying feature responsible for the enhanced potency, given the similarity between the two formats, is not known.

MacroGenics has also ventured into autoimmune indications with the DART technology as shown with CD32B × CD79B bispecific during Dr. Bonvini's presentation. The molecule blocked B cell activation by binding in cis to double-positive cells. Signal inhibition is believed to be accomplished by co-ligation of the two targets which attenuates downstream signaling of Syk.

Nicolas Fouque (NovImmune) presented on a manufacturing platform for the κλ-body bispecific format. The κλ-body consists of an IgG with two light chain types (Kappa and Lambda). Each light chain codes for the specificity against each target antigen, whereas the heavy variable region is passive and serves as a stabilizing scaffold for each light chain. This bispecific format negates the necessity of extraneous linkers, which alleviates added risk related to immunogenicity. Dr. Fouque presented two approaches for antibody discovery to assemble a bispecific: de novo generation of both antibody arms using fixed V_H phage libraries and a sequential approach which takes the V_H from an existing IgG to generate libraries for selection of V_Ls with additional specificity.

The majority of Dr. Fouque's presentation focused on the manufacturing platform generated for this class of bispecific antibodies. NovImmune's strategy for CHO expression of the κλ-body consists of a tri-cistronic vector comprising the heavy chain, κ light chain, and λ light chain on one plasmid. HIC-HPLC was employed to quantify percentages of the κλ-bispecific compared with the κκ and λλ species. Dr. Fouque outlined the typical manufacturing strategy for mAbs as a conventional, three-phase process consisting of capture, polish, and fill/finish steps. For the κλ-body, three capture chromatography columns are employed. Post protein A, a KappaSelect column and LambdaFabSelect column are integrated into the purification process to exclude the λλ and κκ species and maximize quality of yield for the κλ-body. For a scale-up to a 100 L pilot scale, this strategy yielded 77% of κλ-body recovery with greater than 99% purity.

November 28, 2012: Day 2, Track C
Bispecific and Alternatives: Closing Plenary Session

Robert Mabry

The closing plenary session of the meeting was chaired by **Steve Coats** (Medimmune) and consisted of two presentations followed by a discussion with panelists **Paul Parren** (Genmab), **Tariq Ghayur** (AbbVie) and **Werner Meier** (Biogen Idec).

Elena Wolff-Holz (Paul-Ehrlich-Institut, Federal Agency for Vaccines and Biomedicines) provided an excellent broad overview of her thinking on biologics and biosimilars in her talk titled "Current regulatory thinking around biosimilars—A regulator's perspective." She emphasized that the views presented are her own and do not necessarily reflect the views of the Paul–Ehrlich-Institut. Dr. Wolff-Holz started her talk by highlighting the benefits of biologics, such as offering real hope for many unmet needs, particularly complex diseases, target specificity that is not possible with other medicines, their contributions to improved survival rates and improved quality of life. She pointed out that by 2016, 8 of the top pharmaceuticals worldwide will be biologics. She emphasized that the future of biosimilars and innovator biologics should be considered within the context of increasing costs of healthcare. The projected cost increases may not be due to aging populations, but rather due to medical treatments. She gave an overview of the generics (small molecules) and some approved "biosimilars" and explained the current definitions of "generic/biosimilar" in EU to set the stage to discuss the "biosimilar" guidelines for antibodies that are far more complex than some of the approved "biosimilars" such as, erythropoietin and insulin. She highlighted the fact that for biologics "the process is the product" as each company has its own unique manufacturing cell line (or cell type) and manufacturing platform/process.

Within this context, Dr. Wolff-Holz described the structural and functional complexity of the antibody molecule, e.g., the structural/functional components of an antibody (Fab, Fc, heavy chains, light chains, hinge region), multiple interaction sites (target binding affinity/potency, FcR, C1q, FcRn binding). She highlighted the concept of "bridging" studies even for innovator molecules when there is a process change and what such studies entail. She emphasized that bridging studies are basically comparability studies and have three aspects/components: (1) quality assessments, including evaluating impurities, batch consistency, contaminants, aggregates, micro heterogeneity and fragments; (2) non-clinical studies, including tissue cross-reactivity, target binding, potency, toxicity, immunotoxicity; and (3) clinical, including efficacy data, safety data and immunogenicity. Such studies may be necessary when there is a new manufacturing site, upscale production, new up- (fermentation, harvest) or down-stream process changes or new filling process. The product comparability is determined on a case-by-case basis based on scientific principles. Within this context, Dr. Wolff-Holz discussed issues related to process changes for innovator drug and biosimilars. She pointed out that the manufacturer of an innovator biologic has historical data and experience to which a biosimilar manufacturer will not have access, and therefore elucidation of

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the structural/functional properties by "reverse engineering" may be required.

With this backdrop, Dr. Wolff-Holz addressed the issue of biosimilar development by asking the question "how much similarity/comparability do we need? She answered this question by talking about four aspects: (1) product quality assessments, including formulation and differential glycosylation in addition to above mentioned criteria; (2) structural/functional analytic characterization and various techniques employed; (3) preclinical characterization, including specificity and potency evaluation; and (4) clinical comparability. She emphasized that the development of a biosimilar product requires a complete product and process development to match the validated process of the innovator and to ensure that the biosimilar matches its reference product in terms of quality, safety and efficacy. Further, the biosimilar physico-chemical and biological comparability studies establish similarity (to the innovator product) and the preclinical, Phase 1 and Phase 3 comparability data confirms similarity. The critical point she communicated was that the aim of the biosimilar development is not to establish benefit as this has already been demonstrated for the reference product, but the goal is to establish biosimilarity. She also emphasized that the critical principle for development is to test the biosimilar in the most sensitive clinical setting where differences between the biosimilar and reference can be detected easily.

Dr. Wolff-Holz then touched on the question of extrapolating clinical data to other indications approved for the reference product. She mentioned that "extrapolating" to other indications, not formally tested, is a sound principle based on overall scientific evidence and provided anti-TNFs as an example. She discussed the results of 5 approved anti-TNF products (3 full-length mAbs—infliximab, adalimumab, golimumab; PEGylated Fab—certolizumab pegol; TNF receptor-Fc fusion—etanercept) where etanercept did not show efficacy in Crohn disease, but is approved for other indications. In her closing remarks, Dr. Wolff-Holz talked about the draft guideline on biosimilar medicines that have been put together and feedback received on this from various stakeholders. She emphasized this as an ongoing process, that the biosimilar approval process requires a thorough review by highly-qualified experts who are responsible for approval of safe and efficacious medicines. She also briefly touched upon the ongoing work on biosimilar policy within and between various regulatory agencies.

John McCafferty (University of Cambridge) presented an excellent talk titled "Can we cure cancer with antibodies?" He started out by reviewing the analysis of data from the European Cancer Registry–based studies EUROCARE-3 and EUROCARE-4. The three aspects highlighted were: (1) the 5 y survival rates (male and female) for various cancer types; (2) the differences in various countries of Europe in these survival rates; and (3) the potential reasons for these regional and cancer-type specific differences.[1,2] He highlighted the fact that only a subset of patients respond to current therapies. With this backdrop, Professor McCafferty addressed two questions: Can antibodies increase 5-y relative survival rates (up to and beyond 5 y) and how to improve/design treatment of patients who do not respond? To address these questions, he highlighted the genetic complexity of cancer (e.g., breast cancer) by reviewing recent publications where, using high throughput sequencing methods, the genomic and transcriptomic basis of breast cancer was examined by analyzing somatic copy number changes and changes in mutations (driver and non-driver) in the coding exons of protein-coding genes. Professor McCafferty pointed out that these studies reveal considerable heterogeneity with regards to inherited and acquired somatic mutations within a tumor.[3,4] Professor McCafferty further reviewed the data from Gerlinger et al.[5] showing intratumoral heterogeneity as evaluated by profiling multiple spatially separated samples from primary renal carcinoma and associated metastasis sites. In these studies, the genomic heterogeneity was assessed by exome sequencing, chromosome aberration analysis and ploidy profiling. Professor McCafferty highlighted the point that ~60–70% of all somatic mutations were not detected across every tumor region; therefore, this intratumor genomic and protein (expression/function) heterogeneity may provide various escape mechanisms and thereby treatment failures. He also reviewed publications describing the molecular evolution of cancer.[6,7] To further highlight the complexity of cancer treatment with antibodies, Professor McCafferty discussed the recent publications showing the mechanisms of "acquired" resistance to anti–EGFR antibodies.[8-10]

Professor McCafferty then talked about additional levels of complexity in cancer treatment and resistance to treatment. He highlighted the fact that, in addition to intrinsic complexity (genomic), the tumor microenvironment also influences "acquired" resistance. He highlighted studies showing the influence of environmental factors (e.g., growth factors), in particular recent studies showing a role for HGF–cMet axis in inducing resistance to certain therapeutic modalities (e.g., certain kinase inhibitors) as assessed by co–culture (tumor and stromal cell lines) screening assays.[11,12] Finally, Professor McCafferty described efforts in his lab to make antibodies to block the HGF–cMet pathway using the phage display approach and the formation of a new company, Iontas Ltd., to develop antibody–derived therapeutics based on these recent advances in cancer biology. In concluding his talk, Professor McCafferty outlined several critical challenges: (1) improving 5-y survival rates and therapeutic options for non-responders; (2) diversity of molecular mechanisms, intratumor heterogeneity and the various escape routes available to cancer cells, which have mutant genomes. He suggested that, to achieve progress, we consider multiple points of attack and different approaches, such as antibodies as targeting agents (e.g., ADC), immune and bystander mechanisms, combination therapies (antibody + small molecule), targeting the root causes if possible, and developing methods to detect tumors early.

A range of topics were covered during the panel discussion. Holding true to the title, "Do alternative scaffolds or bi/multispecific antibodies hold the greater potential?" A short conversation was held on the competition of scaffolds with antibodies for multi-targeting approaches. The panelists briefly reviewed the historical rationale for the alternative scaffold "rush" that gained rather forceful momentum almost a decade ago. Whether those advantages will hold true for scaffolds and ultimately "replace"

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antibodies is rather unlikely, but areas that reveal an absolute advantage have yet to be determined. The topic of immunogenicity risk frequently accompanies alternative scaffolds, but it was pointed out by an audience member and agreed by one panelist that antibody regions taken outside of the native IgG format no longer constitute a native antibody (e.g., scFvs, other fragment-based approaches). In addition, many scaffolds are based on native human proteins with diversity in solvent-exposed regions and may share the same risk associated with human antibodies. The panelists also agreed that there may be a slight advantage for scaffolds when targeting multiple epitopes on a single target or perhaps combining multiple specificities beyond dual-targeting. In the area of diagnostics, there also may be an advantage for scaffolds based on stability and shelf-life.

Questions were raised with regards to current challenges in developing effective antibody-based therapeutics and the affect of biosimilars on biologics cost. Both the panelists and the audience were of the opinion that with regards to developing effective biologics to benefit patients, we now understand well the technical aspects of making antibody therapeutics; however, the challenges now are in understanding target biology within disease context and translational issues (animal models to human disease). These challenges will be greater for bispecifics because the key to success in this case will be in identifying the most efficacious target pairs (combinations). With regards to the affect of biosimilars, the emerging opinion was that biosimilar (antibodies/fusion proteins) will arrive (and some are already being developed or being marketed in emerging markets); however, as Dr. Elena Wolff-Holz pointed out in her talk, the concerns about similarity/comparability need to addressed for use in developed markets. The impact biosimilars will have on the costs of innovator drugs remains to be seen.

Earlier presentations had reviewed several bispecific antibody formats for CD3-engaging therapeutics, and a question was raised on the difference in potencies observed among different bispecific antibody formats. As presented by Tariq Ghayur earlier in the day, antibody variable regions manipulated from the native IgG format can alter the interaction between the antibody and target and can significantly change mechanism of action. The panelists briefly touched on the differences in distance between antibody moieties as a potential source of disparity among antibody formats. It was postulated that the proximity of target-binding moieties may play a role in the recruitment of T cells to the adjacent target. While affinity to CD3 has also been considered a factor for potency, CD3-engagement potency is not solely affinity-driven and may require a more extensive in vitro-based screening approach to generate best-in-class anti-CD3 antibodies and bispecific antibodies.

Continuing the discussion of CD3 targeting, the panelists were questioned on the potential of T cell therapy using bispecifics to activate cells prior to administration. This approach has been employed in multiple clinical studies (up to Phase 2) and the results have been encouraging. The panelists agreed that the clinical efficacy of this approach has been impressive, yet they were concerned with the regulatory pathway, which may be a daunting. The variables associated with cell culture and standardization of this approach are extremely complex and will require thorough discussions with drug agencies to establish a pathway for approval.

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Note

Summaries were prepared from PDFs of the presentations provided by speakers after the meeting. In the cases when a speaker was not able to share their presentation, detailed summaries are not included, although the speaker's name, affiliation and topic appear in the report.

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