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World Antibody-Drug Conjugate Summit, October 15–16, 2013, San Francisco, CA

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Abbreviations: ADC, antibody-drug conjugate; CRPC, castration-resistant prostate cancer; CMC, chemistry, manufacturing and control; CTC, circulating tumor cell; CD, cluster of differentiation; CMO, contract manufacturing organization; DOE, design of experiment; DAR, drug-to-antibody ratio; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; HER, human epidermal growth factor receptor; IHC, immuno-histo-chemistry; MBC, metastatic breast cancer, M&S, modeling and simulation; mAb, monoclonal antibody; ORR, objective response rate; PD, pharmacodynamics; PK, pharmacokinetics; PFS, progression-free survival; PSA, prostate-specific antigen; PBD, pyrrolobenzodiazepine; QRT-PCR, quantitative reverse transcriptase polymerase chain reaction; TFF, tangential flow filtration; TD, toxicodynamic

The World Antibody-Drug Conjugate (WADC) Summits organized by Hanson Wade are currently the largest meetings fully dedicated to ADCs. The first global ADC Symmit was organized in Boston in October 2010. Since 2011, two WADC are held every year in Frankfurt and San Francisco, respectively. The 2013 WADC San Francisco event was structured around plenary sessions with keynote speakers from AbbVie, Agensys, ImmunoGen, Immunomedics, Genentech, Pfizer and Seattle Genetics. Parallel tracks were also organized addressing ADC discovery, development and optimization of chemistry, manufacturing and control (CMC) issues. Discovery and process scientists, regulatory experts (US Food and Drug Administration), academics and clinicians were present, including representatives from biotechnology firms (Concortis, CytomX Therapeutics, Glykos, Evonik, Igenica, Innate Pharma, Mersana Therapeutics, Polytherics, Quanta Biodesign, Redwood Bioscience, Sutro Biopharma, SynAffix), pharmaceutical companies (Amgen, Genmab, Johnson and Johnson, MedImmune, Novartis, Progenics, Takeda) and contract research or manufacturing organizations (Baxter, Bayer, BSP Pharmaceuticals, Fujifilm/Diosynth, Lonza, Pierre Fabre Contract Manufacturing, Piramal, SAFC, SafeBridge).

Introduction

Among all antibody-related products,[1] antibody-drug conjugates (ADCs)[2] have become increasing important as oncology therapeutics. The first global ADC Summit was organized in Boston in October 2010. In 2011, the second global World ADC meeting, held in Frankfurt,[3] was followed by a similar meeting in San Francisco. These annual events are now the largest ADC-dedicated meetings,[4] with the 2013 San Francisco version attracting ~500 attendees.

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The "ADC discovery" stream showcased the multitude of approaches to construct homogenous ADCs with properties to enable conjugation to a diverse range of payloads with new mechanisms of action. Attendees gained insights into the latest advances in developing targeted cancer therapies and their applications to guide diagnosis, therapy selection, and treatment monitoring. This stream also provided an opportunity to learn more about the unique properties of natural products that make them advantageous as payloads to fuel the ADC development pipeline.

The "development approaches" stream helped attendees gain a better understanding of the efficacy and safety profile of their ADCs by the design of preclinical ADME and biodistribution studies to enable improved translation into the clinic. The emphasis was on understanding how diagnostic tests are being co-developed with ADCs to inform patient selection strategies and identify those who would be better served by pursuing other treatment options. Case studies of pharmacokinetics (PK)/ pharmacodynamics (PD)/ toxicodynamic (TD) modeling at different stages of ADC discovery and development to maximize clinical success were also presented.

In the "optimizing CMC" stream, achieving seamless ADC development, scale-up and manufacturing by avoiding common pitfalls and determining critical scale-dependent process parameters were discussed. Key parameters such as minimizing the effect of high drug load species on ADC physical instability to ensure enhanced stability were discussed. On the practical side, case studies were used to illustrate how to establish a smooth ADC supply chain and ensure each manufacturing step is communicated effectively as requirements for comprehensive manufacturing and CMC management continue to evolve.

Note: Summaries were prepared from PDFs of the presentations provided by speakers after the meeting. In the cases when speakers were not able to share their presentation, detailed

summaries are not included, although the speaker's name, affiliation and topic appear in the report.

October 15, 2013: Plenary Morning Session

In the introductory keynote, **John Lambert** (ImmunoGen) discussed ImmunoGen's approach, with a focus on ADCs in the clinic, including those targeting folate receptor 1 (IMGN853), CD56 (IMGN 901), CD37 (IMGN529), and epidermal growth factor receptor (EGFR; IMGN289). He also discussed ADCs developed with partners, such as the anti-mesothelin antibody BAY94–9343 that is partnered with Bayer.

Melanie Smitt (Genentech) then presented the success story of the recently approved ADC Kadcyla™ (ado-trastuzumab emtansine) developed by Genentech/Roche. This product was approved by the US Food and Drug Administration (FDA) in February 2013 for HER2+ metastatic breast cancer after failure of trastuzumab plus chemotherapy regimens. Ado-trastuzumab emtansine (T-DM1) consists of the validated anti-human epidermal growth factor receptor (HER)2 antibody conjugated with DM1 via the stable linker MCC. Dr Smitt described the data from the clinical development of T-DM1, beginning by the first-in-human Phase 1 study of T-DM1 in HER2+ metastatic breast cancer (MBC) patients after progression on at least one trastuzumab and chemotherapy regimen. The maximal tolerated dose was 3.6 mg/kg every three weeks. She then summarized the results of two Phase 2 studies with T-DM1 as single agent (3.6 mg/kg every three weeks): the proof of concept TDM4258 study (first patient in (FPI) in July 2007) and the TDM4374 study (FPI in August 2008) in 108 and 110 HER2+ MBC patients, respectively, previously treated with trastuzumab and chemotherapy regimens. In the TDM4374 study, as primary endpoint, the objective response rate (ORR) based on independent review facility (IRF) was of 34.5% and the median progression-free survival (PFS), evaluated as one of the secondary endpoints was 6.9 mo. Besides its promising anti-tumor activity, T-DM1 was well tolerated in these two Phase 2 clinical trials.

The first Phase 3 study, EMILIA (FPI in February 2009) began before the data from the TDM4374 Phase 2 study were available (November 2009). The EMILIA study was designed to compare T-DM1 as single agent (3.6 mg/kg every three weeks) in one arm against the combination of capecitabine (Cap) and lapatinib (Lap) in HER2-positive MBC or locally-advanced breast cancer (LABC) patients previously treated by trastuzumab and taxane and who progressed on metastatic treatment or within 6 mo of adjuvant treatment. To accelerate approval, the EMILIA protocol was amended in October 2010 to include overall survival (OS) as co-primary endpoint with PFS by IRF and safety, and to increase the sample size (N) from 580 to 980 patients. The PFS in the T-DM1 arm was 9.6 vs. 6.4 mo for patients receiving Cap+ Lap. The OS after confirmatory analysis was of 30.9 and 25.1 mo for TDM-1 and Cap+ Lap, respectively. The ORR was 43.6% and 30.8% and the duration of response (DOR) was 12.6 and 6.5 mo for TDM-1 and Cap+ Lap, respectively. The nature of the grade $\geqslant$ 3 adverse events (AE) was different between the two arms. The top four grade $\geqslant$ 3 AE were diarrhea (20.7%), hand-foot syndrome (16.4%), vomiting (4.5%) and neutropenia (4.3%) for the Cap+ Lap arm, and thrombocytopenia (12.9%), increased aspartate aminotransferase (AST) (4.3%), increased alanine aminotransferase (ALT) (2.9%) and anemia (2.7%) for the T-DM1 arm. The marketing application submission was completed in August 2012 and Kadcyla™ was approved by FDA in February 2013.

A second Phase 3 study (TH3RESA) was designed to compare T-DM1 as single agent (3.6 mg/kg every three weeks) in one arm (n = 400) against the treatment of physician's choice (TPC) (n = 200) in HER2-positive advanced BC. Co-primary endpoints were PFS by investigator and OS. The PFS in the T-DM1 arm was 6.2 vs. 3.3 mo in the TPC arm. The difference of ORR evaluated as one of the secondary endpoints was of 22.7% between the T-DM1 and TPC arms. The grade $\geqslant$ 3 AEs were of 43.5% and 32.3% for TPC and T-DM1 arms, respectively.

Dr Smitt finished her talk by summarizing a series of other clinical trials including a Phase 2 study in HER2-positive, recurrent locally-advanced breast cancer or MBC patients, where T-DM1 (n = 70) was compared with trastuzumab + docetaxel (n = 67) with primary endpoints PFS and safety. The PFS were 14.2 and 9.2 mo for T-DM1 and trastuzumab + docetaxel, respectively. Any grade > 3 AE were of 46.4% and 89.7% for T-DM-1 and trastuzumab + docetaxel, respectively. A new Phase 3 study, MARIANNE, (n = 1092) was designed to compare T-DM1 + pertuzumab in one arm vs. T-DM1 or vs. trastuzumab + docetaxel or trastuzumab + paclitaxel in HER2-positive progressive or recurrent locally-advanced or untreated MBC patients with PFS by IRF and AEs as primary endpoints. Dr Smitt noted that more than 3000 patients have received T-DM1 in the 18 clinical trials conducted so far.

October 15, 2013: ADC Discovery Stream

Trevor Hallam (Sutro Biopharma) described Sutro's site-specific technology that utilizes unnatural amino acid incorporation into antibodies by cell-free expression, and its application to ADCs. They overcame the difficulties in ribosomal truncation due to the presence of release factor (RF1) by engineering a RF1 variant that is inactivated upon cell lysis. With such a system, the RF1 is available during cell growth when its presence is needed for cell viability, but it is inactivated by OmpT protease upon cell lysis and formation of the cell-free extract. Using this system, incorporation of azido functionality was screened at about 250 positions in an antibody, and ~25 preferred sites were identified. These sites were conjugated with improved copper-free click chemistry. The resulting conjugates showed variable in vitro and in vivo potency depending on the site of conjugation.

Dr Hallam also discussed the use of the cell-free technology to conjugate both IgG1 and IgG2, as well as scFv-Fc fusions, and showed comparable efficacy of the scFv-Fc to the IgG1 version of the ADC. Sutro has also developed a way to incorporate orthogonal chemistry into the expression system. By separating the expression of light and heavy chains, two orthogonal chemistries can be incorporated using the same amber codon.

Using this strategy, conjugation of two different toxins to the same antibody can be achieved at specific sites.

Pavel Strop (Rinat-Pfizer) gave a talk on the effect of the site of conjugation on the stability, PK, and toxicity of ADCs. In the first part of the presentation, Dr Strop described the use of bacterial transglutaminase to make site-specific ADCs. A glutamine tag was introduced at 90 different sites in IgG, and 12 sites that had good biophysical properties and high conjugation yields were selected. These sites were located throughout the IgG, and therefore could be used to study how conjugation site affects the properties of ADCs. Dr Strop also showed that the process is scalable, cleavable and non-cleavable linkers are compatible with TG conjugation, and that diverse cytotoxic compounds can be conjugated.

Two selected sites (one at the C-terminus of heavy chain, the second in the light chain) were conjugated with AcLys-VC-MMAD and compared with a mc-VC-MMAD conventional conjugate. The efficacy of the site-specific conjugates against the M1S1 target [drug-to-antibody ratio (DAR) 2)] was comparable to the conventional conjugate (DAR4) in the BxPC3 mouse xenograft model; however, the site-specific conjugates were better tolerated.[5] In the second part of the talk, Dr Strop showed data comparing the two site-specific conjugates in terms of stability and PK. Data was presented showing that the site of conjugation can have an effect on the drug-linker stability. While no changes were observed in the TG linkage during the in vitro and in vivo stability studies, the ValCit cleavage element appeared to be cleaved in circulation to some extent in the heavy chain conjugate. These findings appeared to be species-dependent, and the differences between the two sites were more pronounced in rats.

The total antibody distribution was also changed based on the site of conjugation. The light chain conjugate showed comparable PK to wild type antibody, while the heavy chain conjugate had faster distribution phase. These findings were also species-dependent and were more pronounced in rats. Dr Strop concluded that the site of conjugation, linker-payload, and the combination of the two all can modify properties of ADCs.

David Jackson (Igenica) discussed site-specific technology that bridges native cysteines in antibodies. The technology utilizes reduction followed by incubation with the bridging moiety. Dr Jackson described the first generation, which was based on dibromomaleimides and carried fluorescein, followed by dithiophenolmaleimide-like compounds for better reactivity. The second generation was tested with PEG11 spacer and both cleavable and non-cleavable version of auristatins. These conjugates were shown to be well-behaved based on binding characteristics, and had somewhat reduced aggregation properties relative to the conventional conjugates. The improvement in aggregation was potentially due to the PEG11 linker. The Herceptin®-based bridged conjugates with comparable loading showed similar efficacy to conventional Herceptin® conjugates in the BT474 model.

Data was also shown for a tubulysin analog T4, which was also conjugated via the bridging technology to an IgG2a antibody and DAR 5 loading. Ramos in vivo data was presented for an undisclosed target for a conventional mcMMAF, bridge-MMAF, and bridge-tubulysinT4. In this efficacy study, the mcMMAF and bridge-MMAF showed comparable efficacy, but the bridge-tubulysinT4 appeared more potent. Dr Jackson also discussed screening and identification of additional bridging linkers that would remove the maleimide component, but no chemical structures were disclosed at this time.

Paul Davis (Quanta Biodesign) described their discrete PEG products (dPEG) and highlighted their capability to manufacture defined PEG moieties from 2 to 300 PEG units in length. Dr Davis described both linear and several branched versions of dPEG, and highlighted their use in modulating PK of proteins and imaging. Incorporation of lysines and tyrosines into the polymers for attachments, as well as bis-maleimide functionality for disulfide bridging, was also discussed.

Florence Lhospice (Innate Pharma) discussed the use of bacterial transglutaminase to create site-specific conjugates to endogenous glutamine Q295. To achieve this, they adopted findings by the Schibli group (ETH Zurich) indicating that, in deglycosylated antibodies, Q295 can be conjugated specifically by transglutaminase.[6] Dr Lhospice discussed their N297S mutant that removes the glycosylation that is typically present in the CH2 domain, and makes Q295 available for conjugation. The conjugation to this residue proceeds well with a single step reaction. N297Q mutant was also discussed. In this construct, conjugation to Q295 and Q297 can be achieved, thus giving DAR4 in IgG1. To achieve more complete conjugation, the DAR4 is typically made in a two-step conjugation, where transglutaminase is used to conjugate azide-containing linker to the Q295/Q297 and in second step is reacted with DBCO containing VC-MMAE.

Dr Lhospice then showed aggregation and in vitro plasma stability results, as well as rat PK results, showing well-behaved ADCs that were stable in vitro and in vivo. Efficacy data was also shown with an anti-CD30 antibody and compared with Adcetris® (brentuximab vedotin). In vitro, the transglutaminase conjugates with DAR4 showed comparable efficacy to Adcetris®, while the DAR2 was somewhat lower (Karpas299). The conjugates were efficacious also in vivo in a Karpas299 mouse xenograft model; however, due to the small starting tumor size and frequent dosing selected for the study, all conjugates showed potent killing and no separation of conjugates was observed. Additional studies with more compounds and different doses/dosing frequency are in progress.

Peter van de Sande (SynAffix) talked about site-specific conjugation that involves glycan remodeling. In this process, SynAffix utilizes undisclosed enzyme(s) to first trim the current glycan in IgGs, and then, in a following step, a second enzyme to incorporate sugar carrying azido, thiol, or chloride handle. The described strained cyclooctyne trastuzumab (BCN-VC-MMAF) conjugate was homogeneous after the trimming step and had picomolar IC_{50}s in-vitro potency in the SKBR3 cell line. Stability in plasma was also shown for 6 d, with very little to no change in drug loading during the experiment. Biodistribution of the glycoengineered conjugate showed the same distribution as wild type trastuzumab. Another conjugate carrying maytansine was

also discussed and comparison to T-DM1 in-vivo in one model showed somewhat better potency.

Dr van de Sande also discussed engineering of glycosylation at other sites, and trimming/incorporating of new sugars into sites at different places in IgG molecule. He also mentioned that it might be possible to incorporate two different sugar functionalities with orthogonal chemistries at two different sites. In summary, Dr van de Sande described a glycoengineering process for making site-specific conjugates that is applicable to IgG1–4, is high throughput amendable, and results in site-specific homogeneous product.

October 15, 2013: Development Approaches

Jay Harper (MedImmune) presented a talk emphasizing the importance of target selection for successful ADC development, and then **Henry Lowman** (CytomX Therapeutics) highlighted the proprietary Probody™ technology platform of the company.

Neil Bender (Weill Cornell Medical College) discussed the importance of imaging as a valuable and critical adjunct to ADC development. He first highlighting how antibody targeted cytotoxicity is simple in concept, but complex in execution, as demonstrated by the decades of failure. He also mentioned how recent clinical successes of ADCs (e.g., brentuximab vedotin, ado-trastuzumab emtansine) have invigorated the field. However, he also explained how several ADCs have shown very narrow therapeutic index in Phase 1, and only two ADCs not based on monomethyl auristatin are actively recruiting patients in Phase 2 studies. He also raised some concerns about the objective response rates for ado-trastuzumab emtansine being ~33%, stating that "closer inspection of ADC data in solid tumors raises some concerns." In his opinion, available data suggest that current ADC technology is precariously balanced and stretched to the breaking point, and there is a substantial room for further optimization of ADCs. He further elaborated on his point by noting that we can glean from the success of ADCs so far that the opportunity for improvements lies within better evaluation of the 3Ts, i.e., target, tumor, and territory.

Dr Bender then mentioned that, although immuno-histochemistry (IHC) is currently a critical analytical tool in target evaluation and patient selection, it provides relatively "bland" and uni-dimensional information. He also detailed few downsides of IHC, such as IHC: 1) can often be archival, 2) requires an invasive procedure, examines only single lesion, 3) is liable to sampling artifact/error, 3) assumes the examined sample represents other sites, and 4) provides at best a semi-quantitative impression of target expression levels. Subsequently, he stated that imaging can provide far richer information compared with IHC, including functional performance. He also detailed a few advantages of imaging, e.g., real-time measurements, non-invasive procedures, ability to assess all the lesions, and multi-factorial functional read out. He further elaborated by stating that, using imaging, one can obtain whole body biodistribution (including tumor) of ADCs, thereby directly confirming if the ADC is "hitting the target." Imaging can also help in obtaining semi-quantitative measurement for the amount of ADC accumulated in tumor, assessing the expression level of ADC target, evaluation of target accessibility by ADC, and measurement of ADC internalization.

Dr Bender further discussed a preclinical case study from Ogasawara et al.[7] as an example for the use of imaging in ADC development. The study was performed on STEAP1 and TenB2 targeting ADCs, which were conjugated to vc-MMAE using site-specific conjugation method, to achieve an average DAR of 2. The authors evaluated the potential of using Zr-89 immunoPET to quantify the delivery ADCs in primary human prostate tumors, where desferrioxamine was used for imaging with Zr-89. ImmunoPET signals were also compared with ADC efficacy, and antigen expression profiles measured using different methods, i.e., IHC, FACS, western, and PCR. The results showed that, for a given cell line, the rank-order of tumor uptake measured by PET imaging was well correlated with the efficacy, where the antibody with the highest uptake showed the best efficacy. However, the absolute antibody uptake required to achieve efficacy varied from one cell line to the other. Although no metric of expression predicted efficacy perfectly, it was observed that the imaging technique provided equivalent results to other conventional techniques for expression measurements. As such, the authors concluded that ImmunoPET imaging adds value in the measurement of factors directly relevant to ADC potency, e.g., receptor expression, tissue penetration, antibody internalization, and cytotoxin accumulation in the tumor.

Lastly, Dr Bender presented his work on the use of imaging in prostate cancer patients who were treated with prostate-specific antigen (PSA)-targeting radiolabeled antibody ^{177}Lu-J591.[8] His results compared imaging scores (0–3+) with the PSA response in patients. It was observed that patients with scores of 0–1 demonstrated 25% response rate, and, of patients with score of 2–3, 58% had major PSA decline, i.e., $\geqslant$ 30%.He concluded by noting that application of current ADC technology to solid tumors requires careful target and patient selection. And, imaging provides an unparalleled, functional tool for preclinical and clinical development of ADCs.

Dhaval Shah (SUNY Buffalo) presented a talk demonstrating how to employ PK/PD modeling and simulation (M&S) to guide the discovery and development of ADCs. As an introduction, he pointed out that any mathematical model is a representation of the system that accounts for its known or inferred properties, and allows for investigation of these properties and, in some cases, prediction of future outcomes. However, all models are approximations, and must be trained and improved using the "Learn and Confirm" paradigm continously. He also noted that M&S is employed at various stages in drug discovery and development, and PK/PD M&S is especially a mainstay across the industry that is routinly employed from optimization of a drug molecule to clinical trial simulations.

Dr Shah then introduced different kinds of PK/PD models that are available to support ADC programs at different stages of drug development. First, he described the "cellular level" PK model for disposition of ADC, which is designed to represent a cell either in an in vitro situation (e.g., 96-well plate) or an in in vivo situation (e.g., a tissue cell). The cellular level model accounts for the known processes regarding cellular disposition

of ADCs, e.g., binding of ADC to cell surface, internalization of antigen-ADC complex, release of the drug within the cell, binding of the drug to its intracellular target, and efflux of the released drug out of the cell. He also mentioned the need to refine the model with respect to intracellular compartments and processing (e.g., endo-lysosomal processing of ADC, lysosomal escape of released drug). He stressed the fact that the physicochemical property of drugs required for ADCs is different than that of traditional drugs, where for ADCs the longer circulation half-life and higher bioavailability of drug is not needed. Dr Shah then described the tumor dispostion model for ADCs.[9] This model is designed to account for tumor and systemic disposition of ADC and released drug simultaneously. The model is a dynamic and interactive model, where continuous changes in tumor size and associated changes in ADC/drug exchnage parmaters between blood and tumor are accounted for. He noted that, although the model is very useful in a priori predicting ADC and drug concnetrations in the tumor, it needs refinement with respect to spatial heterogeneity of the tumor.

Subsequently, he described the possibility of using a physiologically-based PK (PBPK) model for ADCs, which is adapted from the tissue disposition model for monoclonal antibodies (mAbs).[10] This model accounts for whole body dispstion of ADC and released drug simultanously, and can be very useful for investigating non-tumor tageting ADCs and drug-drug Interaction potential for ADCs. Dr Shah also mentioned the transit compartment models used to characterize individual DAR for an ADC.[11] However, he also noted the issues associated with these kind of models, such as: 1) uncertainty about if the drug dissociation rates the same for all DARs, 2) how one differentiates between drug dissociation and ADC degradation, 3) where the dissociation and degradation of ADC occur, and 4) whether the changes in preclinical DAR are applicable for preclinical-to-clinical translation. Finally, Dr Shah brienfly described the PD and TD models employed for ADCs.[12,13] He also highlighted the use of mechanism-based cell cycle models as PD models to characterize the efficacy and toxicity of ADCs. He noted that, the main hurdle in successful PK/PD/TD model development for ADCs is the lack of undersanding about which analytes correlates best with efficacy and safety of ADCs.

Dr Shah then presented a case study demostrating how to use mechanistic PK/PD models for preclinical-to-clinical translation of ADC efficacy, using brentuximab vedotin as an example.[9,14] His work provided a framework for preclinical-to-clinical translation of ADC efficacy, and demonstrated that it is essential to understand and characterize the disposition of ADC and released drug at the cellular and physiological level to predict the clinical outcome of ADC. Subsequently, he presented how to exploit M&S to guide the discovery and clinical development of ADCs. He described several 3-dimensional analyses demonstrating the relationships between different system and drug specific parameters and the efficacy, which can influence clinical outcome of ADCs. The first relationship was between dose of ADC, tumor antigen concentration, and efficacy. This relationship answers precision medicine-related questions, e.g., what cut-off receptor number per cell (antigen concentration) one needs for assuring the efficacy of ADC in patients at a given dose. The second relationship was between dose of ADC, target affinity of antibody, and efficacy. This relationship answers feasibility and antibody optimization-related questions, e.g., at the given dose, what benefit one may achieve by improving the affinity of antibody toward the target, and beyond which point there is a diminishing return on investment. The third relationship he described was between dose of ADC, efflux rate of the released drug out of cell, and efficacy. This relationship answers drug-linker design and drug resistance development questions, e.g., what is the ideal permeability of the released drug, and how does the required dose to achieve efficacy change with the alteration in efflux rate of the drug out of cancer cells. The last relationship was described between ADC dose, tumor growth rate, and ADC efficacy. According to Dr Shah, this was one of the most important relationships that is often ignored by clinicians. It can help one answer questions related to precision medicine and cancer indications, e.g., what dose will be efficacious at the given growth characteristics of tumor in a patient, and what tumor types are slow-growing and responsive to the cure.

Lastly, Dr Shah discussed the use of M&S to understand the underlying system responsible for the disposition of ADC and released drug, using brentuximab vedotin as a case study. Looking into the determinants of drug concentration in systemic circulation, he pointed out that: (1) non-specific clearance of ADC appears to be a higher contributor of released drug in plasma than drug dissociated from intact ADC, (2) drug generated in the tumor does not contribute significantly to the drug concentration in plasma, and (3) over the period of time % contribution of the newly generated payload toward drug plasma concentrations decreases and the contribution of payload coming from the peripheral compartment becomes predominant. Looking into the determinants of drug concentration in tumor interstitium, Dr Shah pointed out that: (1) the released drug distributing from plasma into the tumor contributes notably to tumor interstitial drug concentrations, (2) the drug generated within the cancer cells gradually becomes an important source of released drug in the tumor interstitial space, and (3) the local dissociation of drug from ADC is a very minute contributor to tumor interstitial drug concentrations. And finally, looking into the determinants of drug concentration in tumor cells, he pointed out that: (1) in the initial times, the drug brought in the cells by ADC is the predominant contributor toward intracellular drug concentrations, (2) as time progresses, binding of the drug within the cells seems to be the major contributor for retaining drug within the cell, and (3) passive influx of the payload across the cell seems to be a very minute contributor of intracellular drug concentrations. Dr Shah concluded by noting that there is a need to conduct novel experiments to better understand cellular and whole body disposition of ADC, and to delineate phenomenon like bystander effects and control ADC responses. He also mentioned that whenever bioanalytical measurements are committed with PK/PD modelers' perspective in mind, it provides an opportunity to integrate these measurements in a quantitative manner using mathematical PK/PD/TD models.

Melissa Schutten (Genentech) presented on the determinants of ADC toxicity. She started her talk by providing an introduction to ADC anatomy, and elaborating on the importance of all three components of ADC, i.e., the antibody, the linker, and the potent toxic drug. She also reiterated the rationale behind developing ADCs by mentioning that ADCs can selectively deliver a potent cytotoxic drug to tumor cells via tumor-specific or overexpressed antigens, leading to increased drug delivery to tumor and reduced normal tissue drug exposure. She then described how ADCs are better tolerated than the free cytotoxic drugs by using two case studies. In the first study, a group of rats was intravenously administered with free DM1 at the dose of 2400 $\mu g/m^2$, and was compared with another group of rats that was administered T-DM1 containing an equivalent amount of DM1. It was observed that 100% of the animals died in the group that was administered with the free DM1, whereas all the animals in the T-DM1 treatment group survived following a maximum of 5% body weight loss for a brief period of time. In the second study, a group of monkeys was intravenously administered with free MMAE at the dose of 750 $\mu g/m^2$, and was compared with another group that was administered an ADC containing equivalent amount of MMAE. The toxicity was monitored as a reduction in the monkey neutrophil count, and it was observed that in the free MMAE group there was a significant (~5-fold) reduction in the mean neutrophils counts of the animal, whereas there was no reduction in animals' neutrophil count when MMAE was delivered linked to an antibody. As such, she concluded that ~2–3 times more cytotoxic drug can be given as an ADC.

Dr Schutten then elaborated on the modes of ADC toxicity by dividing them mainly in two categories, i.e., toxicity due to the systemic release of toxin, and unwanted ADC-mediated cytotoxicity. She further divided the sources of systemic release of toxin into instability of the linker and catabolism of ADC. To demonstrate the effect of linker instability of ADC on toxicity, she presented the case study from Polson et al.[15] In this case, anti-CD22 antibody was conjugated to DM1 using linkers with various stabilities, and it was shown that: (1) un-cleavable linkers resulted in slower deconjugation and drug release in the systemic circulation, (2) cleavable linkers resulted in higher magnitude of weight loss in rats compared with non-cleavable linkers, and (3) more stable linkers resulted in reduced systemic toxicity of ADCs in rats as monitored by changes in AST, ALT, WBC, platelet, and neutrophil levels. As a case study to demonstrate the effect of ADC catabolism on ADC toxicity, Dr Schutten presented results showing increase in toxicity of trastuzumab-mc-vc-PAB-MMAF ADC by increasing the DAR. She also presented data from Junutula et al.[16] comparing THIOMAB-drug conjugates (TDC) with ADC, where it was shown that TDC resulted in slower catabolism of antibody compared with ADC, resulting in better toxicity profile for TDC conjugate.

To explain the unwanted ADC-mediated cytotoxicity, Dr Schutten first focused on the toxicity resulting from targeted binding of ADC to normal tissues expressing antigen. She presented a case study with the ADC-targeting leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5), which is a marker for colon stem cells and postulated marker for colon cancer stem cells. It was observed that the LGR5-targeting ADC resulted in an on-target (intestinal stem cell-targeted) toxicity, leading to blunted or fused villi. She also presented data from CD22-targeting ADCs, where target-dependent depletion of replicating B cells by anti-CD22-vcMMAE ADC was observed in the monkeys. Using anti-CD22 ADCs, it was also shown that the targeted toxicity on B-cell depletion was more profound when drug mechanism of action was independent of cell proliferation (i.e., DNA damaging agent) compared with cell cycle-specific microtubule inhibitors like MMAE. Dr Schutten also mentioned that off-target (cross-reactive) binding of ADC to normal tissues and non-antigen-mediated ADC uptake (e.g., Fc-mediated uptake, pinocytosis) can also result in unwanted ADC-mediated cytotoxicity.

Dr Schutten concluded by noting that, for ADCs, there is a fine balance between efficacy and toxicity. And, the choice of linker, cytotoxic drug, and antibody, are all important determinants of ADC safety, PK, and efficacy. In her opinion, ADC toxicity is usually antigen-independent and ADC/drug-dependent. However, she also acknowledged that antigen-dependent toxicity can/may occur for ADCs. Lastly, she stated that linker stability, DAR, and site of drug conjugation, also affect systemic toxicity of ADCs.

Omar Kabbarah (Genentech) talked about using predictive biomarkers for ADC development. He started by mentioning that, compared with conventional chemotherapy, targeted therapies like ADCs offer an attractive precision healthcare opportunity. Where one can treat patients who are more likely to respond, and avoid treating patients who are not. He then introduced DSTP3086S, a six transmembrane epithelial antigen of the prostate (STEAP1)-targeting ADC, being developed for the treatment of metastatic prostate cancer. He elaborated on the target by noting that STEAP1 is expressed in the majority of human primary and metastatic prostate tumors, and its putative secondary structure suggests its role as a channel or transporter. He also elaborated on DSTP3086S by detailing that it is an ADC containing humanized anti-STEAP1 antibody conjugated to the cytotoxic agent MMAE via a protease-cleavable peptide linker.

Dr Kabbarah then discussed using STEAP1 expression as a predictive biomarker for the ADC. He noted that it is important to monitor the disease and antigen expression during treatment because the status of disease at the beginning of the treatment can be different than the disease status at the time of initial diagnosis, mainly due to the development of resistance, disease heterogeneity, and disease evolution. Thereafter, Dr Kabbarah detailed the components of STEAP1 biomarker development plan, where simultaneous use of IHC, ImmunoPET, quantitative reverse transcriptase (QRT)-PCR, and circulating tumor cell (CTC) analysis was proposed. Owing to its well-established reputation as a companion diagnostic platform and ability to assess both archival and fresh tissues, IHC was included. ImmunoPET was included because it can detect overall disease burden and can also provide valuable PD end-points. QRT-PCR was included because it is quantitative, provides wide dynamic range, and it is sample quality-independent. And, CTC analysis

was included because of the possibility of using liquid biopsy of different lesions, and ability to monitor disease status during the treatment.

Going forward Dr Kabbarah presented the Phase 1 clinical trial design for the treatment of metastatic castration-resistant prostate cancer (CRPC) using DSTP3086S. Eligibility criteria for including patients into the clinical trial were metastatic CRPC, measurable disease per RECIST v1.0 or PSA progression according to PCWG2 Criteria, and ECOG score of 0–2. The treatment included single-agent administration every 3 wk until disease progression or unacceptable toxicity, and the primary objectives of the trial were evaluation of the safety and tolerability of DSTP3086S and determination of MTD and recommended Phase 2 dose (RP2D) for the ADC. A standard 3+3 dose escalation scheme was implemented, ranging from the dose of 0.3 mg/kg to 2.8 mg/kg. The expansion cohort at MTD (RP2D) was scheduled to be 20 patients, with archival STEAP1 IHC score of 2+/3+ and $\leqslant$ 2 prior chemo regimens, and a mandatory fresh tumor biopsy was recommended for those patients. At the time of Dr Kabbarah's presentation, enrollment into the clinical trial for the escalation cohorts was completed, 27 patients had discontinued study treatment, and enrollment into expansion cohorts was ongoing.

Dr Kabbarah then briefly presented the development of STEAP1 IHC assay for companion diagnostics, and comparison between IHC and prototype QRT-PCR assay in Phase 1 dose escalation. He mentioned that they observed generally good, but not complete, concordance between QRT-PCR and IHC, and there was 50% attrition in ability to assess STEAP1 by QRT-PCR due to inadequate tumor tissue. Subsequently, he presented data from the Phase 1 trial of DSTP3086S, which were limited to the patients in the dose escalation cohorts only. He showed the best percentage change in PSA levels for each patient in all the dose cohorts. In general, higher doses resulted in decline in the PSA levels and lower doses resulted in increased PSA levels. Higher STEAP1 expression also resulted in decreased PSA levels, but this trend was not unanimous, and, in some dose group, patients with lower STEAP1 expression profile demonstrated greater reduction in PSA levels compared with higher expressing patients. Dr Kabbarah presented a case study of 60 y old man who was diagnosed with prostate cancer in 2008, and was initially treated with prostatectomy, followed by leuprolide acetate and bicalutamide treatment upon recurrence. The patient manifested a CRPC in 2011, and was treated with ARN-509 (androgen receptor antagonist), abiraterone acetate, and docetaxel, with progressive prostate cancer. However, the patient demonstrated a partial response after 4 cycles of DSTP3086S, and continued on the treatment until cycle 7, when the disease showed progression.

In his conclusion, Dr Kabbarah showed data on the CTC from the trial. He noted that, based on the work from De Bono et al.,[17] the baseline for CTC was defined as 5 circulating tumor cells per 7.5 mL of blood, and a decrease in CTC number of <5 cells per 7.5 mL at any time after the first treatment was considered efficacious response. The results from the clinical trial were presented in the form of a relationship between maximum % PSA change from baseline and log CTC change for each evaluable patient, and a Pearson correlation coefficient of 0.66 and Spearman rank order coefficient of 0.68 was observed. These results suggest that there was a reasonable agreement between the two different biomarkers employed for the evaluation of patient response to the ADC. Dr Kabbarah also compared the results from PSA, CTC, and clinical ORR analysis, and, although there was a trend toward agreement between these three methods, results were not conclusive. He also demonstrated how time-dependent changes in CTC following ADC treatment can be used as PD biomarker for establishing PK/PD relationships, and for monitoring disease status to detect the progression in early stages. He also noted that status of STEAP1 expression in CTCs at baseline and on treatment/progression, and prostate cancer and resistance genes in CTCs that emerge at time of progression, provide valuable information regarding the nature of the disease. Dr Kabbarah concluded by summarizing that DSTP3086S is currently in Phase 1 clinical development in metastatic prostate cancer, and assessment of STEAP1 as a predictive biomarker for the efficacy of ADC is ongoing using a variety of approaches. According to the results so far, 2 PRs were observed in patients with IHC 2+ or 3+ tumors, and CTC responses correlated with PSA and RECIST responses in evaluable patients. Additonally, in conjunction with PSA levels, CTCs are being used to monitor for disease progression, and for discovery of mechanisms related to the development of resistance to the ADC.

October 15, 2013: Optimizing CMC

Laurent Ducry (Lonza) discussed timelines associated with the technical transfer of a process to a contract manufacturing organization (CMO).[18] Depending on the amount of work that took place prior to the transfer, one to six months will be needed for the process development and scale-up. Thereafter, he went on presenting typical process development activities, indicating that these should focus on achieving the desired product quality, but that manufacturability is also important to prevent scale-up issues at later stages of the project. Investigation of the drug-to-antibody ratio and most suitable co-solvent were recommended as up-front laboratory activities, followed by a design of experiment (DOE) approach to develop the remaining parameters of the modification and conjugation reactions. In the next step, development of the purification process was discussed in detail. According to Dr Ducry, a well-developed tangential flow filtration (TFF) process is in most cases sufficient to reduce the amount of non-conjugated toxin to an acceptable level. The TFF process can, however, not remove aggregates. If aggregate formation cannot be prevented during the conjugation process, a chromatography purification step will most likely be needed.

Lonza's small scale GMP assets (10–60 L vessel size) and FDA-approved large scale assets (100–600 L vessel size) were presented in the conclusion slides, together with the conjugation plant under construction which will double the company's conjugation capacity when starting operation in spring 2014.

Damon Meyer (Seattle Genetics) gave a presentation on Seattle Genetics' efforts to extend its technical repertoire to site-specific conjugation of pyrrolobenzodiazepine (PBD) payloads to

engineered cysteines. Site-specific conjugation enables selection of conjugation sites for desirable biopharmaceutical properties. The presentation focused on SGN-CD33A, a PBD-based conjugate with IC_{50} of 3–68 ng/ml depending on the cell line, and 300 μg/kg in xenograph mice. The conjugation process consists of three reactions. Reduction of capped engineered cysteines, as well as inter-chain disulfide bridges, using an excess of TCEP is followed by selective re-oxidation of the inter-chain disulfide bridges with dihydroascorbic acid, and finally conjugation of two PBD payloads to the unprotected engineered cysteines. Dr Meyer commented that the PBDs are so potent that a review of the health and safety measures proved necessary. DoE and reaction time-course took place to develop the reaction conditions. The excess of PBD could not be fully removed by TFF and a carbon filtration was introduced to sufficiently clear-up drug-related contaminants. The remaining amount of unconjugated PBD could thus be reduced to ~4% of what was originally present in the feed through one carbon filtration (91% ADC recovery), and to ~1% with two carbon filtrations (76% ADC recovery). The process was successfully scaled-up by the CMO partner and very comparable quality attributes were achieved. This ADC is currently undergoing clinical trials.

Cynthia Wooge (SAFC) presented some of the challenges faced by CMOs, namely varied products, development status, timing requirements and communication flow. Standardized communication tools are used at SAFC to facilitate communication with the client. From an organizational perspective, analytical development and process development are under one management to quickly coordinate both activities. Platform analytical methods are optimized for each therapeutic protein. Regarding manufacturing equipment, glass and stainless steel conjugation vessels are used (up to 100 L available), whereas disposable purification options are preferred in order to gain flexibility.

Maria Elena Guadagno (BSP Pharmaceuticals) discussed ADC manufacturing complexity and facility design. An ADC manufacturing facility requires a combination of both biological manufacturing and highly toxic compound capabilities and expertise. At BSP, manufacturing is performed within classified areas (class C for conjugation activities and class A for fill/finish activities), while facility and equipment design ensure occupational exposure levels below 10 ng/m^3. The ADC supply chain is extensive and expensive, generally requiring fine-tuning of multiple vendors and many project management hours to ensure successful project execution. BSP's fill/finish capabilities currently cover development up to commercial supply for both lyophilized and liquid vials (2 to 100 ml vial size), with the necessary cleaning procedures and analytical capabilities. In the near future, conjugation capabilities will become available to offer conjugation and fill/finish at the same site, thus simplifying the supply chain.

Klaus Kaiser (Bayer) presented new manufacturing concepts to enhance safety and quality, and then **Matthew Hutchinson** (Genentech) discussed ADC process development and scale-up for late stage manufacturing. **Maximilian Yeh** (Evonik) concluded the session with a discussion of a contract manufacturing approach to bring an ADC from the lab to the clinic and ultimately to the market.

October 15, 2013: Plenary Afternoon Session

Tim Lowinger (Mersana Therapeutics) presented the Fleximer Platforms, which consist of a clinically-validated biodegradable polymer, with a broad array of customizable linker chemistries (conjugation to lysine or cysteine) matched to therapeutic payloads. For example, this platform allowed the conjugation of 3 to 4 Fleximers per mAb (trastuzumab) via lysine residues, with up to 10 drugs (vinca) per Fleximer, with no aggregation. The cysteine bioconjugate platform leads to highly stable cysteine-based ADCs (cross-linkage of cysteines, DAR = 20) that are highly active, selective and well-tolerated in preclinical tumor models.

George Badescu (PolyTherics) described the site-specific ThioBridge™ technology. The resulting ADCs are highly stable because the hinge disulfide bonds are re-bridged. The resulting ADC also has the benefits of reduced heterogeneity and better stability in serum. Both mAb and Fab fragment conjugates were exemplified.

Robert Pettit (Arizona State University) gave a presentation on novel cytotoxic molecules isolated from natural organisms that are used to produce novel classes of ADCs. For example, pancratistatin induces apoptotic cell death of human cancer cells, but fibroblast and endothelial cells remained unaffected. He also summarized the discovery of dolostatins and auristatin derivatives.

October 16, 2013: Plenary Morning Session

Dan Pereira (Agensys) presented the rationale for targeting CD37 in non-Hodgkin's lymphomas (NHL) and chronic lymphocytic leukemia (CLL) with an ADC and the in vitro and in vivo preclinical data of AGS67E, which is a fully human IGg2κ anti-CD37 antibody conjugated to MMAE. The investigational new drug application will be submitted early next year.

Ed Reilly (AbbVie) described ABT-414, an ADC that comprises a unique EGFR-binding antibody (ABT-806) with tumor-specific binding properties that limit effects of the toxin on normal tissues. He then presented the ABT-414 in vivo activity in preclinical tumor models overexpressing wild-type EGFR or EGFR vIII mutant (A431, U87, tumor fragment from glioblastoma patient). The activity was correlated with EGFR expression. ABT-414 is now in Phase 1 clinical studies in solid tumors and also in glioblastoma in combination with temozolomide plus or minus radiotherapy.

William Fanslow (Amgen) presented the background of the CD27L target and the choice for an ADC approach. He then summarized the preclinical AMG172 in vitro and in vivo pharmacology studies in renal cell carcinoma models. A two-part clinical Phase 1 study of AMG172 was initiated in refractory clear cell renal cell carcinoma patients.

October 16, 2013: ADC Discovery Stream

Frank Koehn (Pfizer) started the stream by giving a talk on leveraging natural products as a source of cytotoxic ADC payloads. **Juhani Saarinen** (Glykos) then showcased a new site-specific conjugation using hydrophilic linkers and original payload derivatives. **John Flygare** (Genentech) elaborated on novel toxins and warhead payloads for use in ADCs, and **David Miao** (Concortis Biosystems) discussed technologies to advance conventional ADCs to multifunctional ADCs.

Kevin Pinney (Baylor University) discussed small-molecule tubulin-binding analogs as potential payloads for ADCs, and then **Karyn O'Neil** (Centyrex, Johnson and Johnson) reported proof-of-concept for exploiting the biophysical properties of Centyrins for targeted delivery. The final presentation of the stream was given by **Vaughn Smider** (The Scripps Research Institute), who discussed cow antibodies as a new structural class of antibody using ultralong CDR3s.

October 16, 2013: ADC Development Approaches

In this session, **Jan Pinkas** (ImmunoGen) gave a talk on advancing preclinical development of ADCs, and **Dave Colcher** (City of Hope) discussed use of radiolabeled antibodies and antibody constructs for preclinical and clinical Applications. **Paul Parren** (Genmab) then presented a case study on advancing toward the clinic as soon as possible based on the pre-clinical development of a therapeutic ADC targeting tissue factor. **William Olson** (Progenics) showcased biomarker strategies for selecting patients and tracking treatment response in prostate cancer, **Barbara Hibner** (Takeda) discussed progress toward developing more predictive preclinical ADC Models, and **Jiang He** (University of Virginia) reported data on tumor-targeting single-chain antibody fragments for cancer imaging and targeted therapy.

October 16, 2013: Optimizing CMC

Yilma Adem (Genentech) started the stream with a discussion of the mechanism of ADCs' physical instability and the role of drug payload. **Lisa Hardwick** (Baxter Biopharma Solutions) then presented a case study for ADC lyophilization process development. She started with an overview of Baxter's experience, capability, and capacity for ADC finished product manufacturing, and the analytical methods available for ADC finished product analysis at Baxter. For the case study, the role of residual moisture was studied using a special approach. This approach - the "Thief Sampling" approach during "step-wise" secondary drying was demonstrated to have advantages over general approach (equilibration at controlled relative humidity) for stability studies of lyophilized product. With this approach, the residual moisture was measured using a non-destructive near-infrared spectroscopy method with a calibration curve. Having an estimate of residual moisture for each vial allowed a relationship between the level of residual moisture and the rate of loss of product integrity to be established. For one ADC, an example of correlation between water content (during secondary drying) and glass transition temperature (Tg) was given. The water content was also linked to the stressed stability data. A postulated mechanism for the residual water content on the loss of product integrity was discussed and related to the crystallization of sucrose during cake collapse.

Nitya Ray (Progenics) presented ADC manufacturing strategy from early-phase clinical development to Phase 3 and/or commercial for a prostate specific membrane antigen (PSMA) ADC. PSMA ADC is composed of a human anti-PSMA mAb linked to cytotoxic drug vcMMAE using conventional cysteine conjugation chemistry. He provided an overview for the manufacturing processes for PSMA mAb: cell culture, mAb purification, and PSMA ADC conjugation reaction. For the conjugation process, key process parameters were determined for two quality attributes, "ADC Molar Ratio" and "Aggregation" using JMP linear model. Several ADC scale-up strategies were discussed, which included: (1) insight into target product profile; (2) focus on product quality with strong scientific and engineering principles (such as systematic studies to establish interrelations between process parameters and product quality attributes); (3) focus on development speed, cost and supply chain by building process platform and using holistic approach to process development; and (4) minimize surprises in process scale-up with appropriate scale-down models. Finally, he highlighted crucial Phase 3 process improvements for PSMA ADC in several areas: improved bioreactor productivity with high-expression cell line; implemented platform purification process with increased yield; improved process robustness with systematic DOE studies; developed a UF/DF process with improved manufacturing efficiency; and optimized PSMA ADC finished product formulation.

Mark Wright (Piramal Healthcare) gave a presentation on challenges in ADC process development and scale up. The first part of his talk was an overview of Piramal Healthcare in several areas: conjugation experience with ADC manufacturing, capabilities and expansion plans for ADC supplies, regulatory history, and its alliance with Fujifilm Diosynth Biotechnologies. The majority of his presentation focused on discussion of case studies for ADCs, which included two basic conjugation processes (partial reduction chemistry and lysine chemistry), process parameter development, and scale up strategies. He started with general process considerations, such as: applying quality-by-design principles and DOE approaches; planning for process development based on risk, timeline, and material availability. For process development, he discussed various parameters (e.g., pH, ionic strength, linker/ reductant equivalents and kinetics, protein concentration, agitation, hold time stability) that could affect the partial reduction/modification and conjugation processes. He also discussed in-process controls used for monitoring the processes. In addition, he noted considerations for chromatography and TTF purification process development. Examples of reaction chemistry kinetics, DOE study on DAR, and membrane size selection were given. For scale up, he discussed several strategies, which included: batch scales, equipment choice, and other process considerations such as scalability of steps and mixing.

Finally, he discussed the process economics for both small and large scale production. For small scale, most cost was associated with the production of mAb, but for large scale, process itself (such as membrane, column, hardware, waste disposable and other consumables) becomes a significant source of cost.

David Rabuka (Redwood/ Catalent) presented a talk on site-specific ADC generation using SMARTag™ Technology.[19-24] SMARTag™ Technology is a platform technology developed by Redwood Bioscience for generating site-specific ADCs. SMART stands for specific modifiable aldehyde recombinant tag, which leverages a formylglycine-generating enzyme to form an aldehyde tag to enable the site-specific conjugation on proteins. A new type of proprietary cytotoxin-linkers that reacted selectively with aldehyde tags was used for generating the ADCs. This new platform technology allowed optimization of ADCs in several areas: producing homogeneous drug product with batch-to-batch reproducibility, controlling drug loading for 2, 4, or 6 DARs, and enabling the study of ADC structure-activity relationship for optimization of therapeutic index. He showed detailed data on the effect of aldehyde tags on physical properties of proteins. The data indicated that aldehyde tags can be placed on a broad region of mAb with minimum/no effects on aggregation, antigen binding, internalization, melting temp, and FcRN binding. He then discussed the advantages of using the new linker, which enabled new conjugation chemistry through formation of C-C bond using a proprietary HIPS chemistry. This new chemistry provided enhanced plasma stability that can be applied to a variety of payloads. Finally, he discussed the in vitro cytotoxicity and in vivo efficacy data for SMARTag™ HER2 ADCs. Potent in vitro cytotoxicity was observed with various linkers and payloads with little positional dependence and significant tumor growth inhibition was observed with payload placement at selected positions for this ADC.

Godfrey Amplett (ImmunoGen) gave a presentation on conducting late stage phase appropriate analytics and assay development. Although scheduled for this session, the presentation by **Michele Dougherty** (FDA) titled "Expectations on Comparability Changes from Phase I to Post Marketing Phase" was cancelled due to the US government shutdown.

April Xu (Pfizer) gave a presentation on analytical development and characterization for early stage ADCs. She started with illustrations of varieties of ADC conjugation chemistries and complexities in ADC constructions, and noted analytical development challenges for ADCs to deal with the heterogeneities arisen from both mAb and linker/payloads, as well as variations in conjugation chemistries. Dr Xu then discussed the strategies for analytical method development and characterization for early stage ADC projects. The strategies included the use of platform mAb methods where applicable and building tool boxes for quality attributes linked to conjugation chemistry. She provided detailed examples of various tool kits available for ADC analysis, which included: 1) DAR determinations by UV spectroscopy, LCMS of reduced ADCs, RP-HPLC for reduced ADCs, and HIC analysis; 2) determination of drug load profile by HIC, iCIEF, and LCMS; 3) determination of free drug and related impurities by RP-HPLC; 4) platform peptide mapping approach for ADC identification and characterization for sites of conjugation. She concluded with a discussion of other tool kits for ADC analysis that may need to be optimized depending on specifics of the ADCs.

October 16, 2013: Plenary Afternoon Session

Nancy Whiting (Seattle Genetics) gave a keynote presentation on ADC design and development. Having achieved proof-of-concept for ADCs in the clinic, the focus on developing more ADCs will lead to rapid improvement such as, combination of ADCs, new cytotoxic agents, refining optimal drug-load, novel linkers to increase specificity and new conjugation methods. The activities of brentuximab vedotin (cAC10-Val-Cit-MMAE) targeting CD30 were described. CD30 is expressed on Hodgkin lymphoma (HL) and systemic anaplastic large cell lymphoma (sALCL) with a very low expression on normal cells (activated B and T lymphocytes and natural killer (NK) cells)). In clinical studies, the effect of conjugation was demonstrated in HL, with an ORR of 75% and 0% for brentuximab vedotin (anti-CD30 ADC, n = 102) and SGN-30 (anti-CD30 mAb, n = 35), respectively. In Phase 2 studies, the ORR in HL and sALCL were 75% and 86%, respectively, and the complete response (CR) rates were 34% and 57%, respectively. 94% and 97% of patients achieved tumor reduction in HL and sALCL, respectively. The most common grade $\geqslant$ 3 adverse events (AE) observed in Phase 2 trials were peripheral sensory neuropathy and neutropenia. The estimated 24-mo survival rates were 65% and 63% in HL and sALCL, respectively. FDA granted brentuximab vedotin accelerate approval in 2011 for HL and sALCL indications; the European Commission and Health Canada also granted approvals in October 2012 and February 2013, respectively.

Dr Whiting then discussed the combination of brentuximab vedotin (administered from 0.6 to 1.2 mg/kg, every two weeks) with adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) n = 25 or AVD, n = 26, in newly diagnosed patients with advanced stage HL. In this Phase 1 study, the most common grade $\geqslant$ 3 AEs observed in both cases were neutropenia and anemia, pulmonary toxicity was only observed in the ABVD combination group. CR response was 95% and 96% in the ABVD and AVD cohorts, respectively. Brentuximab vedotin (1.8 mg/kg, every three weeks) was also combined with cyclophosphamide, doxorubicin, prednisolone (CHP) (CHOP without vincristine) in a Phase 1 study as frontline treatment of sALCL (n = 19) and other CD-30-positive mature T-cell and NK-cell lymphomas (n = 7). The most common grade $\geqslant$ 3 AEs observed were neutropenia, peripheral sensory neuropathy, dyspnea and nausea. CR was 84% and 100% in the sALCL and other diagnoses, respectively. Besides HL and sALCL, other CD30-expressing malignancies could potentially benefit from brentuximab vedotin treatment.

Dr Whiting gave an overview of their pipeline of 6 ADCs in Phase 1 clinical studies, including those targeting CD70 (SGN-75), nectin-4 (ASG-22ME), CD19 (SGN-CD19A), CD33 (SGN-33A), SLITRK6 (ASG-15ME) and LIV-1 (SGN-LIV1A). SGN-CD19A is an anti-CD19-mc-MMAF ADC. Two Phase 1 studies were initiated in January 2013 in B-ALL and

B-NHL. CD19 is a pan-B cell marker expressed on pro-B, pre-B, immature B, mature B and activated B-cells. Both studies are divided into two parts: part 1 will estimate the MTD and part 2 will refine the dosing regimen.

Dr Whiting concluded her talk with three examples of new technology in preclinical and early clinical evaluation. The first example was engineered cysteine antibodies (EC-mAbs) with the substitution of S239C on heavy chain, which leads to uniform two drugs per antibody, increase of stability in vitro, reduction of ADCC activity of the mAb and of aggregation with novel payloads. The second example was a first-in-class PDB-ADC. SGN-CD33A is composed of a humanized mAb targeting CD33 with two engineered cysteine residues, a mc-Val-Ala linker and a PDB drug SGD 1882, which is a potent DNA cross-linker resistant to multi-drug resistance (MDR) pumps. SGN-CD33A mediated cytotoxicity against a large panel of AML cell lines and primary AML samples in vitro and antitumor activity (a single dose of SGN-CD33A at 0.3 mg/kg) in MDR+ TF1-α sc. AML model in vivo. A Phase 1 study of SGN-CD33A is currently enrolling patients with CD33-positive myeloid malignancies. The final example presented was the self-stabilizing linkers (hydrolysis of maleimide part of ADC) with robust improvement of stability in vitro and a minimal drug loss during extended circulation in vivo. The self-stabilizing linkers increased antitumor activity in some in vivo models and decreased neutropenia observed in vivo.

Seattle Genetics has multiple collaborations with major pharmaceutical companies to develop ADC against a large panel of targets such as CD22, CD79b, STEAP1, MUC16, NaPi2b, ETBR, GPNMB, PSMA, AGS-16, GCC, 5T4, TF and other undisclosed targets. Dr Whiting concluded her talk by saying that ADCs are a rapidly-evolving therapeutic option for cancer. Characteristics will be further defined and optimized in the next several years and technological advances are likely to include novel chemotypes, mechanisms and sites of conjugation, and modified antibodies.

David Goldenberg (Immunomedics) detailed Immunomedics' ADC platform designed for targeted delivery of SN-38, a potent active metabolite of irinotecan. An optimized linker was chosen to maximize efficacy in vivo, consisting of an acid-labile linkage for intracellular release. Six SN-38 molecules are linked per IgG and the ADC showed good stability in vitro and in vivo. Dr Goldenberg then presented IMMU-132, a Trop-2-targeted ADC. Trop-2 is a pan-epithelial cancer antigen involved in cancer aggressiveness and metastasis. It is expressed in ⩾ 80% of lung, colorectal, breast, prostate, pancreatic and gastric cancers, as revealed by IHC study. The mAb (RS7) is a humanized IgG1κ that can elicit ADCC activity. CL2A is a cleavable linker (pH-sensitive). After conjugation of 6 moles of SN-38/IgG, the antibody binding and the drug activity are preserved. IMMU-132 preclinical in vivo efficacy was demonstrated in various human xenograft models (lung, pancreatic, colon, breast and gastric cancers) having different Trop-2 levels. GLP toxicological study was done in cynomolgus monkeys. There was no evidence of clinically significant toxicity to any Trop-2-expressing normal tissue. An IMMU-132 Phase 1 study was initiated in advanced epithelial tumors of different cancers (colorectal cancer (CRC), triple negative breast cancer (TNBC), small cell lung carcinoma, gastric, pancreatic, ovarian, prostate and renal cancers) in patients who failed standard therapy. The grade ⩾ 3 AEs after injection of 8 mg/kg (n = 7) or 10 mg/kg (n = 6) were neutropenia (n = 1) in both cases, anemia (n = 1) in the 10mg/kg arm and fatigue (n = 1) in the 8mg/kg arm. IMMU-132 shows a decrease of 51% of lesions compared with baseline in a TNBC patient after 20 wk follow-up.

IMMU-130 and IMMU-131 ADCs target CEACAM5 and CD22, respectively. The humanized anti-CEACAM5 mAb was first used as ^{131}I-radioconjugate for CRC therapy, confirming specific tumor/CEA localization and demonstrating that targeting occurs even with high levels of antigen in circulation. Two Phase 1 studies are ongoing with IMMU-130, with a major response seen in one heavily pre-treated patient (overall response > 7 mo) and no grade ⩾ 3 AEs, and no anti-antibody response after 18 treatments. Dr Goldenberg concluded by noting that the efficacy of IMMU-131 was demonstrated in the Ramos B-cell lymphoma xenograft model in mice. Two ADCs with SN-38 have been developed and shown to be tolerable and efficacious in diverse solid tumors. This ADC technology has also shown efficacy preclinically with anti-lymphoma/leukemia mAbs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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