World Antibody Drug Conjugate Summit Europe
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The World Antibody Drug Conjugate Summit Europe, organized by Biorbis/Hanson Wade was held in Frankfurt, Germany on February 21–23, 2011. Antibody drug conjugates (ADCs), also called immunoconjugates, are becoming an increasingly important class of therapeutics as demonstrated by the attendance of nearly 100 delegates at this highly focused meeting. Updates on three ADCs that are in late-stage clinical development, trastuzumab emtansine (T-DM1), brentuximab vedotin (SGN-35) and inotuzumab ozogamicin (CMC-544), were presented by speakers from ImmunoGen, Genentech, Roche, Seattle Genetics and Pfizer. These ADCs have shown encouraging therapeutic effects against solid tumors (T-DM1) and hematological malignancies (SGN-35, CMC-544). The key feature of the new generation of ADCs is the effective combination of the cytotoxicity of natural or synthetic highly potent antineoplastic agents, tumor selective monoclonal antibodies and blood-stable optimized linkers. Early clinical data for ADCs were showcased by Progenics Pharmaceuticals (PSMA ADC, Celldex (CDX-011) and Biotest (BT-062). Takeda, MedImmune and sanofi-aventis outlined their strategies for process development and analytical characterization. In addition, presentations on duocarmycin based-ADCs, alpha emitting immunoconjugates and antibody-conjugated nanoparticles were given by representatives from Syntarga, Algeta and the University of Stuttgart, respectively.

Opening Remarks

Alain Beck (Centre d’Immunologie Pierre Fabre), chairman of the summit, opened the meeting with an introduction to antibody drug-conjugates (ADC). ADCs, also called immunoconjugates are composed of a recombinant antibody covalently bound by a synthetic linker to a highly cytotoxic drug. The main objective is to combine the pharmacological potency of small (300 to 1,000 Da) cytotoxic drugs with the high specificity of monoclonal antibodies (mAbs) for tumor-associated antigens. Antineoplastic drugs, such as doxorubicin, daunomycin, vinca-alkaloids and taxoids, have demonstrated their ability to kill cancer cells, but generally with limited selectivity and high toxic effects on normal cells, thereby yielding marginal therapeutic indices. On the other hand, approved naked antibody, e.g., rituximab, trastuzumab, cetuximab, bevacizumab, panitumumab, alemtuzumab and ofatumumab, have demonstrated their therapeutic utility in malignancies, but treatment in combination with small cytotoxic drugs is often needed to achieve significant clinical efficacy. Since the use of mAbs as single agents is sub-optimal, many strategies to improve efficacy are being investigated, including enhancement of intrinsic Fe-linked effector functions by glyco-engineering and use of bispecific antibodies, polyclonal antibodies and conjugates.

Covalent conjugation of mAbs to drugs using synthetic chemical linkers is not a new concept. The use of ADCs in animal models was reported in the 1960s and in the 1980s, clinical trials with murine IgG-based ADCs were conducted. To date, the clinical success of immunoconjugates has been very limited compared with that of naked IgGs. Gemtuzumab ozogamicin (Mylotarg; Pfizer), an anti-CD33 mAb conjugated to calicheamicin, was approved by the US Food and Drug Administration (FDA) in 2000 for the treatment of patients with acute myeloid leukemia (AML). Gemtuzumab ozogamicin is a heterogeneous mixture of 50% conjugates (0 to 8 calicheamicin moieties per IgG molecules, with an average of two or three, randomly linked to solvent-exposed lysyl residues of the antibody) and 50% unconjugated antibody. This first generation ADC product was voluntarily withdrawn from the US market in 2010. Despite this setback, the extensive recorded data and the lessons learned for this first-in-class ADC helped to pave the way for the next generation immunoconjugates. At least 15 promising new immunoconjugates are currently investigated in clinical trials.

Challenges and Opportunities of Antibody-Drug Conjugates

Gregory Landes (Takeda) discussed challenges and opportunities in the field of ADCs. He started with an overview of lessons learned so far, detailed the structural features of ADCs that are designed to generate potent anti-tumor activity and illustrated the opportunities for future development. Based on a PubMed-based survey of the literature that included more than 500
hundred papers published since 1974, three successive periods of excitement, disappointment and re-invigoration of the field were identified. Highlights in the history of antibodies and specifically ADCs, include the first report of an ADC (1974), publication of the method for generation of monoclonal antibodies (1975), the first report of human anti-mouse antibody (HAMA) response in a clinical setting (1982), publication of a first clinical study of a mouse/human chimeric antibody with improved pharmacokinetics (PK) and reduced occurrence of HAMA (1989), the report of the first humanized antibody in a clinical trial that showed no HAMA and a plasmatic half-life similar to that of native human IgG (1996), publication of clinical activity in AML by an anti-CD33 calicheamicin immunoconjugate (1999), Phase 1 data for IgG (1996), publication of clinical activity in AML by an anti-CD33 calicheamicin immunoconjugate (1999), Phase 1 data for T-DM1 showing clinical activity and mild toxicity (2010) and Phase 1 data for SGN-35 that showed induction of durable objective response and tumor regression in CD30+ lymphoma patients (2010).

Dr. Landes summarized the key features of a successful ADC, which include a potent cytotoxic drug active in many tumor types, a target-specific antibody with moderate to high affinity for the disease target (5 nM to 10 pM), linker chemistry that confers high stability of prodrug in systemic circulation while enabling activation upon cellular internalization, effective conjugation chemistry to achieve a drug/antibody ratio (DAR) that maintains antibody specificity while providing critical targeted cytotoxicity within the therapeutic window and, last but not least, a reproducible, quality-based, scalable production process for drug and linker, conjugation and purification that yields a stable, high-quality product.

Auristatin-Based Antibody-Drug Conjugates

Peter Senter (Seattle Genetics) gave a keynote presentation on empowered antibodies as cancer therapies. MAbS such as trastuzumab, rituximab, cetuximab, bevacizumab, panitumumab and ipilimumab have demonstrated significant activities in a wide variety of tumor indications through mechanisms such as direct signalling, antibody-dependent cell-mediated cytotoxicity (ADCC) and complement fixation. Approaches are currently underway to enhance the activities of these and other mAbs targeting tumor antigens by enhancing their effector functions or by utilizing them for selective drug delivery.

Several technologies have been explored to enhance the ADCC activities of mAbs, including mutagenesis of Fc domains and modification of the carbohydrate regions on antibody heavy chains. These approaches require either engineered constructs or cell lines and are not amenable for selecting mAbs from among large panels at early stages in development. A unique approach to optimize ADCC activities utilizing inhibitors of fucosylation of the branched heavy chain carbohydrates was described by Dr. Senter. A series of fucose derivatives that inhibited various enzymes involved in carbohydrate fucosylation were identified. When the fucose derivatives were used in the mAb production process, the resulting defucosylated mAbs had ADCC activities that were between 10 and 50 times greater than the parental mAbs due to stronger binding of the antibodies to FcγRIII on natural killer cells.

Dr. Senter also provided an update on Seattle Genetics’ ADCs. A totally synthetic anti-mitotic drug, monomethylauristatin E (MMAE), was conjugated to mAb cysteine residues through a peptide linker that is readily cleaved by intracellular proteases. This conjugation strategy is distinguished from earlier methodologies due to the conditional stability of the linker, the highly potent drug component and the conjugation technology in which the drugs are attached mainly in the antibody hinge region.

The activities of brentuximab vedotin (cAC10-Val-Cit-MMAE, SGN-35), one of the most active ADCs ever reported, were described by Dr. Senter. cAC10-Val-Cit-MMAE consists of an anti-CD30 mAb (cAC10), attached to MMAE with a valine-citrulline (val-cit) dipeptide linker. The conjugate is highly active in several preclinical models of CD30 positive malignancies and is well-tolerated. Based on these data, a Phase 1 study in Hodgkin disease and anaplastic large cell lymphoma (ALCL) patients was undertaken and the response rate was sufficient to warrant further clinical evaluation at a dose of 1.8 mg/kg every 3 weeks. In the Phase 2 studies, the objective response rates in Hodgkin disease and ALCL were 75 and 86%, respectively. This represents the most active ADC yet reported. A biologics license application (BLA) was submitted to the FDA in February 2011.

Several other presentations featured the auristatin drug class as a component of ADCs. William Olson (Progenics Pharmaceuticals) described the activities of an anti-prostate specific membrane antigen (PSMA) mAb-val-cit-MMAE ADC in prostate cancer. This particular ADC is of special interest because the target antigen is also known to bind to neovascularization on many carcinomas. Patients with PSMA-positive prostate cancers were administered ADC at doses up to 1.8 mg/kg every 3 weeks. Evidence of response based on decreases in serum PSA, circulating tumor cells and bone pain was obtained.

Thomas Hawthorne (Cellnex Corporation) provided a clinical overview of CDX-011, an anti-GPNMB-val-cit-MMAE ADC that recognizes antigens overexpressed on breast cancer and melanoma. Some degree of normal tissue cross-reactivity in skin, mammary glands and the oesophagus also occurs. Responses were obtained in patients with both types of tumors, with neuropathy, skin rash and hand/foot syndrome as common adverse events. Some observed trends were that patients with higher antigen levels had stronger responses and more frequent dosing was more efficacious, but also more toxic.

Jihong Wang (MedImmune) described the compositional analysis of a mAb-val-cit-MMAE ADC made according to the methods used for the other auristatin ADCs described in the meeting. The conjugate contained an average of four drugs/mAb, with a binomial distribution consisting of mainly even numbered drug/mAb ratios. This result was due to placement of the drugs on mAb cysteines that were generated through disulfide reduction. The drugs were mainly located on the heavy-heavy chain disulfides, with minor species between the heavy-light chains. Upon incubation in buffers, some hydrolysis of the maleimide adducts occurred, but the drug did not detach from the mAb. In
contrast, on incubation in serum, drug loss from the ADC took place with a half-life in the range of 4–7 days.

Michael Buckley (Takeda) showcased an analytical method platform to support discovery of ADCs and optimal antibody/drug combinations. Dr. Buckley discussed critical antibody attributes for optimization, comparison of different drug-linker combinations on the same mAb (with auristatin and duocarmycin), use of a large panel of orthogonal analytic methods to understand in vivo model results and evaluation of formulation and stability. In particular, he described substitution chemistry and its relationship to product profiles. Hierarchy in the susceptibility of interchain disulfides to reduction is a critical parameter because disulfide bonds between the heavy and light chains are more susceptible than disulfide bonds between the two heavy chains to reduction. Microheterogeneity within hinge region disulfide bonds is also important. Analytical data show that the drug to antibody ratio (DAR) appears to drive predictable product profiles. Target DAR determines also the relative amount of unconjugated antibody in the final drug product. The consequences of the presence of unconjugated antibody are dependent on its intrinsic therapeutic activity.

In the studies presented, in vivo and in vitro functionality trumped analytics development. For the right combination of antibody and antigen, two or four drugs/molecule yielded equivalent functionality despite differences in DAR and the amount of unconjugated antibody. Functional attributes of the naked antibody are also highly important, as are the requirement for high purity and high affinity antibody starting materials. In addition, the best combination of antibody, drug linker must be determined experimentally.

Maytansinoid-Based Antibody-Drug Conjugates

Ravi Chari (ImmuNoGen, Inc.,) briefly reviewed the first generation ADCs that incorporated anti-cancer drugs such as methotrexate, vinblastine or doxorubicin and lessons learned from their poor activity in clinical trials. One reason for the lack of success of these early ADCs was insufficient potency of the cytotoxic drug. Because of the limited number of antigens on the cell surface, inefficient internalization processes, and the reported low tumor localization of antibodies in patients, Dr. Chari suggested that effector molecules should have potency in the $10^{-8}$ to $10^{-11}$ M range. He discussed the suitability of several drug classes that are antibody-accessible and amenable to linker attachment. Antibody-antigen pairs are highly potent DNA alkylators/crosslinkers that are synthetically accessible and amenable to linker attachment. Antibody-IGN conjugates were potent and specific in vitro, even against mdr cells and cells that express a low antigen number. Antibody-IGN conjugates were stable in circulation in vivo and maintained biological activity in circulation. Dose-dependent anti-tumor activity was demonstrated in tumor xenograft models in vivo.

Gail Phillips (Genentech) presented an account of the pre-clinical and clinical activity of trastuzumab emtansine (T-DM1). Evaluation of a panel of linkers revealed that the nature of the linker (disulfide or thioether) connecting the maytansinoid drug with trastuzumab did not significantly affect in vitro potency or in vivo efficacy. Thus, a thioether linker was selected for T-DM1 based on the improved tolerability of the conjugate in mice. In vitro evaluation in four different HER2-amplified breast cancer cell lines showed that T-DM1 was more potent than trastuzumab. T-DM1 also maintained all the functional properties of trastuzumab, including inhibition of HER2 extracellular domain shedding, the ability to suppress PI3K signalling and mediation of ADCC. However, unlike trastuzumab, T-DM1 was able to induce apoptosis in three different HER2+ cell lines. T-DM1 also showed greater anti-tumor activity than trastuzumab in both trastuzumab-sensitive and insensitive HER2-positive breast tumor xenograft models. T-DM1 caused complete tumor regressions lasting >125 days after a single injection of conjugate.

Dr. Phillips presented clinical data on T-DM1 either as a single agent or used in combination. In a Phase 2 clinical trial in HER2+ metastatic breast cancer patients (n = 110) who were previously treated with a number of chemotherapeutic agents (an anthracycline, a taxane, capcitabine, lapatinib, trastuzumab), treatment with T-DM1 as a single agent resulted in an objective response rate (partial response + complete response) of 34.5% and a clinical benefit rate (partial response, complete response and stable disease >6 months) of 48.2%. To support further clinical evaluation of T-DM1 in combination with other therapeutic agents, in vitro and in vivo combination studies were conducted. Combination of T-DM1 with two different PI3 kinase inhibitors (GDC-0941 or GDC-0980) resulted in additive to synergistic activity in cell viability assays in vitro. Both combinations produced enhanced apoptosis and evidence of suppression of the PI3K pathway in vitro and improved efficacy in vivo. Based on these results, a Phase 1b dose escalation study of T-DM1 in combination with the PI3K inhibitor GDC-0941 was initiated.

T-DM1 was also combined with pertuzumab, an antibody that prevents ligand-induced HER-HER3 dimerization. This
combination was synergistic in vitro and highly active in vivo (MDA-MB-475 and Calu-3 xenografts). In a global single arm study combining T-DM1 (3.0 or 3.6 mg/kg q3w) and pertuzumab (840 mg loading dose, then 420 mg q3w), the overall response rate was 57.1% in first-line patients (n = 21) and 34.8% in relapsed patients (n = 46). Combination studies of T-DM1 with chemotherapeutic agents (vinorelbine, 5-FU or docetaxel) showed additive antitumor activity in preclinical models. The combination of T-DM1 with docetaxel is being evaluated in a multicenter Phase 2 study in patients with HER2+ metastatic breast cancer.

Fabric Branle (Hoffmann-La Roche) then presented additional data for T-DM1, including the clinical development strategy and results from a Phase 2 randomized trial. T-DM1 is undergoing a broad clinical development program consisting of two Phase 3 trials: (1) T-DM1 compared with capecitabine + lapatinib (EMILIA study) and (2) a first-line study comparing T-DM1 with the regimens of T-DM1 + pertuzumab and trastuzumab + a taxane. In addition, several Phase 1b combination studies are on-going. Dr. Branle summarized the results of a Phase 2 study in previously treated HER2+ metastatic breast cancer patients. In this multi-institutional, open-label, single-arm Phase 2 trial, patients (n = 110) had prior exposure to an anthracycline, a taxane, capecitabine, lapatinib and trastuzumab and two HER2-directed regimens in the metastatic setting. T-DM1 was administered at an intravenous dose of 3.6 mg/kg q3w, with a primary endpoint of overall response rate (ORR). The objective response rate by an independent review facility was 34.5% with a clinical benefit rate (CBR) of 48.2%, and the median progression free survival (PFS) was 6.9 months. T-DM1 was well-tolerated by patients at the dose and schedule tested with no dose limiting cardio toxicity or new safety signals. Based on these results, a full approval strategy.

T-DM1 is now undergoing testing in a two arm study (EMILIA; T-DM1 vs. lapatinib + capecitabine) in patients (n = 580) who received prior therapy that included a taxane and trastuzumab. Dr. Branle presented results from another 2-arm study evaluating T-DM1 compared with the combination of trastuzumab + docetaxel in patients (n = 137) with HER2-positive, recurrent locally advanced breast cancer or metastatic breast cancer. The key primary endpoint of this study was PFS, with secondary endpoints of ORR, CBR and overall survival. Data presented on 67 patients receiving T-DM1 and 70 receiving trastuzumab + docetaxel indicated that the ORR was 47.8% for the T-DM1 arm and 41.4% for the combination arm, while the CBR was 55.2% and 57.1%, respectively. In patients who had received prior trastuzumab or taxane therapy, the response to T-DM1 was significantly higher than that observed for the trastuzumab + docetaxel combination (ORR of 54.2% vs. 35.5%, respectively). There was significantly greater incidence of grade 3 or higher adverse events (AE) in the combination arm (75% AE) compared with the T-DM1 arm (37.3% AE). The nature of the AEs was also different, with the three most common occurrences in the combination arm being alopecia, neutropenia and diarrhea, while in the T-DM1 arm patients experienced nausea, fatigue or pyrexia. Interestingly, alopecia was noted in 45/68 patients in the combination arm and only 1/67 patients in the T-DM1 arm. Neutropenia was also more pronounced in the combination arm, with an incidence in 57.4%, compared with 7.5% in the T-DM1 arm.

Dr. Branle concluded that the ORR was slightly higher for T-DM1 compared with the combination arm. T-DM1 appears to have a favorable overall safety profile compared with the combination of trastuzumab + docetaxel in first line metastatic breast cancer, with the incidence of Grade ≥3 AEs in the T-DM1 arm half that observed in the trastuzumab + docetaxel arm. T-DM1 was not associated with an increased risk of cardiotoxicity compared with the trastuzumab + docetaxel combination. No new safety signals were observed with T-DM1 in this study population compared with previous T-DM1 studies.

Christoph Uherek (Biotest) presented preclinical and clinical data on BT-062, an anti-CD138 antibody-maytansinoid conjugate for the treatment of multiple myeloma. CD138 represents a good ADC target because it is expressed in a vast majority of multiple myeloma patients, but not expressed on healthy blood or bone marrow cells. Three different linker/maytansinoid combinations were evaluated in preclinical studies. The conjugate with a cleavable, hindered disulfide link (nBT-062-SPDB-DM4) had the highest in vivo activity and was selected for clinical evaluation. Immunohistochemical analysis revealed that some binding occurred on non-tumor cells of primarily epithelial origin. To rule out potential skin toxicity, primary keratinocytes were tested in vitro and found to be insensitive to BT-062.

Dr. Uherek then presented data from a Phase 1 dose escalation study in patients with relapsed/refractory multiple myeloma. Patients were treated with a repeated single dose every 3 weeks using an escalation scheme (10 mg/m² to 200 mg/m²). In the maximum tolerated dose (MTD: 160 mg/m²) cohort of 12 patients, there was 1 PR and 1 minor response and a clinical benefit rate of 58%. BT-062 was generally well-tolerated, with most AEs of mild to moderate intensity. There was no clinical evidence of myelosuppression. Data from this study suggest that a more frequent dosing regimen might be beneficial, and such a trial is now on-going.

In discussing potential combination trials, Dr. Uherek showed in vitro and in vivo data on the activity of BT-062 in combination with various chemotherapeutic agents currently used in multiple myeloma. Based on the additive to synergistic activity of the combination of BT-062 and lenalidomide observed in vitro, lenalidomide was chosen for in vivo evaluation. This combination showed additive to synergistic activity in the MOLP-8 multiple myeloma xenograft model. To expand the tumor indication for BT-062, immunohistochemical analysis was conducted on tumor arrays. Tumors of the pancreas, breast, head and neck, bladder and lung were confirmed as additional tumor types that express CD138. Four different established tumor xenografts derived from primary human tumors (pancreas, lung, bladder
and mammary) were treated with BT-062 and complete long-term tumor regressions (>50 days) were obtained in each case.

Yelena Kovtun (ImmunoGen, Inc.) discussed antibody-drug conjugates designed to overcome multidrug resistance (mdr) in cancer. Cytotoxic agents of different families (taxanes, vinca alkaloids, doxorubicin, calicheamicin, dolastatins and CC-1065) were identified as substrates of the ABC transporter MDR1 (P-glycoprotein). Maytansinoids and antibody-maytansinoid conjugates (AMCs) were found to be substrates for MDR1, but not the other ABC transporters MRP1 and BRCP in cell culture. Data for two polar linkers (PEG4mal and sulfo-SPDB) that can enhance the potency of AMCs against multidrug resistant cancer cells were presented. AMCs bearing either a short polyethylene glycol (PEG4) spacer or a standard thioether link between the antibody and the maytansinoid showed similar potency on cells devoid of MDR1 expression. However, AMCs containing the polar PEG4-spacer were more effective than the standard conjugates designed to overcome multidrug resistance (mdr) in cancer. Cytotoxic agents of different families (taxanes, vinca alkaloids, doxorubicin, calicheamicin, dolastatins and CC-1065) were identified as substrates of the ABC transporter MDR1 (P-glycoprotein). Maytansinoids and antibody-maytansinoid conjugates (AMCs) were found to be substrates for MDR1, but not the other ABC transporters MRP1 and BRCP in cell culture. Data for two polar linkers (PEG4mal and sulfo-SPDB) that can enhance the potency of AMCs against multidrug resistant cancer cells were presented. AMCs bearing either a short polyethylene glycol (PEG4) spacer or a standard thioether link between the antibody and the maytansinoid showed similar potency on cells devoid of MDR1 expression. However, AMCs containing the polar PEG4-spacer were more effective than the standard conjugates in arresting MDR1-expressing tumor cells in the G1/M phase and killing them in vitro. The enhanced in vitro potency of the AMCs with the PEG4-spacer also translated into greater efficacy in several different MDR1-expressing xenograft tumor models.

The polar linker sulfo-SPDB that provided a disulfide-linked AMC bearing a charged sulfonic acid substituent in the linker was also discussed by Dr. Kovtun. AMCs prepared with this linker showed similar potency towards MDR1-negative cells compared to those with the standard non-charged disulfide (SPDB) linker. However, the AMCs with the sulfo-SPDB linker were more potent towards MDR1-expressing cells in culture and in a tumor xenograft model. Dr. Kovtun concluded that incorporation of polar linkers in ADCs may offer a means of overcoming the phenomenon of multidrug resistance in cancer.

Stephane Cornen (sanofi-aventis) gave a presentation of the analytical tools used for the characterization of ADCs, with a focus on the analysis of antibody-maytansinoid conjugates. The antibody component of an ADC is itself heterogeneous, with attributes such as pyrogglutamic acid formation, deamidation, methionine oxidation and glycation. The list of assays expected by regulatory authorities for ADCs includes drug-to-antibody ratio, drug distribution pattern, residual drug, unconjugated antibody, cytotoxicity and process related impurities (e.g., residual solvents). Dr. Cornen identified mass spectrometry (MS) as a critical analytical tool and displayed data from a “top down” approach wherein the mass spectra of a deglycosylated ADC is directly collected and a “bottom up” approach in which the ADC is first subjected to enzymatic digestion, followed by MS analysis of the peptide fragments. He stated that MS analysis was a good tool to monitor the effect of process changes on ADCs and showed data demonstrating: (a) similar mass spectral profiles in three side-by-side before and after process changes (top down analysis) and (b) the same lysine residues were modified before and after process changes (bottom up analysis).

Dr. Cornen also highlighted the benefits (fast, quantitative, reproducible) of using image capillary isoelectric focusing analysis to monitor charge heterogeneity in ADCs. Formation of aggregates, if present in ADCs, can be reversible, irreversible or covalent and Dr. Cornen suggested that multiple orthogonal methods would be needed to monitor them. For soluble aggregates in a stressed (40°C, 4 weeks) sample, the data showed good correlation between size exclusion chromatography (SEC) and velocity sedimentation (SV-AUC). For subvisible/visible particles, microscopy imaging analysis was deemed to be the preferred option. For example, Dr. Cornen showed that the reversal of aggregation by the addition of Tween 80 to samples subjected to shaking stress could be monitored by this method. Dr. Cornen also described methods to assess the stability of ADCs under various stress conditions. He showed that differential scanning calorimetry was a powerful tool to measure thermal denaturation and could also serve as a means of screening of the buffers and excipients used for formulation of ADCs. In concluding his talk, Dr. Cornen discussed assays for monitoring free maytansinoid release, if any, from antibody-maytansinoid conjugates, including reverse phase HPLC with UV detection and a HISEP protein column. In one case, these assays were used to detect leachables from sample bags used to store drug substance.

**Calicheamicin-Based Antibody-Drug Conjugates**

John Di Joseph (Pfizer) gave an update on inotuzumab ozogamicin (CMC-544), which is a CD22-targeted cytotoxic agent composed of a humanized, hinge-stabilized IgG4 antibody covalently linked to N-acetyl-calicheamicin dimethyl hydrazide (DNA-disrupting agent) via the acid labile acetylphenoxy butanoic linker. The sites of conjugation of the parental G544 antibody were investigated. A total of 78 lysines (22 in the light chain, 56 in the heavy chain) in IgG4/kappa are potential sites for conjugation. Experimental data showed that four lysines (3 in the CH2 domain, 1 in the CH3 domain) are preferred sites for conjugation to calicheamicin and the data indicated an estimated drug loading of 6 moles calicheamicin/mole antibody on average. CD22 is a B-lymphoid lineage-specific differentiation antigen expressed on both normal and malignant B cells. CMC-544 binds CD22 with subnanomolar affinity and, upon binding, is rapidly internalized, which delivers the conjugated calicheamicin moiety inside the cells. This preferential intracellular delivery of the cytotoxic drug leads to DNA damage that results in cellular apoptosis.

A therapeutic combination of rituximab and CHOP is extensively used in the treatment of B-NHL. It may be advantageous to add a targeted chemotherapeutic agent such as CMC-544 to the CHOP combination to gain additional therapeutic benefit, especially during the treatment of aggressive BCL. The addition of rituximab may not change the safety profile of the CMC-544/rituximab treatment combination, but may provide greater antitumor activity.

Safety, tolerability and preliminary efficacy data for anti-CD22 inotuzumab ozogamicin as monotherapy in a Phase 1 study and in combination with rituximab in a Phase 1/2 study, were presented. In the Phase 1 study of 50 patients, the MTD was determined to be 1.8 mg/m² (~50 μg/kg based on the mAb) administered every 4 weeks. The half-life was 12–30 h based on data for the first dose and this increased with dose and cycle.
Activity was observed in follicular (68.8% ORR, 30% complete response) and diffuse large B cell lymphoma patients (33% ORR). Adverse events (thrombocytopenia, bone marrow suppression, liver enzyme elevation) were manageable and reversible. The study is designed with two parts: (1) dose-escalating, with 3–6 patients per cohort; (2) two-arm, expanded cohort with inotuzumab ozogamicin administered at the MTD. Preliminary results in non-rituximab refractory patients are encouraging, with overall response rates in the 75–85% range and complete responses in the 50–60% range for both FL and DLBCL patients. CMC-544 has also demonstrated good activity in multicenter Phase 2 studies as either monotherapy or in combination with rituximab. Toxicities were primarily hematopoietic and were manageable. Phase 3 studies have started.

Duocarmycin-Based Antibody-Drug Conjugates

Vincent de Groot (Syntarga) presented data on combinations of DNA-damaging duocarmycins and suitable linker technologies as an alternative payload technology. Syntarga B.V. is an emerging ADC company that has entered into a number of new research collaborations with biopharmaceutical and biotechnology companies. ADCs undergoing evaluation by the company’s collaborators comprise the newest Syntarga Linker-Drug chemistries linked to collaborator antibodies. Syntarga is leveraging its proprietary technologies and expertise to generate and commercialize, alone and with partners, a portfolio of next-generation ADC products. Syntarga develops duocarmycins toxins that are potent as free drugs and as ADCs in vitro against multidrug resistant cell lines. The drug is not toxic in vivo as free drug at molar levels much higher than ADC efficacious doses. Thus, even if unintentionally released from stable linker, toxicity may be low. The linkers have demonstrated high stability in human plasma for all DNA alkylator-linked L-Ds and DNA alkylator-linked L-Ds are more stable than DNA binder-linked L-Ds. The aim for the company is to transition from “linker-drug discovery” to ADC product, i.e., to select the best linker-drug and target/mAb combination and to advance the first ADC to the clinic. To date, Syntarga payload ADCs are safe at high dose and highly efficacious at low dose. Dr. de Groot presented results of preclinical development, including drug potencies, ADC and payload stabilities in plasma, cleavage kinetics and in vivo therapeutic window aspects for ADCs directed against HER2.

Alpha Emitter Immunoconjugates

Thomas Ramdahl (Algeta) gave an update on the development of novel alpha-emitting radio immunoconjugates with potent antineoplastic activity. Algeta’s lead product, which is based on radium-223, is a first-in-class, highly targeted alphanapharmaceutical under clinical evaluation for improvement of survival in patients with bone metastases from advanced cancer. The drug, partnered with Bayer Schering Pharma AG, is in a global Phase 3 clinical trial (ALSYMPCA) to treat bone metastases resulting from prostate cancer. Algeta is a world leader in the medical application of alpha emitters and the development of alpha-pharmaceuticals, a new class of cancer therapeutics that utilizes the attractive properties of alpha-particle emitters to destroy cancer cells. The company has developed a substantial expertise in radiochemistry, nuclear medicine and medical oncology. Alpha-particle emitters offer the potential to deliver potent and localized destruction of cancer cells with minimal effect on surrounding normal cells. These properties suggest that alphanapharmaceuticals will be well-tolerated and convenient to use. Radium-223 and thorium-227 are the two most promising alpha-emitting elements (radionuclides) considered suitable for development as therapeutics. Radium-223 is included in Alpharadin®, Algeta’s lead alpha-pharmaceutical candidate and thorium-227 is incorporated in its TH-1 targeted delivery platform.

Bone metastases are frequent consequences of different cancers, causing debilitating pain, decreasing patient quality of life and reducing life expectancy. Alpharadin® (radium-223 chloride) combines a natural affinity for bone metastases with the potent and localized tumor cell-killing activity of alpha-particle emission, which has minimal effect on surrounding normal cells. Development of Alpharadin is most advanced as a treatment for bone metastases resulting from castration-resistant prostate cancer. In Phase 2 studies, Alpharadin® demonstrated strong evidence that it can prolong patient survival times, improve quality of life and offer a benign safety profile.

Antibody-Conjugated Nanoparticles

Roland Kontermann (University of Stuttgart) discussed antibody-conjugated nanoparticles for targeted drug delivery. The aim of work with this drug class is improvement of the effectiveness of tumor targeting and development of potent targeted carrier systems. The expected benefits of using immunoliposomes include improved selectivity and therapeutic efficacy. Immunoliposomes are generated by coupling antibodies to the liposomal surface. This allows active tissue targeting through binding to tumor cell-specific receptors. Instead of whole antibodies, single-chain variable fragments (scFv), which represent the smallest part of an antibody containing the entire antigen-binding site, may be an interesting alternative.

Preparation of type II scFv immunoliposomes by the conventional coupling method, as well as the post-insertion method, was discussed by Professor Kontermann. Methods to analyze binding of these immunoliposomes to antigen-expressing cells, as well as internalization through receptor-mediated endocytosis, were also presented. Targeting liposomes to cells by attaching antibodies to the liposomal surface allows targeted delivery of drugs incorporated or encapsulated in the liposome.

As with more conventional ADCs, several covalent coupling methods are available for the generation of immunoliposomes, including the generation of thioether, disulfide, carbamamide, amide and hydrazide linkages between antibodies and the liposome surface. Depending on whether antibodies are coupled to the lipid bilayer or inserted polyethylene glycol chains, immunoliposomes can be grouped into three types: (1) type Ia immunoliposomes (antibody coupled to the lipid bilayer of non-PEGylated liposomes); (2) type Ib immunoliposomes (antibody coupled to
the lipid bilayer of PEGylated liposomes; and (3) type II immunoliposomes (antibody coupled to the distal end of the PEG chain incorporated into the lipid bilayer).

Different antibody formats such as whole antibodies, Fab’ fragments and scFv have been employed for the generation of immunoliposomes. However, immunoliposomes prepared from whole antibodies have been shown to be immunogenic and are rapidly cleared from circulation through Fc-mediated uptake by macrophages (Kupffer cells of the liver). These drawbacks can be circumvented using Fab’ or scFv molecules as ligands. ScFvs are small and can be easily modified through genetic engineering. For instance, they can be genetically modified to expose an additional cysteine residue. Compared to coupling through amino or carboxyl groups, this allows very defined and site-directed coupling, which does not interfere with the antigen-binding activity of the antibody fragment. Two approaches to generate immunoliposomes have been described in the literature: (1) the conventional method, in which antibody molecules are coupled directly to the liposome surface; and (2) the post-insertion method, where the ligands are first coupled to micelles prepared from functionalized lipids that are then inserted into preformed liposomes. The post-insertion method offers the advantage of independent liposome preparation (including drug loading) and scFv coupling, with each step performed under optimal conditions or even using commercially available drug-loaded liposomes such as Doxil (liposomal doxorubicin).

In summary, Professor Kontermann showed that encapsulation of drugs into liposomal carriers extends plasma half-life and protects the drug from degradation and undesired side effects. Liposomal drugs passively accumulate in tumors; insertion of ligands, e.g., genetically engineered antibody fragments, enables targeted delivery to tumor cells in vitro and in vivo. Efficient drug release (extra- or intra-cellularly) is central to further improve of antibody-conjugated nanoparticles for targeted drug delivery.

Chairman’s Closing Remarks

Alain Beck (Centre d’Immunologie Pierre Fabre) concluded the meeting by noting that the effective combination of the cytotoxicity of natural or synthetic highly potent antineoplastic agents with monoclonal antibodies through blood-stable optimized linkers is the key feature of a new generation of ADCs. Compared with naked antibodies, the development of these conjugates for oncology indications takes longer and is more complex because many more parameters have to be finely tuned. As a sign of technological maturity, three ADCs, trastuzumab emtansine, inotuzumab ozogamicin and brentuximab vedotin, have reached late stage clinical development and have shown encouraging therapeutic effects against both solid tumors and hematological