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MEETING REPORT

Antibody-drug conjugates: Design and development for therapy and imaging in and beyond cancer, LabEx MAbImprove industrial workshop, July 27–28, 2017, Tours, France

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ABSTRACT

The annual "Antibody Industrial Symposium", co organized by LabEx MAbImprove, MabDesign and Polepharma, was held in Tours, France on June 27–28, 2017. The focus was on antibody-drug-conjugates (ADCs), new entities which realize the hope of Paul Ehrlich's magic bullet. ADCs result from the bioconjugation of a highly cytotoxic drug to a selective monoclonal antibody, which acts as a vector. Building on knowledge gained during the development of three approved ADCs, brentuximab vedotin (Adcetris®), ado trastuzumab emtansine (Kadcyla®) and inotuzumab ozogamicin (Besponsa®), and the many ADCs in development, this meeting addressed strategies and the latest innovations in the field from fundamental research to manufacturing.

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Introduction

On June 27, 2017, the meeting *Antibody-drug conjugates: Design & development for therapy & imaging in and beyond cancer* (http://ais2017.fr/) was opened by **Denis Requier** (vice-president of Polepharma, Tours, France), **Priscilla Domaingue** (communication manager, MabDesign, Lyon, France), **Thibault Coulon** (auxiliary mayor of Tours, France) and **Pr. Marie-Claude Viaud-Massuard**, president of the scientific committee of the Antibody Industrial Symposium (GICC CNRS UMR 7292, Labex MabImprove, University of Tours, Tours, France), who welcomed participants and thanked the organizers, and both institutional and industrial sponsors. The aim of this meeting was to bring together people with many skills and various centers of interest around antibody-drug conjugates (ADCs), from chemistry to biology and from different institutions in academia, industry and the clinic. ADCs are specialized targeted therapies, combining the excellent selectivity of monoclonal antibodies (mAbs) and the high potency of the payload, connected through linker technologies.[1] The topics covered in this symposium were very diverse. Indeed, overview presentations of research in the ADC field, as well as new target searches, and new applications were presented by different speakers. New linkers, new payloads and new bioconjugation technologies leading to the next generation of ADCs were described, and the challenges in scale-up and manufacturing were discussed. Importantly, the subject of ADC safety, and efficacy in offering new opportunities for the treatment of patients who are non-responsive, resistant or relapsed was also discussed.

Keynote session I: ADCs, empowering cancer therapy

The first session dealt with the challenge of improving cancer therapy using ADCs and gave a comprehensive overview of the field. It was chaired by **Dr. Nicolas Joubert** (GICC CNRS UMR 7292, Labex MabImprove, University of Tours, Tours, France) and started with a talk from **Dr. Ravi Chari** (ImmunoGen, Waltham, USA). He introduced the aim that has driven ADC research for decades: improving the therapeutic index by lowering the minimum effective dose and increasing the maximum tolerated dose. He described the development of the first ADC against solid tumors, Kadcyla®, an anti- human epidermal growth factor receptor-2 (HER2) ADC with a maytansinoid payload, underlining the process of drug and linker selection. Many challenges must be overcome for a payload to be suitable for use in an ADC: aqueous solubility, stability in blood stream, amenability to chemical modifications, and high potency; criteria that were met by maytansine. Several *in vitro* and *in vivo* studies were presented, highlighting the differences of activity *in vivo* of three different disulfide linkers with varying cleavage rates, and a non-cleavable linker, depending on the model and target.[2] There was a particular focus on the demonstration of a bystander killing effect for ADCs with cleavable

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linkers, leading to positive results on co-culture models mimicking heterogeneous tumors. Dr. Chari then introduced a new ADC, mirvetuximab soravtansine. This ADC, which targets folate receptor-α, uses a new linker with a sulfonic moiety to increase its hydrophilicity, and is linked to a maytansinoid payload.[3] First trial results were very encouraging, with an objective response rate (ORR) of 47% and a median progression-free survival (PFS) of 6.7 months. Mirvetuximab soravtansine is now ongoing a Phase 3 study in platinum-resistant ovarian cancer.

Even though maytansinoids have proven to be effective in ADCs, payloads with new mechanisms of action and increased potency compared to classical tubulin inhibitors are needed. Dr. Chari presented a new class of payload, indolinobenzodiazepines (termed IGNs), displaying better potencies against some tumor cell lines compared to maytansinoids. Chemical investigations on the core showed that switching one imine for an amine changed the crosslinking character of the di-imine into the alkylating character of the mono-imine. This resulted in reduced toxicity observed *in vivo* while conserving an equivalent potency (pM range) for the mono-imine IGN compared to the di-imine IGN.[4] Combining a sulfonic cleavable linker to this new deoxyribonucleic acid (DNA)-alkylating IGN, the ADC obtained had an improved therapeutic window and demonstrated bystander killing effect. Using this molecular construction on an anti-CD33 mAb, the resulting ADC IMGN779 showed promising activity in models with high and moderate expression of the antigen. It has now entered a Phase 1 study for patients with acute myeloid leukemia. A question from the audience highlighted the problems of mAb choice when making an ADC. Dr. Chari explained that the key parameter is its ability to deliver the payload efficiently.

To complete this first session, **Dr. Rakesh Dixit** (MedImmune Inc, Gaithersburg, USA), summarized some of the advancements in ADCs, and the future challenges. He began by presenting the long distance traveled from the principle of the « magic bullet » as defined by Paul Ehrlich in 1913, to the first US Food and Drug Administration (FDA)-approved ADCs. Gemtuzumab ozogamicin (Mylotarg®) was FDA-approved in 2000, but withdrawn in 2010 due to an excessively high dose used in therapy, leading to severe toxicity. It is worth noting that Mylotarg was re-approved, specifically for newly diagnosed adult refractory/relapsed acute lymphoid leukemia patients, with a lower recommended dose and dosing schedule. Brentuximab vedotin (Adcetris®) and ado-trastuzumab emtansine (Kadcyla®) were FDA-approved in 2011 and 2013, respectively.

Today, three different generations of ADCs have been developed, with improved structural characteristics (conjugation and linker), toxicity, and homogeneity. Mylotarg®, as a first-generation ADC, presented an unstable cleavable hydrazone linker, and was associated with targeting and instability issues during plasma circulation, leading to a high toxicity profile. Then, Kadcyla®, as a second-generation ADC, integrated significant enhancements, including a more stable non-cleavable linker, which reduced off-target toxicity, resulting in an improved therapeutic index. Low therapeutic index, however, is still the major problem for most ADC development. Dr. Dixit also highlighted the lack of concordance between preclinical and clinical trials. Indeed, the preclinical efficacy could be over- or underestimated because of the lack of relevant murine tumor models with high translational value. Moreover, ADCs face different mechanisms of tumor resistance, which are more significant than expected, including the downregulation of the receptor (one of the many factors responsible for the current failure of anti-HER2 therapies in breast cancer treatment) or its rapid recycling back to the surface without lysosomal targeting.

To overcome such issues and improve the therapeutic index of ADCs, third-generation ADCs were developed. The necessity of producing ADCs with homogeneous drug-to-antibody ratios (DARs) was underlined, as heterogeneous DARs leads to heterogeneous drug administration. Thus, third-generation ADCs are produced using site-specific conjugation technologies, leading to highly homogeneous DARs (2 or 4). The loading warheads are very potent cytotoxic molecules, including pyrrolobenzodiazepines (PBDs) or tubulysin,[5] with low pM potencies, which allow the bystander effect. Some of these ADCs are mutated in the Fc fragment to avoid Fc-Fcγ receptor interaction, leading to reduced toxicity in blood cells (*e.g.*, reduction in thrombocytopenia) without limiting tumor antigen targeting, internalization or lysosomal trafficking.

This next generation of ADCs was illustrated with MEDI-4276,[5] an anti-HER2 biparatopic ADC developed by MedImmune, with tubulysin warheads. Its biparatopic structure induces HER2 clustering, leading to a higher internalization rate than Kadcyla®, to limit recycling. MEDI-4276 is a site-specific ADC. Fc mutations allowed a homogeneous DAR 4, and a reduced Fc-Fcγ receptor interaction.[6] MEDI-4276 also showed superior efficacy *in vivo* in a low HER2-expression model compared to ado-trastuzumab emtansine. Even in an ado-trastuzumab emtansine-unresponsive model, MEDI-4276 at 3 mg/kg led to complete tumor regression. Encouraging results were also shown in a low HER2-expression model of breast cancer patient-derived xenograft (PDX) with a potential for superior safety, and no overt evidence of thrombocytopenia or dose-limiting toxicities. Dr. Dixit finished by describing very promising combination treatments exploiting synergistic effect between ADCs and immune-oncology agents (*e.g.*, immune checkpoint inhibitors).

Session II: Discovery of novel ADCs targets and formats

There is a pressing need to explore new targets and ADCs formats in order to improve their success rate and field of action. They were discussed in the second session chaired by **Maxime Lampilas** (Sanofi, Paris, France).

Dr. Roger R. Beerli (NBE-Therapeutics AG, Basel, Switzerland) presented a site-specific enzymatic conjugation technology called sortase-mediated antibody conjugation (SMAC) technology™, which allows the replacement of C-terminal affinity tags on the light and heavy chains with drug-bearing linkers.[7] Affinity tags ease purification, allowing removal of unconjugated mAb or underconjugated ADCs to yield homogeneous DAR 4 ADCs. The diversity of sortases provides the opportunity for dual-payload ADC production, exemplified with an amanitin/maytansine DAR 4 (2+2) ADC. The new

anthracyclin PNU-159682, an *in vivo* metabolite of nemorubicin with a 3-log increased potency compared to doxorubicin, was bioconjugated with SMAC technology™ onto trastuzumab, and the resulting ADC displayed a half maximal effective concentration (EC_{50}) on the SK-BR-3 cell line of 2.8 ng/mL. The linker stability in serum was demonstrated *in vitro* and *in vivo*, and this stability was attributed to an absence of albumin transfer. This ADC was active *in vitro* in the low HER2-expressing cell line T-47D and *in vivo* on Herceptin®- and Kadcyla®-resistant models.[8] Dr. Beerli presented results obtained in an orthotopic breast cancer model, in which sustained survival and induction of an antitumor immune response (mediated by increased activation of CD8 T cells) were observed. Some ongoing work demonstrated that PNU-159682-loaded ADCs could induce an upregulation of numerous genes implicated in inflammation and immune response. NBE-Therapeutics is developing a diversified pipeline of anthracyclin-based ADCs in several indications, and the company aims to start a first-in-human study in 2019. After questions, Dr. Beerli indicated that toxicity profile of PNU-159682 still needed to be fully addressed, but that it was dependent on DAR and conjugation site.

Dr. Renaud Burrer (Histalim, Montpellier, France) presented the new platform TissueScreen, a tissue-based approach, which can be used without *a priori* knowledge, to identify relevant proteins for antibody-based therapy. Indeed, tumor tissues are the most relevant source of new targets, comprising expression levels and post-translational modifications with a focus on membrane proteins. Moreover, a proteome atlas of 37 organs from 3 healthy donors, used in European Medicines Agency/FDA tissue-cross reaction (TCR) studies, was generated. The use of this atlas allowed minimization of the risk of failure at later stages due to cross-reactivity. The process involved multiple steps. Step 1 was strategy definition by a medical and scientific committee, which was composed of scientific experts, a pathologist, a medical expert and a project manager. This step consisted of definition of the opportunities by bibliography, cancer subclasses, available treatments and the strategy planning. In Step 2, studies on bio-banks allowed access to clinical information. In Step 3, characterization of tumor tissues from ten donors was done by mass spectrometry (MS), allowing the identification of thousands of protein specific fragments. Data mining, which was Step 4, allowed analysis enrichment by data selection and validation, and by requests in databases such as Gene oncology, leading to a synthesis. The target candidates were filtered by TCR data and function evaluation to obtain hits, which were confirmed in Step 5 during the lead selection. This consisted of extension to 50 other cases, and protocol validation for multiplex mass-spectrometry. Absolute quantification and ranking resulted in one or more lead(s). The antigen expression confirmation in tissues was achieved using antibodies. At this step, it was possible to generate the antibody of interest, if it did not yet exist, to confirm the expression. At this point, Histoselect® technology was used for the immunohistochemistry (IHC) protocol validation. The last step was immunohistochemical confirmation by tissue expression and cross reactivity. The TissueScreen pipeline comprised different indications, including pancreas adenocarcinoma and triple-negative breast cancer. Others Histalim technologies were also presented, such as Histoprofile®, consisting of a high-throughput multispectral IHC with six biomarkers on the same biopsy section, and Histoselect®, which permit the screening of 1000 mAb clones per day on custom tissue microarrays.

The next speaker was **Dr. Thierry Chardès** (IRCM Inserm U1194/ICM Hospital/University of Montpellier, Labex MabImprove, Montpellier, France) who presented the development of an ADC targeting HER3, used in synergy with radiation therapy in pancreatic cancer. The mAb developed by Dr. Chardès' team, 9F7-F11, displayed intrinsic biological properties, induced tumor regression *in vivo* in different models,[9,10] and was efficiently internalized (50% after 2 h at 37 °C). The corresponding monomethylauristatin E (MMAE) ADC, produced through first-generation maleimide bioconjugation, was evaluated alone and in combination with radiation treatment. The latter was examined due to recent data indicating the ability of MMAE to provoke arrest in G2/M cells, and cause DNA damage in cells, making them radiosensitive.[11] The anti-HER3-MMAE ADC induced cell accumulation in G2/M phase, apoptosis, DNA double-strand breaks, and a decrease in colony formation. Combination with irradiation intensified these effects *in vitro* and enhanced growth inhibition of pancreatic cancer cells *in vivo*, with a 37- or 44-day benefit compared to control, and a 23- or 36-day benefit compared to the ADC alone on two pancreatic cancer models with 2 Gy irradiation. Western blot experiments allowed the elucidation of the molecular events responsible for this synergism, pointing towards: (1) a slight decrease of phosphorylation of nibrin (NBS1) and ataxia-telangiectasia mutated (ATM), the two main drivers of DNA repair; (2) an increase of γH2A histone (γH2AX) expression; (3) a stronger inhibition of the phosphorylation of HER3 and of the downstream AKT signaling; (4) an increase of caspase 3 cleavage, a marker of apoptosis. Dr. Chardès concluded his talk with prospects of increased therapeutic window, and lowered doses to achieve the same effect when MMAE-ADCs are used in combination with radiation therapy.

Dr. Thomas Sandal (Crescendo Biologics, Cambridge, United Kingdom) presented a new format of antibody, the Humabody® and its use in Humabody®-drug conjugates (HDCs). Humabodies® are the smallest fragments of antibody, made from a fully human V_H building block of 12–14 kDa, and are produced in microbial systems in high yield (9 g/l). Humabodies® are very stable and can be combined to form a diverse range of multi-valent or multi-specific constructions. These antibody fragments are obtained by immunization of a transgenic mouse, permitting the avoidance of hybridoma fusion and allowing use of phage display selection. Crescendo Biologics' pipeline contains, for example, a programmed cell death 1 (PD-1)xPD-1 biparatopic, and a PD-1xlymphocyte activation gene 3 (LAG3) trispecific Humabody®. Compared to ADCs, HDCs are smaller, permitting better tumor penetration, and have a short half-life that can be extended by association with a serum albumin (SA)-binding Humabody®. The DAR is also more defined than that of an ADC with a controlled conjugation. In a mouse model, a Tc99-labeled anti-prostate specific membrane antigen (PSMA) Humabody® was mainly present in the tumor and the kidney, whereas the equivalent IgG was also found in circulation. Dr. Sandal also showed that the tumor uptake for Humabodies® is faster than for IgGs, with a better tumor/

background ratio. Even the bispecific construction (anti-murine SA Humabody®) still showed faster tumor uptake, and presented a larger distribution volume than the IgG. The bispecific construction against PSMA and murine SA demonstrated an impressive tumor accumulation, with more than 20% of the injected dose present in the tumor 24 h after treatment, in contrast to the IgG (~6%). Dr. Sandal underlined the importance of the Humabody® combination for cell internalization efficacy. Moreover, in a cell-killing assay, MMAE-conjugated constructions were tested and one of the bispecific anti-MSA/anti-PSMA Humabodies® (DAR 2) showed better potency than the equivalent IgG (DAR 4). Nevertheless, this capacity was dependent on the Humabody® structure. In mouse models, HDCs are potentially better tolerated than corresponding ADCs. The combinational potency of Humabodies® and the wide range of available linkers offers a great opportunity for the field of oncology. Indeed, Dr. Sandal presented a biparatopic Humabody® against PD-1, associated with an anti-murine SA fragment. This construction showed a superior activity compared to pembrolizumab in a mouse tumor model. After a question from Dr. Chari, Dr. Sandal explained that the affinity of Humabodies® depends on the target and can be very different, with a range from pM to μM. It is more a question of low on-rate and off-rate, which permits significant tumor penetration, and also a question of Humabody® combination.

The last presenter of this session was **Dr. Ekkehard Moessner** (Roche Innovation Center, Basel, Switzerland), who explained the development of a new interleukin (IL)-2-based immunocytokine: carcinoembryonic antigen (CEA)-IL2v. IL-2 is an important cytokine for the proliferation, activation and differentiation of immune effector cells. However, treatments using IL-2 have shown high systemic toxicity with vascular leak syndrome, and a dysregulation balance between T-cell effectors and regulatory T-cells. A novel variant of IL-2 (IL2v) was designed in order to abolish CD25 binding, while maintaining IL-2R$\beta\gamma$ interaction and activation of effector cells, leading to antitumor potency with reduced toxicity. The immunocytokine CEA-IL2v was built by fusion of one monomer of IL2v with an antibody targeting CEACAM5 (a validated tumor antigen) with a high affinity (390 pM). This antibody presented Fc mutations, preventing Fcγ receptor and C1q binding. Published results showed that CEA-IL2v led to reduced regulatory T-cell activation, whereas proliferation was unchanged for natural killer (NK) and CD8+ T-cells, with the same tumor lysis as CEA-IL2wt alongside an up-regulation of CD25+ NK-cells. The therapeutic synergy of this immunocytokine was shown, *in vitro*, in combination with an antibody-dependent cell-mediated cytotoxicity (ADCC)-competent antibody (cetuximab), with a significant increase of tumor cell and CD25-positive NK-cell lysis with 1 ng/ml of cetuximab.[12] In mice, CEA-IL2v led to peripheral T- and NK-cell expansion and immune effector cell recruitment in a mouse tumor model, with an increased CD8+/CD4+ T-cell population. Moreover, CEA-IL2 at 0.25 mg/kg combined with programmed death ligand 1 (PD-L1) at 10 mg/kg significantly improved the survival of tumor-bearing mice compared to each treatment alone. This combination was tested at low dose in Phase 1 clinical study: T-cell infiltration and an increase in tumor T-cell CD8:CD4 ratio was observed in several patients. IL-2 is also implicated in autoimmune diseases through activation of regulatory T-cells. A novel variant IL-2m was designed to present a reduced affinity to IL-2R$\beta\gamma$. Then, one IL-2m variant was fused with each heavy chain of an IgG, forming IgG-$(IL\text{-}2m)_2$. This immunocytokine stimulated regulatory T-cells with high selectivity, without modification of CD8+ T- and NK-cell activation, and led to an expansion of both CD4+ and CD8+ regulatory T-cell populations after a single dose of 30 and 100 μg/kg in cynomolgus monkeys. To conclude, treatment with this immunocytokine should allow an increase in regulatory T-cell numbers and restore immune homeostasis.

Pitch session

A pitch session addressing new ADC formats especially for diagnosis and immunomonitoring followed session 2. The chair was shared by **Prof. Dr. Andreas Pahl** (Heidelberg Pharma, Ladenburg, Germany) and **Dr. Saïd El Alaoui** (Covalab, Lyon, France).

The first pitch was given by **Dr. Gokhan Yahioglu** (Antikor Biopharma Ltd, Stevenage, England). He presented their work on fragment-drug conjugates (FDCs) as an alternative to ADCs in solid tumors, using OptiLink™ technology on single-chain variable fragments (scFv).[13] Optimization of the structure allowed easy bioconjugation of all lysines, achieving highly potent single FDC entities with DARs up to 12 without hampering the solubility of the construct. Small format conjugates present rapid and efficient tumor uptake and an optimum pharmacokinetic profile. Demonstration of an MMAE-bearing anti-HER2 scFv with a DAR of 8 showed outperformance of MMAE-bearing trastuzumab with a DAR of 4 in terms of (1) efficacy, especially with large tumors; (2) tumor uptake, with an hour-scale for the FDC compared to a day-scale for the ADC; and (3) rapid clearance leading to no observable liver, kidney or hematological toxicity. He concluded his pitch by underlining the promise of this bioconjugation platform in difficult solid tumors.

Dr. Mathieu Cinier (Affilogic, Nantes, France) followed with his talk on Nanofitins® as tunable targeting agents for both imaging and therapy. Nanofitins® are very small binding proteins designed for therapeutic application with low predicted immunogenicity, as well as good tissue and tumor penetration. Their fast clearance profile can be easily modulated to match the requirements of their application *via* a proprietary half-life extension domain. Nanofitins® are naturally highly stable to temperature (Tm > 70 °C) and to various chemical conditions such as high and low pH (0–12). All these different features make them attractive bioconjugate formats. Protein engineering, genetic fusion or chemical reactions can be performed at either the *C*-terminus or *N*-terminus, giving the opportunity to pair Nanofitins® with radioelements, toxins, enzymes, nanoparticles, mAbs or half-life extension modules. A case study with anti-epidermal growth factor receptor (EGFR) Nanofitins® was presented with demonstration of their selectivity for both murine and human EGFR (affinities in the nM range). Internalizing Nanofitins® were identified using a truncated portion of *Pseudomonas* exotoxin A (Pe38) toxin-based assay. Upon 18F labelling, an anti-EGFR Nanofitin® allowed fast and high contrast imaging of tumors *in vivo* with no accumulation in healthy organs.[14,15]

The next speaker, **Mr. Bruno Tillier** (Synthelis, La Tronche, France), introduced a cell-free protein expression technology. Proteins are expressed from the cellular extract of any cell line after DNA matrix and reagent enrichment: prokaryotic and eukaryotic, and even mammalian in order to conserve post-translational modifications. The possibility of recovering disulfide bonds upon production made it possible to use this technology for antigen, functional antibody and antibody fragment production, but also for the incorporation of unnatural amino-acids to allow more defined DAR in future ADCs.[16] Mr. Tillier presented their unique cell-free expression platform based on protein-presenting proteoliposomes. By presenting antigens for phage display at the surface of these proteoliposomes, they were able to provide a new mAb selection platform, exemplified with the successful selection of an anti-C-X-C chemokine receptor type 4 (CXCR4) nanobody, as well as new immunization reagents used for the development of an anti-CXCR4 antibody. He emphasized the speed, robustness, scalability and adaptability of this process for various applications in the antibody and ADC field.

Dr. Jonathan Sjögren (Genovis AB, Lund, Sweden) then presented GlyCLICK^{TM}, a technology that combines two enzymatic approaches and click chemistry, without any prior mAb engineering required. The first step consists of antibody deglycosylation using GlycINATOR, an endo-β-N-acetylglucosaminidase. Interestingly, this enzyme requires the native IgG form and is able to deglycosylate IgG1–4 from several species.[17] The second step used β-1,4-galactosyltransferase I, GalT (Y289L) licensed from Thermo Fisher, which adds a modified galactose residue, UDP-GalNaz containing an azide group. This azido moiety is then used for conjugation with an alkyne label by click chemistry.[18] Currently, this technology allows the conjugation of dyes, affinity tags, metal chelators, or any alkyne-carrying conjugate of choice using the site-specific azide activation of the antibody. The obtained conjugated antibodies had site-specific conjugation of the Fc, with a DAR of 2.0, with no reduced antigen binding. The applications include *in vivo* imaging, as ^{89}Zr-DFO-trastuzumab is used in positron emission tomography scanning or as a tool for site-specific ADCs development. A question was asked about making DAR 4 conjugates, which is possible by conjugating two drugs per glycan.

Next, **Dr. David Bonnafous** (McSAF, Tours, France) made a brief presentation of the activities of the new CRO McSAF, which specializes in bio-organic chemistry and bioconjugation. He presented their development of chemical tools for bioconjugation based on proprietary disulfide rebridging heterocyclic bioconjugation scaffold from the expertise of IMT lab (GICC, CNRS UMR 7292, Université de Tours).[19] Using this technology, site-specific conjugation and controlled DARs can be achieved on a wide range of native mAbs with increased homogeneity of the final ADC compared to currently FDA-approved ADC products. He concluded with an overview of all the ADC projects currently ongoing, for fields inside and beyond oncology, accessible with this technology within their research and development portfolio.

Dr. Jean Viallet (Inovotion, La Tronche, France) then introduced their new *in vivo* pre-screening in chick embryos for the evaluation of a large range of molecules, including mAbs and ADCs, in addition to treatment types with synergistic or sequential effects. They developed a new grafting technology of classical human tumor cells onto the chorioallantoic membrane (CAM) of chick embryos. This grafting determines the efficacy of the compound through quantification of tumor weight after dissection, and the extent of metastatic invasion after real-time polymerase chain reaction (qPCR) analysis of the low CAM to detect nodules. In addition, some insights about future toxicity effects can be gained by rating embryo death and checking body abnormalities. Histological comparisons of several tumors confirmed the similarity between tumor growth in this technology and in mice. Finally, an ADC study gave the same results in this pre-*in vivo* model as in mice.[20] He reminded the audience that this assay can be done in one month with low quantities of compounds, high reliability and no need for an ethical committee, while also being affordable. This makes it a perfectly sensible assay to eliminate low value molecules before going to mice.

As discussed by **Dr. Severine Giltaire** (ImmunXperts SA, Charleroi, Belgium), toxicity can be driven by immunogenicity and immunotoxicity, hence increasing the need for early evaluation of these factors. She emphasized the problem of clinical adverse events caused by an immune response to a treatment. Many factors are implicated, but some are specific to ADCs, including the risk of epitope spreading, its hapten-like structure, and its modification with a linker-payload. Dr. Giltaire's company has developed techniques at several levels to assess this unwanted immunogenicity in drug candidates. She focused on *in vitro* assays either on peripheral blood mononuclear cell (PBMC) for late memory responses by analysis of proliferation, or whole blood, PBMC, and dendritic cells for early innate responses by analysis of cytokines signatures. The impact of aggregation of nivolumab and trastuzumab on immunogenicity was demonstrated by the increase of IL-8 and MCP-1 production. Their PBMC proliferation assays allowed them to rank the immunogenicity potential of several candidates. The combination of both of these gives opportunities for safety profile engineering, candidate selection, analysis of specific population subset responses, understanding of immunological mechanisms, and biosimilar comparison.

Dr. Oréda Boussadia (EpiVax Inc, Clifford St Providence, USA) presented an *in silico* protein screening and optimization platform: Interactive Screening and Protein Reengineering Interface (ISPRI). This platform enables the immunogenicity prediction of biologics, such as ADCs, and identifies individual T cell epitopes and epitope clusters that could lead to anti-drug antibodies (ADA) production. ISPRI analyzes amino acid sequences and scores the immunogenicity, predicting for both class I and II human leukocyte antigen (HLA) binding, with a high degree of correlation.[21] Contrary to other immunogenicity prediction tools, the contribution of regulatory T cell epitopes is also included in ISPRI analysis. With potential T cell epitopes identified, ISPRI is also capable of proposing strategies to deimmunize the biologics by suggesting amino acid residue changes at various positions in the sequence. Information provided by ISPRI facilitates the movement of ADCs to the clinic with improved prospects and reduced risks.

Next, **Dr. Serge Desmoulins** (Agilent Technologies, France) described DAR determination of ADCs in serum by a sample preparation platform. Current techniques for protein

quantification in biological matrices are: (1) ligand-binding assays, applicable for antibodies and ADCs but without DAR follow-up, (2) MS-based techniques for peptide quantification, applicable for antibodies and ADCs but without DAR follow-up, and (3) MS-based techniques for intact protein, applicable for antibodies, ADCs quantification and DAR follow-up for pharmacokinetic studies. In MS, the intact protein approach is the only technique applicable to DAR follow-up. However, mAb quantitation using liquid chromatography-mass spectroscopy (LC/MS) is a challenge because of issues including specificity, sensitivity, and challenges in sample preparation. An analysis technique for quantitation of an intact ADC (ado-trastuzumab emtansine) in rat serum was then presented. The first step is an immune-capture with an AssayMAP Bravo, which consists of capturing the ADC from the serum on an immobilized antigen. Then, the captured ADC is deglycosylated with PNGase F, and eluted. Afterwards, LC/quadrupole time-of-flight (Q-TOF) analysis is carried out. Serial dilutions of the ADC in rat serum allowed the acquisition of quantitation curve for deglycosylated ADC. Then, the LC/Q-TOF allowed the DAR determination. After questions, Dr. Desmoulins explained that this technology is still relevant for ADCs with site-specific conjugation in order to check the ADC functionality.

To conclude the first day, **Dr. Jean-Marc Joumier** (Easy-Chelators, Brest, France) presented a new tool to produce antibody-radionuclide conjugates (ARC) for cancer diagnostics and imaging. Classically used 1,4,7,10-tetratazacyclododecane-1,4,7,10- tetraacetic acid (DOTA) chelators are not sufficiently stable, and after only 2 h post injection in mice, the copper is completely free from its DOTA cage. To overcome this drawback, they developed new C-functionalized chelators bearing carboxylic moieties that can be bridged to enhance their stability towards metal chelation.[22] These display high complexation rates, enhanced thermodynamic stabilities, good selectivity between Cu and Zn, and biological as well as acidic inertia, while retaining all the properties of both the vector and the chelator. He finally reiterated the opportunities these tools afford for the localization and monitoring of new vectors in process development.

Session III: Accelerate the development of the next-generation ADCs

The third session discussed new site-specific conjugations and payloads, and was chaired by **Dr. Alain Beck** (Centre d'Immunologie de Pierre Fabre, Saint Julien-en-Genevois, France).

The session began with **Dr. Saïd El Alaoui** (Covalab, Villeurbanne, France) who presented a new bacterial transglutaminase Q-tag substrate for site-specific ADC conjugation.[23] Transglutaminase catalyzes the formation of a covalent bond between a free amino group and the carboxamide group of a protein or peptide glutamine. The best microbial transglutaminase (TGm) substrates were identified by screening different glutamine-containing peptides. These sequences were then C-terminally fused with each heavy chain of an anti-HER2 antibody, and enzymatic conjugation was performed with an amine-containing payload: AlexaFluor®488-cadaverine. No difference in ADC antigen binding was observed by enzyme-linked immunosorbent assay (ELISA) and Biacore. With the anti-HER2 antibody, the conjugation was better with: (1) the optimum glutamine donor substrate (Q-tag1) than with known peptide substrates like LLQG, because of the substrate enhanced affinity, and (2) a mutated antibody presenting lysine depletion, which avoids non-specific conjugation. Conjugations made in this way are homogeneous and reproducible.

Dr. El Alaoui then showed that the sequence environment, the conformation of the antibody, and the type of spacer can influence the conjugation, as illustrated with the improved conjugation of AlexaFluor®488-cadaverine when the Q-tag1 was C-terminally present in the heavy chain compared to light chain. Payload optimization was made with the development of linkers bearing drugs such as MMAE, DM1 or DM4. A DAR 2 was obtained with an AlexaFluor®488-cadaverine payload, the MMAE payload was grafted on native anti-HER2 antibody with a DAR of 1.5, whereas the MMAE payload on a lysine-depleted anti-HER2 antibody afforded a DAR of 1.74. *In vitro* toxicity was evaluated and the lysine-deleted MMAE-containing ADC showed potent cytotoxicity on the SK-BR-3 cell line, in the same range as free MMAE. However, it was not better than ado-trastuzumab emtansine (DAR 3.5), and this may be explained by the difference in DAR. In mice, the MMAE anti-HER2 ADC was removed from the blood by the liver after 24 h. Finally, a pharmacokinetic study showed that this ADC was stable in mice, and *in vivo* cytotoxicity studies are in progress. A question was asked about the scale-up of enzymatic conjugation reaction. Dr. El Alaoui answered they are currently evaluating this parameter as the TGm is highly sensitive to changes, and the use of a mutated enzyme may be required.

Continuing in this theme, **Dr. François Romagné** (MI-mAbs, Marseille, France) presented another transglutaminase site-directed coupling technology. Dr. Romagné introduced the importance of obtaining homogeneous ADCs with regards to the DAR. TGm is one way to obtain this homogeneity. The described method requires an aglycosylated antibody obtained either by enzymatic deglycosylation, by N297S mutation to produce a DAR 2, or by N297Q mutation to obtain a DAR 4. The one-step approach for antibody conjugation consisted of directly adding the drug on a primary amine-containing linker to the mAb with TGm. However, the two-step approach consisted of the conjugation, by TGm, of a primary amine-containing linker with a reactive moiety, which permits crosslinking of the drug in the second step. The one-step approach with MMAE led to a DAR 3.7. The two-step approach led to a DAR 4 without intermediate species, with an azide spacer and a dibenzocyclooctyne-derivatized MMAE using fewer drug equivalents and time than the one-step approach.[24] The two-step approach allowed conjugation of hydrophobic payloads, as illustrated with ADC-PBD synthesis. Moreover, the intermediate species is stable, making that method versatile and adaptable to different toxins and triggers. ADCs obtained this way showed good *ex vivo* stability in the plasma of several species. An ADC based on an anti-CD30, cAC10q, with a DAR 4 was compared to Adcetris®. Both ADCs were stable in rat in regards to both DAR and total antibody concentration. Moreover, the TGm-ADC showed better clearance profile than Adcetris®, which was cleared twice as fast as TGm-ADC. The latter had better tumor uptake with a favorable tumor-to-organ ratio and limited off-target accumulation. Dr. Romagné

described the results of a xenograft model in severe combined immunodeficiency (SCID) mice, in which animals were treated with a single iv dose of either 0.3 or 1 mg/kg ADCs. At the highest dose, Adcetris® and TGm-ADC showed similar tumor regression. Nevertheless, TGm-ADC was a little more potent with regards to tumor volume regression at 0.3 mg/kg. Interestingly, TGm-ADC was better tolerated in rat with a maximum tolerated dose greater than 60 mg/kg, compared to 20 mg/kg for Adcetris®. On another hand, TGm-ADC showed better efficacy with other antibodies and drugs than ADCs obtained by lysine or unpaired cysteine conjugation. To conclude, this technology led to the development of MI-mAbs that permit *in vitro* and *in vivo* evaluation to allow the best mAb and toxin selection with regards to a given target.

Following this, **Prof. Dr. Andreas Pahl** (Heidelberg Pharma, Ladenburg, Germany) presented antibody-targeted amanitin conjugates (ATACs) currently under development at Heidelberg Pharma. ATACs are based on a new payload, amanitin, isolated from mushroom, which inhibits ribonucleic acid (RNA) polymerase II. Due to its hydrophilicity, amanitin does not display a bystander killing effect, which is an advantage with regards to safety concerns according to Prof. Dr. Pahl. Moreover, ATACs presented a pM EC_{50} on murine non-dividing embryonic stem cells, confirming their activity on quiescent cells and making amanitin a payload of choice. Solid-phase peptide synthesis of this hydrophilic bicyclic octopeptide gave access to gram-quantity of GMP-quality product. He first presented a proof of concept for ATAC using a random lysine bioconjugation on trastuzumab. *In vitro* results showed low pM activities on high-expressing cell lines and a low nM one on a trastuzumab-resistant cell line (JIMT-1). *In vivo* results on JIMT-1 xenograft model demonstrated the superiority of the trastuzumab-based ATAC compared to Kadcyla® and this was confirmed in a Kadcyla®-resistant PDX model, where a complete remission was observed after a single dose of ATAC. Activity was also demonstrated on a heterogeneous model even if there was no direct bystander killing effect of amanitin. A study to understand these mechanism of action is currently ongoing. The proof of concept complete, they moved on to site-specific homogeneous ATACs with the ThioMab™ technology. After a screening, engineered cysteines were incorporated at positions involved in FcγR binding to reduce toxicity. Site-specific DAR 2.0 ATAC showed a pM *in vitro* activity similar to random DAR 4.9 ATAC.

Prof. Dr. Pahl then presented HDP-101, their clinical candidate, targeting B-cell maturation antigen (BCMA) in multiple myeloma. It was active in the pM range on different BCMA-expressing cell lines. *In vivo* experiments using a luciferase transfected cell line (MM1.S-Luc) showed a 2-month remission after a single dose of 0.1 mg/kg and a 3- to 4-month remission at a single dose of 1 mg/kg. Toxicity studies on monkey ruled out liver toxicity (alanine aminotransferase levels) and showed a transient increase, but no accumulation, of lactate dehydrogenase (LDH). In addition to these results, Prof. Dr. Pahl presented the identification of tumor protein 53 (TP53) deletion as a biomarker for the use of ATAC in therapy. Indeed, they demonstrated that a diminution of target's copy number correlated with an increase of the activity, hence improving the therapeutic index and helping the choice of cohorts for clinical evaluation.[25]

Continuing on the topic of new payload development, **Dr. Marie-Priscille Brun** (Sanofi, Vitry-sur-Seine, France) presented Sanofi's work on new tubulin binders named cryptophycins. Derived from C1, the first cryptophycin isolated from cyanobacteria, the C52 macrocyclic depsipeptide displayed better *in vitro* potency compared to classical tubulin binders such as DM4 or MMAE. Several ADCs with cleavable and non-cleavable linkers were produced through lysine technology.[26] *In vitro* potency of ADCs including a cleavable Val-Cit linker were 10-fold more potent than the reference ADCs on the market. *In vivo* efficacy in xenograft and PDX models with a single dose administration of 3 mg/kg translated into complete remission. However, pharmacokinetic studies led to the observation of a macrocycle metabolization without deconjugation in mice, but not in cynomolgus monkeys. Tandem MS analysis and *in vivo* metabolite identification led to the elucidation of the metabolization pathway. Chemical optimization of C52, through conversion of ester to amide and tuning of steric hindrance on sensitive sites, solved this issue while preserving potency. Second-generation cryptophycin ADCs were stable towards metabolization in mice, and maintained a pM potency *in vitro* with several new payloads. In a MDA-MB-231 xenograft model, tumors were eradicated at low dose and ADCs showed a promising therapeutic index, warranting further development of this cytotoxic payload for an ADC approach.

Session IV: Challenges in ADC developability, scale-up and manufacturing

Chaired by **Dr. François Romagné** (MI-mAbs, Marseille, France), session 4 focused on the challenges associated with ADC developability, scale-up and manufacturing.

The first speaker was **Dr. Alain Beck**, who gave a keynote presentation about multilevel, state-of-the-art analytical methods for ADC structural assessment. Second- and third-generation ADCs were obtained *via* exploitation of new properties associated with a better knowledge of structure and the related effects on function and safety. Dr. Beck first discussed the detailed characterization of antibody structure. All possible macro- and micro-variants of IgGs were published by the FDA.[27] Different analytical technologies allowed assessment of the primary structure and micro-variants of mAbs, as intact or IdeS-digested and reduced structures, by 2D-LC-MS, native MS, ion-mobility, capillary electrophoresis (CE)-MS, top-down sequencing, or hydrogen-deuterium exchange (HDX)-MS. IdeS digestion is currently the best compromise for getting information at the middle level. For illustration, the *N*-glycan profiling of cetuximab, on Fc and $F(ab')_2$ was resolved quantitatively and qualitatively. IgG charge variants, representing dozens of variants, needed to be resolved based on cationic exchange chromatography, to discriminate critical variants from others. One improvement is 2D-LC-MS technology, which comprises in one dimension the cationic exchange chromatography, and a reverse phase (RP) LC to remove salt and further analyze each peak. RPLC is classically used; however, a new orthogonal method, hydrophilic liquid interaction chromatography, permits full resolution of the Fc by inversion of the peak elution order. The second part of the talk discussed the ADC multilevel structural analysis. Indeed, a lysine-conjugated ADC presents 7

main isoforms, which could correspond to around 4 million regio-isomers. ADCs could be analyzed at different structural levels: native ADCs by native MS, hydrophobic interaction chromatography (HIC) and size-exclusion chromatography (SEC), at the middle scale, by IdeS cleavage and reduction and also by denaturing RP-HPLC and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). At the bottom-up scale, ADCs are analyzed by peptide mapping. Moreover, small molecular drugs are analyzed by LC-MS, LC-MS/MS, giving insight into deconjugation and metabolism.

Native MS, ion mobility-MS (IM-MS) or HDX-MS can be used to characterize higher order ADCs. Native MS permitted unambiguous structural assessment of PBD-based ADCs, with DAR and drug loading distribution. Dr. Beck then discussed details of IM-MS, which gives information about both the mass and the molecule shape, and is useful to assess drug loading and glycoforms. He used a 2D-LC system with HIC as the first dimension, and RPLC-MS as the second, allowing direct information retrieval regarding ADC structure, degradation products, and DAR. In the case of bioanalysis, this technique is useful for ADC serum stability assessment after an immune-precipitation step and reduction. Alternatively, another technique to resolve the structural characterization of ADCs is CE-MS on native ADC or after IdeS digestion, which gives information about the light chain, Fc and Fabs. CE-electrospray ionization (ESI)-MS/MS, on the other hand, can be used for peptide mapping and drug localization. Studies on the third-generation molecules were done by native MS/IM-MS, which showed a higher homogeneity than brentuximab vedotin and ado-trastuzumab emtansine. The challenge was also to evaluate residual levels of small molecular drugs. The sensitive 2D-LC/MS can be used with a solid phase extraction in the first dimension to remove all antibodies and ADCs, and then a RPLC in the second dimension. Dr. Beck concluded by underlining the importance of antibody and ADC characterization at multi-level of structure at a very early stage of development.

Translational skills are essential for successful development of an ADC, and **Mr. Régis Lelou** (Lonza, Basel, Switzerland) highlighted the different steps to follow to achieve success. From his point of view, it is very important that the contract manufacturing organization (CMO) partner already has experience in lab to clinic translation for a wide range of technologies and payloads. For a CMO, the first step is to reproduce, in mL scale reactors, the reaction and adapt parameters if necessary. Then, the process development can begin with a focus on quality and manufacturability of bioconjugation and purification steps. Finally, fine tuning of the process is needed to assure the fit between the developed process and the manufacturing plan. Throughout these steps, a strong analytical platform is necessary in order to control the process and the final product. Mr. Lelou noted that the current trend is towards single-use technology, which increases adaptability and decreases the need for cleaning. Nonetheless, one must verify that the single-use items are compatible with the conditions used to manufacture the ADC.

Dr. Eric Devic (GTP Technology, Labège, France) and **Dr. François D'Hooge** (Novasep, Le Mans, France) then discussed the need for good symbiotic collaboration between R&D and manufacturing. Dr. Devic started with the presentation of a partnership to produce vaccines, but also several IgG or engineered mAbs. The optimization of transfer procedures between the two partners led to a decrease in the time needed to deliver the drug product, and enhanced flexibility in term of time, services and budget. Then, Dr. D'Hooge underlined the many challenges faced within ADC manufacturing. He reiterated that CMOs aim to simplify these for the customer by providing development and manufacturing of the ADC parts like mAbs, cGMP toxins, and linker production, as well as offering bioconjugation scaling facilities. The combination of R&D, production, quality control, and quality assurance units provides a favorable environment for technology transfer. He confirmed the necessity of having a broad analytical platform that includes chromatography, high resolution MS, capillary electrophoresis, and aggregate analysis to achieve controlled processes and products.

The last speaker of session 4 was **Dr. Klaus Kaiser** (Bayer, Wuppertal, Germany) who presented how to accelerate ADC development. Small molecules have traditionally had low success rates in moving from Phase 1 to launch. In contrast, mAbs may have success rates in the range of 20–25% (unpublished data, The Antibody Society). This success rate pattern could be the same for ADCs. For Dr. Kaiser the main point was to have a very good target in order to transform a small molecule into a precise weapon. As an example, mesothelin, a 40 kDa cell surface glycoprotein with unknown function is overexpressed in ovarian, pancreatic cancer as well as mesothelioma. An ADC was developed, composed of anetumab (an anti-mesothelin antibody) and ravtansine (DM4), a microtubule-affecting maytansine, conjugated *via* a cleavable *N*-succinimidyl-4-(2-pyridyldithio)butanoate linker. This ADC presented an average DAR of 3. *In vitro*, its cytotoxicity depended on tumor cell mesothelin expression. Furthermore, in a xenograft model of mesothelioma, anetumab ravtansine (10 mg/kg and 2.5 mg/kg) showed a better effect on tumor volume than the classical treatments cisplatin (3 mg/kg) and pemetrexed (100 mg/kg).[28] Dose escalation studies led to a final maximum tolerated dose of 6.5 mg/kg, and showed promising results in patients with mesothelioma with regards to the tumor size and response. Bayer organized a manufacturing platform as an integrated approach for antibody production and immunoconjugation at their Wuppertal site, to ensure capacities for clinical supplies, production of highly potent substances, and final ADC production. Key factors to take into account are: (1) minimizing toxic waste, (2) minimizing risks factors associated with highly toxic compounds, using glove boxes and "ready-to-use" disposable pieces of equipment, and (3) lowering capital investment. To conclude, all process steps for clinical manufacturing of an ADC were developed and fully integrated in a short timeframe. A comprehensive set of predictive scale-down models were developed as early as possible.

Session V: Moving ADCs forward into the clinic: safety and efficacy

The last session of this meeting dealt with safety and efficacy concerns related to the ADC field. It was chaired by **Dr. François Lefoulon** (Servier, Orléans, France).

The first speaker was **Dr. Philip Howard** (Spirogen, London, UK). He presented the latest update on PBD dimer warheads, which present a new mechanism of action. Indeed, they bind to the DNA without distorting it, avoiding resistance mediated by protein repair mechanisms, especially in cancer cells. Hence, ADCs loaded with PBD dimers (APCs) are active on cisplatin-resistant cell lines. Moreover, the flexibility of the PBD dimer platform makes it possible to modulate potency and physico-chemical properties by changing the site of linker fixation (C-2, C-7 or N-10) or the nature of the link to form the dimer. Different PBD dimers-containing linkers are available, and the first presented (talirine) was conjugated to trastuzumab to induce a complete tumor remission on a BT-474 model, outperforming Kadcyla® and equivalent ADCs conjugated with auristatins E and F at 1 mg/kg.[29] However, talirine possesses a high logP and a low maximum tolerated dose which led to development of tesirine, a PBD-containing linker with a lighter aromatic scaffold and a similar single digit pM activity. Moreover, functionalization of the PBD dimer at the N-10 position with a cleavable Val-Ala linker hindered its activity until enzymatic release, making tesirine a formal prodrug and increasing its maximum tolerated dose. Tesirine conjugated to the anti-human delta-like protein 3 (DLL3) rovalpituzumab induced a complete remission in a small cell lung cancer PDX model and eradicated stem cells compared to other treatment. Results from a Phase 1 study were very promising, with a 75% clinical benefit rate, and this APC is currently undergoing evaluation in several Phase 3 studies of small cell lung cancer patients.

Another application of PBD dimers with cleavable linker concerns non-Hodgkin lymphomas. A talirine derivative, containing the Val-Ala dipeptide, was conjugated to an engineered anti-CD70 antibody.[30] Complete tumor remission was achieved with a dose of 1 mg/kg on a multi-drug resistant model (HEL92.1.7) and 3 mg/kg on a Thomsen-Friedenreich epitope (TF1-α) model, whereas Mylotarg® had no activity. Dr. Howard then presented some non-cleavable linkers accessible thanks to the synthetic flexibility of PBD dimers. Buchwald, Huisgen or Sonogashira reactions were conducted to afford new payloads. Their evaluation demonstrated the advantage of introducing an alkyne when binding to DNA. A trastuzumab conjugate bearing the non-cleavable alkyne PBD dimer was evaluated on the SK-BR-3 cell line *in vitro* and did not display a bystander effect, whereas the tesirine version does. A cleavable alkyne linker did not improve the efficacy *in vivo* on NCI-N87 model but increased toxicity. Altogether, he demonstrated the interest of PBD dimers as warheads for ADCs, for liquid and solid tumors, with APCs currently in Phase 3 studies. Once again, he demonstrated that a good combination of antibody, linker technology, and payload is essential for good efficacy and tolerability.

Dr. Charles Dumontet presented the mechanisms of resistance to ADC. He reminded the audience that resistance is a lack of clinical response, graduated with different levels of disease development: complete response, partial response, progressive disease, and stable disease. Moreover, even in the case of a partial response, there are still cells resistant to chemotherapy responsible for a relapse, which explains the disappointing overall survival rates for patients receiving Adcetris® and Kadcyla®. He pointed out the differences of chemoresistance between resistant cell lines obtained by continuous exposure to the molecule or by chemical alteration. Resistant cells are obtained *in vitro* either by continuous increased concentration or by pulsatile concentrations of ADC, whereas *in vivo* resistant models are obtained by exposure of the tumor-bearing mouse to suboptimal doses of ADCs. Once the tumor size increased, it is implanted in naïve mice, until it becomes resistant to treatment. The most explored in such a model is the mechanism of resistance to ado-trastuzumab emtansine. Indeed, resistance takes place at each step: target binding, internalization, lysosomal degradation, release of Lys-maleimidomethylcyclohexane-1-carboxylate (MCC)-DM1, tubulin binding and dynamics, induction of apoptosis and ADCC. For example, trastuzumab and Kadcyla® encounter resistance linked to antibody/antigen interaction.[31] Resistance mechanisms are linked to inactive intracellular concentrations of conjugates, below the efficient antitumor concentration. Inhibition of the ABC transporter family or activation of lysosome to cytosol transporters can overcome resistance to certain payloads.

To conclude, Dr. Dumontet presented the development of *in vitro* resistance models to ado-trastuzumab emtansine on esophageal adenocarcinoma HER2-positive cells (OE-19 cell line). A multi-drug resistance gene mutation (MDR-1) inhibitor, cyclosporine A (CspA), was used on one cell line to avoid P-glycoprotein (a member of ABC family) overexpression. Both cell lines (with and without CspA) developed resistance to ado-trastuzumab emtansine, with no alteration of the antigen or its accessibility to the ADC. Moreover, there was no alteration of ABC transporter activity and expression, and no development of resistance to other anticancer agents. These resistant models present an unusual aspect of non-reduced antigen expression and no ABC overexpression, with a new aspect of altered cell adhesion and sensitivity to prostaglandin. These cells models are of interest in order to understand resistance mechanisms and evaluate novel non-cross-resistant agents, even if it is still important to test clinically relevant samples. The main issue is the access to biological samples before and after treatment to understand why a patient does not respond.

Prof. Gilles Paintaud (GICC UMR7292 CNRS, Labex MabImprove Tours, France) presented an overview of the data collected from the few ADCs that have completed late-stage clinical trials.[32] He first presented data concerning progressive deconjugation of Kadcyla® over time and the difference in pharmacokinetics between Kadcyla® (half-life of 3.5 days) and Herceptin® (half-life of 12.5 days). A study differentiating the species in Kadcyla® demonstrated the quantitative influence of individual factors of pharmacokinetic variability such as weight, albumin, aspartate aminotransferase (AST) or tumor burden, and the impact of DAR, showing that high DAR species had lower half-lives.[33] Meta-analysis proved that a differentiating quantification of species was not necessary and that measuring Kadcyla® (as a total) residual concentration gave a significant factor for survival. Meta-analysis also confirmed the increase in PFS and overall survival, as well as the decrease of grade 3 or higher side effects upon Kadcyla® treatment, even though it affected platelet proliferation and induced hepatic toxicity. Safety concerns also arose from Adcetris® clinical trials and were not predicted from the preclinical toxicity studies in monkeys or rats. Even though this ADC obtained good

results in Phase 2, there was no difference in overall survival in Phase 3.[34] The last ADC he addressed was Besponsa®, which showed complete remission in 80% of cases, compared to 30% for the standard of care, but no improvement of overall survival. Patients were more prone to stem cell transplantation but the same safety concern as for Mylotarg® emerged, with hepatobiliary toxicity and several deaths due to treatment.[35] He concluded by reminding the audience that ADC clinical trials are done after several chemotherapy lines, on patients with advanced diseases, and that it is very important to keep in mind the benefit-over-risk ratio. He insisted on the importance of making well-designed clinical trials. Finally, he finished his talk by suggesting an individual dose optimization considering the influence of ADC concentrations on efficacy and safety.

The last presentation was **Dr. Olivier Marcq** (Agensys, Santa Monica California, USA) who presented the importance of ADC process development to control safety-related Critical Quality Attributes (CQAs). The next generation of ADCs is being developed in order to increase the therapeutic index, and he focused on means to maintain the widest possible therapeutic index through good development. The toxicity is linked to the therapeutic index through e.g. the antibody design and target selection. In order to increase the latter, FcγR and FcRn interactions, and antigen expression on normal cells, needs to be minimal. Moreover, linker design and conjugation site selection defines the stability and mode of release (off- and on-target toxicities) and has a direct impact on overall toxicity. On the other hand, payload-driven toxicity, with a specific mode of action and eventual premature release or recirculation, is also important. Formulation composition, purity and aggregation all contribute to increased ADC toxicity.

Dr. Marcq then illustrated the difficulties of maintaining the ideal ADC characteristics during the development using loaded species distribution (monitored, for instance, by HIC). Indeed, initially, the target ADC is an ideal ADC made of a single species (i.e., all molecules with the same DAR) or, more realistically, a species with a very narrow distribution range, due to process variability or lack of selectivity of the conjugation reaction. The aim of process development is to consistently produce an ADC not too far from the ideal HIC distribution across scales. A drift could lead to a modification of the therapeutic index. To ensure the widest possible index, efficient clinical development is required regarding dose and regimen optimization, target population identification and selection, as well as disease stage (maximum tolerated dose and minimum effective dose determination). A controlled process is necessary to deliver consistent quality, and establish release specifications, based on process capability, which are narrow enough that the therapeutic index is maintained across clinical stages. This is achieved with rigorous process development, analytical characterization and formulation development. Dr. Marcq illustrated his points with the examples of an MMAE unpaired cysteine IgG1 ADC that presented different maximum tolerated doses due to the DAR species present,[36] and a MMAE-bearing ADC with a pegylated glucuronide linker and DAR 8 that showed efficacy and good tolerance *in vivo*, demonstrating the impact of conjugation technology.[37] The FDA is now requiring monitoring of DAR distribution in addition to average DAR in the authorization process. DAR distribution and the structural determination of DAR 4 classical cysteine ADC (DAR 4A: heavy-light chain or DAR 4B: heavy-heavy conjugates) underlined the impact of the difference of structure on the therapeutic index in animal models, as well as the potential impact of the control of process parameters.[38] Aggregates and DAR distribution challenges can be overcome by controlling conjugation process parameters, purification and formulation. As a process development expert, Dr. Marcq's advice was to invest early in critical process development to understand and optimize your ADC and to have the best knowledge and control of the product for clinical trials.

Outsourcing roundtable

Dr. Florence Lhospice (Innate Pharma, Marseille, France) chaired a roundtable composed of **Dr. Arnaud Martin** (Sanofi, Paris, France), **Régis Lelou** (Lonza, Basel, Switzerland) and **Christophe Aussourd** (Servier, Paris, France) about the key features of selecting a good CMO. They underlined the difficulty of finding the right balance between flexibility and GMP requirement. The critical aspect that emerged from this roundtable was communication. Indeed, all participants agreed on the importance of having face-to-face meetings to deeply understand technological challenges, and customer needs, but also to set intellectual property and regulatory considerations. They insisted on the time schedule, saying that it should be done very early on the project to prevent any delay in the development.

Conclusion

This successful 5th edition of the conference presented the latest advancements in ADC technologies, including new payloads, new targets, new bioconjugation tools and analysis platforms. The complete overview of research and development, from the very start to GMP production, gave attendees (industrial and academic) the keys to understanding the different steps involved in making an ADC program successful. **Pr. Hervé Watier** (GICC CNRS UMR 7292, CHU Tours, LabEx MAbImprove, Tours, France), **Dr. André Pèlegrin** (IRCM, LabEx MAbImprove, Montpellier, France) and **Priscilla Domaingue** (communication manager, MabDesign, Lyon, France) closed the meeting with the announcement of the next Antibody Industrial Symposium, "Targets for mAbs", which will be held in Montpellier in 2018.

Abbreviations

ADC	Antibody-drug conjugate
ADCC	antibody-dependent cell-mediated cytotoxicity
APC	antibody PBD conjugate
ATAC	antibody targeted amanitin conjugates BCMA, B-cell maturation antigen
CAM	chorioallantoic membrane
CD	cluster of differentiation
CE	capillary electrophoresis
CEA	carcinoembryonic antigen
CMO	contract manufacturing organization

CspA	cyclosporine A
CXCR4	C-X-C chemokine receptor type 4
DAR	drug-to-antibody ratio
DNA	deoxyribonucleic acid
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-acetic acid
EC_{50}	half maximal effective concentration
EGFR	epidermal growth factor receptor
F(ab')$_2$	fragment antigen binding
Fc	fragment crystallizable
FcγR	gamma Fc receptor
FcRn	neonatal Fc receptor
FDA	Food and Drug Administration
FDC	fragment drug conjugate
GMP	Good Manufacturing Practices
HDC	Humabody® drug conjugate
HDX	hydrogen-deuterium exchange
HER	human epidermal growth factor receptor
HIC	hydrophobic interaction chromatography
HPLC	high performance liquid chromatography
IgG	immunoglobulin G
IHC	immunohistochemistry
IL	interleukin
IM	ion mobility
LC	liquid chromatography
mAb	monoclonal antibody
MMAE	monomethylauristatin E
MS	mass spectrometry
NK	natural killer
PBD	pyrrolobenzodiazepine
PBMC	peripheral blood mononuclear Cell
PD-1	programmed cell death 1
PDX	patient-derived xenograft
PFS	progression free survival
PSMA	prostate specific membrane antigen
qPCR	real-time polymerase chain reaction
Q-TOF	quadrupole time-of-flight
RP	reverse phase
SA	serum albumin
scFv	single chain variable fragment
SMAC	sortase-mediated antibody conjugation
TCR	tissue-cross reaction
TGm	microbial transglutaminase

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