MABS
2023, VOL. 15, NO. 1, 2211692
https://doi.org/10.1080/19420862.2023.2211692

Taylor & Francis
Taylor & Francis Group

MEETING REPORT

10th antibody industrial symposium: new developments in antibody and adoptive cell therapies

Ana Antunes[a], Luis Alvarez-Vallina[b,c], Federico Bertoglio[d], Nicolas Bouquin[e], Stéphanie Cornen[f], Francis Duffieux[g], Pierre Ferré[h], Raphaëlle Gillet[e], Christian Jorgensen[i,j], Mark B Leick[k], Bernard Maillère[l], Hélène Negre[m], Mireia Pelegrin[n], Nicolas Poirier[o], Dietmar Reusch[p], Bruno Robert[q], Guy Serre[r], Alain Vicari[s], Martin Villalba[n], Christoph Volpers[t], Gavin Vuddamalay[a], Hervé Watier[u], Thierry Wurch[v], Lennart Zabeau[w], Stefan Zielonka[x], Baolin Zhang[y], Alain Beck[z*], and Pierre Martineau[q*]

[a]MabDesign, Lyon, France; [b]Cancer Immunotherapy Unit (UNICA), Department of Immunology, Hospital Universitario 12 de Octubre, Madrid, Spain; [c]H120-CNIO Cancer Immunotherapy Clinical Research Unit, Spanish National Cancer Centre (CNIO), Madrid, Spain; [d]Technische Universität Braunschweig, Institute of Biochemistry, Biotechnology and Bioinformatics, Department of Biotechnology, Braunschweig, Germany, Current address; [e]REGIMBEAU, Paris – 69006 Lyon, France; [f]Innate Pharma, Marseille, France; [g]Large Molecules Research, Sanofi, Vitry-Sur-Seine, France; [h]Compugen Ltd, Holon, Israel; [i]IRMB, université de Montpellier, Inserm U1183, Montpellier, France; [j]Unité d'immunologie clinique et de thérapeutique des maladies ostéoarticulaires, département de rhumatologie, hôpital Lapeyronie, Montpellier, France; [k]Cellular Immunotherapy Program, Cancer Center, Massachusetts General Hospital, Boston, MA, USA; [l]Université de Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé, SIMoS, Gif-sur-Yvette, France; [m]Institut de Recherches Internationales Servier, Suresnes, France; [n]IRMB, Univ Montpellier, INSERM, CNRS, Montpellier, France; [o]OSE Immunotherapeutics, Nantes, France; [p]Pharma Technical Development Analytics Biologics, Roche Diagnostics GmbH, Penzberg, Germany; [q]IRCM, INSERM, U1194 Univ Montpellier, ICM, 208, rue des Apothicaires, Montpellier, France; [r]Institut Toulousain des maladies infectieuses et inflammatoires - INFINITY- Inserm, CNRS, Université Toulouse III, Toulouse, France; [s]Calypso Biotech SA, Plan-les-Ouates, Switzerland; [t]Michalski Huettermann & Partner, Frankfurt/Main, Germany; [u]CEPR, INSERM U1100 Université de Tours, et CHU de Tours, Tours cedex, France; [v]Evotec, Toulouse, France; [w]Orionis Biosciences BV, Ghent, Belgium; [x]Protein Engineering and Antibody Technologies, Merck Healthcare KGaA, Darmstadt, Germany; [y]Office of Biotechnology Products, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA; [z]Biologics CMC & Developability, Institut de Recherche Pierre Fabre, St Julien-en-Genevois Cedex, France

ABSTRACT

The annual "Antibody Industrial Symposium", co-organized by LabEx MAbImprove and MabDesign, held its 10th anniversary edition in Montpellier, France, on June 28–29, 2022. The meeting focused on new results and concepts in antibody engineering (naked, mono- or multi-specific, conjugated to drugs or radioelements) and also on new cell-based therapies, such as chimeric antigenic receptor (CAR)-T cells. The symposium, which brought together scientists from academia and industry, also addressed issues concerning the production of these molecules and cells, and the necessary steps to ensure a strong intellectual property protection of these new molecules and approaches. These two days of exchanges allowed a rich discussion among the various actors in the field of therapeutic antibodies.

ARTICLE HISTORY
Received 31 March 2023
Revised 3 May 2023
Accepted 4 May 2023

KEYWORDS
Antibody engineering; Antibody industrial symposium; antibody-related molecules; biotherapeutics; cell-based therapies; congress; Intellectual property; research translation into clinic; therapeutic antibodies

Introduction

Antibody Industrial Symposium (AIS) focuses on antibody-based therapies, from antibody discovery to testing and optimization in patients. In 10 years, participation in the AIS increased from few people to more than 400 participants from 14 countries in 2022. The AIS is now a well-recognized meeting in France and through-out Europe, still with the objective to stimulate discussions about antibodies and also other biotherapeutics among people with different scientific backgrounds, from academia to industry. Antibody-based therapies now comprise a very wide field, and their applications as well as new approaches are growing very rapidly. A single meeting cannot cover all these developments, but the AIS aims to balance general sessions targeted to newcomers and emerging companies and sessions on specific topics.

After two years of COVID-19 pandemic, the meeting was again in-person, allowing direct interactions, including informal discussions during lunch/coffee breaks and poster sessions. This was also an opportunity to validate the new format of the meeting, with two parallel sessions. The 10th Antibody Industrial Symposium (AIS), co-organized by LabEx MAbImprove and MabDesign, was held in Montpellier, France, on June 28–29, 2022 (https://aiscongress.com). Labex MAbImprove (https://mabimprove.univ-tours.fr/en/) is a French network of academic research laboratories with the common objective of improving therapeutic antibody

CONTACT Alain Beck alain.beck@pierre-fabre.com Biologics CMC & Developability, Institut de Recherche Pierre Fabre, St Julien-en-Genevois Cedex, France; Pierre Martineau pierre.martineau@inserm.fr IRCM, INSERM, U1194 Univ Montpellier, ICM, 208, rue des Apothicaires institution, Montpellier, France
*Equal contribution & corresponding authors.

development and use. MabDesign (https://www.mabdesign.fr/en/) aims to structure the French biotherapy sector by promoting networking and support the stakeholder's skills development.

Naked antibodies against specific tumor targets, such as rituximab and trastuzumab, have revolutionized therapies in the past 25 years. This topic is still important and has been broadened to include all new antibody-related molecules, such as antibody-drug conjugates (ADC), bi- and multi-specific antibodies, and antibody fragments (*Novel monoclonal antibody targets – Bispecific antibodies – Fc-fusion proteins/Rare diseases session*). A second breakthrough was the development of immune checkpoint inhibitors that target the CTLA-4 and PD1 pathways. This is a very active topic that was discussed in a specific session: *Antibody-based immunotherapies: latest developments*. More recently, the clinical success of chimeric antigenic receptor (CAR)-T cells has extended the use of antibodies from soluble molecules to receptors to tweak the immune system. The emergence of these new approaches is now regularly covered by the AIS in a session called *Adoptive cell therapy & Gene editing*. Producing these new complex molecules and cells involves developing specific approaches and tackling new challenges linked to their production and validation. This topic was covered by the session *Analytical & Characterization*. Lastly, successes and failures at the clinical stage must be monitored, and this was covered by the session *Driving innovative drugs to clinical success*.

An important aim of the AIS has always been to foster discussion between academic and industrial scientists. An exciting aspect of this field is the quick translation of basic research findings into the clinic. This can be accomplished only if intellectual property (IP) is properly considered early in the process. Therefore, since 2019, the AIS has an IP session on trends and issues linked to patent protection in the field of biologics (*Intellectual Property: Where do we go from now?*). This year, the AIS welcomed experts from IP law firms based in France, United Kingdom, and Germany. Using patent law decisions in Europe and the USA and the most recent court rulings as examples, these experts discussed current issues in the protection of antibodies and provided insights into upcoming changes in European patent laws.

The 10th AIS anniversary also marked the creation of the AIS Awards to recognize major scientific contributions in the therapeutic antibody field. They are given to scientists who have continuously worked to advance this field with the objective of developing better treatments for patients. It was an honor to attribute the first AIS Awards to three experts who significantly contributed to the development of therapeutic antibodies with improved quality, safety and efficacy: Prof. Hervé Watier from Tours University Hospital, Dr. André Pèlegrin from Inserm and University of Montpellier, and Dr. Alain Beck from Pierre Fabre Group. We would also like to acknowledge their initial input and strong continuous involvement in AIS establishment as a major monoclonal antibody conference in France.

Plenary introductory keynotes

Dr. Peter Slavny (IONTAS, UK) described a mammalian display system with a particular emphasis on the improvement of IgG potency, specificity, functionality, and manufacturability. To construct libraries in cells, a single-gene copy must be incorporated in each cell to allow signal deconvolution. P. Slavny's group systematically tested several approaches to introduce site-directed cleavage and recombination: zinc-finger nucleases, transcription activator-like effector nucleases (TALENs), CRISPR/Cas, and I-*Sce*I meganuclease. Using TALENs and CRISPR/Cas, they successfully generated libraries with a 2–5% efficiency and a final diversity of ~1 million clones. Each cell contained a single integration, as confirmed by co-transformation with two different antibodies. The system allows the selection by fluorescence-activated cell sorting (FACS) using full-length IgG. P. Slavny described the power of this system for improving affinity. Starting from an anti-programmed death ligand 1 (PD-L1) antibody with a modest affinity of 75 nM, they successfully modified the molecule to an affinity of 2 nM, better than that of nivolumab (6.7 nM), a clinical reference.[1] They also noted that the signal level is directly proportional to the *in vitro* biophysical properties of the IgG in solution. Starting from poorly stable and badly behaving antibodies, they constructed mutant libraries expressed at the mammalian cell surface and then sorted by cytometry the cells expressing high IgG amounts at their surface. Poly-reactivity and aggregation propensity were reduced in the selected clones.[2] This system allows selecting desirable binding properties in the final format and is sensitive to important biophysical characteristics, such as aggregation propensity and poly-specificity, thereby enabling the early detection and elimination of problematic antibodies in the initial discovery phase.

Dr. Mark Leick (Massachusetts General Hospital, USA) discussed CAR-T cells that target CD70 for acute myeloid leukemia treatment and translational investigations on the relapse and resistance to commercial CD19-targeted CAR-T cells for aggressive lymphomas. In acute myeloid leukemia, M. Leick's group identified a novel resistance mechanism in which the ligand-based targeting mechanism undergo proteolytic cleavage by tumor-secreted enzymes. By modifying putative proteolytic cleavage sites, they could circumvent proteolysis and improve CAR-T cell performance.[3] Next, they modified their anti-CD70 CAR to secrete a CD33-targeted T-cell engaging antibody molecule (TEAM) that outperforms their original optimized CD70-targeted CAR-T cells. Finally, M. Leick described an ongoing translational investigation in which single-cell RNA sequencing is used to analyze serial samples from a cohort of patients with aggressive lymphoma treated with CAR-T cells. This analysis allowed the identification of a novel resistance mechanism via the increase of CAR-T regulatory cells (higher in non-responders than responders). This finding was recapitulated in *in vivo* models.[4]

Novel monoclonal antibody targets – Bispecific antibodies – Fc-fusion proteins/Rare diseases

Dr. Hulin Jin and **Dr. Berend Neuteboom** (Merck KGaA, Germany) described the mechanisms of the faster clearance and shorter half-life of avelumab compared to other anti – PD-L1 monoclonal antibodies (~4 days vs ~21 days for atezolizumab). Avelumab is an IgG1 approved as monotherapy for metastatic Merkel cell carcinoma and advanced urothelial carcinoma. They compared avelumab and atezolizumab. Both antibodies recognize PD-L1 with comparable affinities (~0.2–0.4 nM) and cross-react with cynomolgus monkey and mouse targets. In both antibodies, a series of mutations abrogating antigen or FcγR binding were also compared. In cynomolgus monkeys and mice, they demonstrated that avelumab internalization is essentially due to CD64 (FcγRI) binding *in vitro*, but that both PD-L1 and FcγRs are involved. Interestingly, atezolizumab harboring wild-type Fc was not internalized by FcγRs *in vitro*, and was eliminated slightly faster *in vivo* compared with the FcγR binding – deficient (N297A) variant. Concerning clearance in the mouse, avelumab with a functional Fc was the fastest eliminated molecule and the PD-L1 binding-deficient variant the slowest. Avelumab harboring a FcγR binding-deficient Fc presented an intermediate clearance rate. These data clearly show the effect of both antigen and FcγR binding on the half-life of these monoclonal antibodies in animal models.[5]

Dr. Luis Alvarez-Vallina (12 de Octubre Hospital, Madrid, Spain) presented innovative work on T-cell redirection using secreted bispecific molecules.[6] The redirection of T cell activity toward cancer cells by targeting cell surface-expressed tumor-associated antigens by bispecific T cell-engaging antibodies and CARs has revolutionized the treatment of hematologic cancers. Anti-CD19 CAR-engineered autologous T cells induce remission in patients with B-cell acute lymphoblastic leukemia; however, many patients will relapse, particularly those with $CD19^+$ cancer cells. Therefore, new therapeutic strategies are clearly needed. L. Alvarez-Vallina's group developed an immunotherapy that combines aspects of antibody- and cell-based strategies: immunotherapy, based on the endogenous secretion of T cell-engaging antibodies (STAb). They compared regular anti-CD19 CAR-T cells and their STAb-T19 T-cells that secrete an anti-CD19 × anti-CD3 T cell-engaging antibody, and showed higher cytotoxic effect and lower escape in a leukemia model *in vitro*. Both strategies displayed similar efficacy in short-term murine models. On the other hand, efficacy was higher in a long-term patient-derived xenograft mouse model, in which STAb-T19 cells efficiently eradicated cancer cells, whereas CAR-T19 therapy was followed by leukemia relapse. This better efficacy seems to be due to the lack of CD19 down-modulation, and also to the recruitment of the endogenous T-cell repertoire by the secreted STAb molecules.

The two next speakers presented artificial intelligence (AI)-based approaches to screen monoclonal antibodies using different and complementary strategies. **Dr. Dilyana Dimova** (Sanofi, Germany) described their in-house high-throughput engineering platform that allows generating very large panels of variants with high-quality data. Using this dataset of up to 10,000 variants and 100,000 datapoints, they could train AI models to extract significant patterns and develop virtual screening workflows. They applied this approach to optimize their new multi-specific format that is called Cross-Over-Dual-Variable Domain (CODV), which represents the basis for the construction of bivalent-bispecific, tetravalent-bispecific, and trivalent-trispecific molecules with multiple applications.[7] This approach is particularly valuable for the optimization of multivalent molecules that potentially lead to a very high number of variants. D. Dimova illustrated the problem to produce CODV molecules that represent 77×77 (5,929) variants. They experimentally constructed and produced 1,867 COVDs and used 1,494 COVDs to train their machine learning model, which then predicted the expression of the 373 remaining variants. The model reached an accuracy of 80%. The model was further improved by incorporating additional COVD-IgG variants (>6,000) with their expression level and structural data, thus reaching an accuracy and a precision of 0.92 and 0.91, respectively. These impressive results are partly explained by the high quality and highly focused collected dataset. This is not an easy task and cannot be obtained in more modest structures (academic or private). **Dr. Astrid Musnier** (MAbSilico, France) described a different approach that combined public databases and an in-house developed docking algorithm for the discovery and design of monoclonal antibodies. Their bioinformatic approach addresses most of the initial needs in a discovery pipeline, including candidate selection, epitope mapping, and affinity prediction. This complete pipeline can deliver the first binders in only 10 days.

Dr. Stefan Zielonka (Merck Healthcare KGaA, Germany) gave a talk on natural killer (NK) cell redirection by targeting the natural cytotoxicity receptor NKp30. NKp30 is an activating receptor expressed on NK cells and its ligand, B7-H6, is upregulated on tumor cells, but absent on most normal tissues. S. Zielonka showed that epidermal growth factor receptor (EGFR)-overexpressing tumor cells are efficiently bridged to NK cells *via* NKp30 using different bi- and multifunctional antibody-based NK cell engagers (NKCEs), thus eliciting very potent tumor cell lysis.[8,9] In the first project outlined by S. Zielonka, multifunctional EGFR-targeting immunoligands were developed based on B7-H6. For tumor targeting, they used the Fab arm derived from a humanized version of cetuximab, whereas for NKp30 triggering, their affinity optimized B7-H6. They demonstrated that immunoligands harboring affinity-matured versions of B7-H6 are substantially more potent in mediating tumor cell eradication than the immunoligand based on wild-type B7-H6. The former molecules were as potent as cetuximab, but displayed a different cytokine release profile. This may result in targeted inflammation of solid tumors. In the second part of his talk, S. Zielonka discussed multifunctional EGFR-targeting NKCEs based on camelid-derived NKp30-directed single-domain antibodies. These molecules were very potent in triggering NK cell-mediated lysis. Moreover, co-engagement of FcγRIIIa further increased tumor cell killing. Importantly, Zielonka's group also identified NKp30-targeting single-domain antibodies that do not interfere with the natural NKp30-B7-H6 signaling, a feature that might be beneficial for therapy.[10]

Although very potent in some cancer subtypes, T-cell checkpoint blockade with approved anti-PD-(L)1, -CTLA4,

or -LAG3 antibodies is insufficient to induce durable anti-tumor responses in most patients. **Dr. Pierre Ferré** (Compugen, Israel) presented results on TIGIT and PVRIG, novel T-cell inhibitory-receptors discovered by computational analysis. Both compete with the co-activating receptor DNAM-1 for binding to their shared ligands, PVRL2 and PVR. Compugen identified PVRIG and TIGIT as key parallel and complementary inhibitory pathways in the DNAM-1 axis that also intersects with the well-established PD-1 pathway.[11] Compugen developed a science-driven, biomarker-informed clinical program to evaluate the blockade of different combinations of members of these pathways in different tumor types. During the meeting, P. Ferré presented the preclinical rationale and key translational findings of early clinical studies on COM701, a potential first-in-class anti-PVRIG human IgG4 antibody, and COM902, a potential best-in-class anti-TIGIT human IgG4 antibody. He described preliminary data on pharmacokinetics, receptor occupancy, and pharmacodynamics. Unlike Fc-active anti-TIGIT antibodies, COM902 did not deplete major TIGIT-expressing T and NK cells, thus maximizing its potential anti-tumor capacity. Besides TIGIT, blocking PVRIG may be the "missing piece" in tumors with different degrees of inflammation that do not respond to the current immunotherapy. The latest results on COM701 showed that PVRIG has a unique dominant expression on early memory T cells that have increased proliferative potential, while its ligand PVRL2 is abundantly expressed across dendritic cell types. Therefore, COM701 could increase T-cell expansion and T-cell numbers also in tumors with lower inflammation levels. Accordingly, emerging translational data showed an increased immune infiltration and activation in tumor and serum samples of patients treated with COM701 ± an anti-PD1 antibody (nivolumab).

Diogo Rodrigues Ferreirinha and **Ed McGowan** (Charles River, UK) described an integrated pipeline to develop antibodies and other molecules (bispecific antibodies, ADC, CAR) and bring them into the clinic with the best chances of success. First, they can screen several highly diverse and already optimized antibody libraries against any target and reformat them into the therapeutic format for final testing. Then, the best candidates can be biophysically characterized to select the most stable and well-behaving molecules. This is followed by *in vitro* pharmacology studies to identify clones with the required functions and properties, such as affinity, epitope competition, species cross-reactivity, antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC). The next step is to analyze the specificity and off-target binding using a large collection of cells that express almost all possible antigens. Finally, *in vivo* experiments can be performed in animal models, including patient-derived xenografts. All the steps are independent and can be applied to any antibody.

Chen Yuning (Sino Biological, USA) presented their recombinant antibody platform that can be used to produce any antibody format, including bispecific antibodies and pentavalent and hexavalent IgM molecules. This can be done at a very high throughput in the case of IgG and VHH-Fc fusion molecules, as demonstrated by the production of 600 antibodies at the 0.3 mg scale in the framework of a COVID-19 program.

Adoptive cell therapy & gene editing

Dr. Manel Juan (Head of the Immunology Service, Hospital Clínic de Barcelona, Spain) described the generation of a new autologous CAR targeting CD19 (ARI-0001). This CAR obtained similar clinical results than commercial CARs used in the same clinical settings and was approved by the Spanish Agency of Medicines and Medical Devices (AEMPS). ARI-0001 was granted a hospital exemption to be used as advanced therapy medicinal product (ATMP), leading to its marketing authorization under specific conditions. These are: the exemption is only applicable to individual patients treated in the hospital (mainly academic centers that developed the ATMP and are supported by the national competent authority and limited to European Union (EU) member states; the exemption is intended for >25-year-old patients with relapsed or refractory $CD19^+$ acute lymphoblastic leukemia. This authorization is the first step in the development of and access to completely academic CAR T-cell products in the EU and can be used as an intermediate step before obtaining a centralized marketing authorization by the European Medicines Agency (EMA). The cooperation between academic partners, who must follow strict standards of traceability, pharmacovigilance, and quality, should favor the accessibility of this product and the approval of other academic ATMPs. M. Juan's team is completing a clinical trial on an anti-B-cell maturation antigen (BCMA) CAR-T for multiple myeloma, with a solid clinical response.[12]

Dr. Tuija Kekarainen (Scientific Director, Kuopio Center for Gene and Cell Therapy (KCT), Finland) started her talk emphasizing that the biological nature and complex mode of action of cell and gene therapies pose many challenges in the field. These include establishing appropriate *in vitro* and *in vivo* proof of concept systems, robust manufacturing systems and specific assays for product and process characterization. An additional challenge is the scale-up processes to secure the production of gene and cell therapy products for large indications. KCT, with international academic and industry collaborators, uses traditional and 'omics' methodologies to support the R&D of gene and cell therapies. T. Kekarainen shared recently published data on how transcriptomics studies can contribute to the development of cell therapy products. Such studies allow the development of "Process" solutions to find the safe and best solution for automated, closed processes that can ensure robust and reproducible product manufacturing.[13]

Dr. Leonie Alten (Scientific Development Manager, Twist Bioscience, Germany) described Twist Biopharma own DNA technology to write synthetic libraries and provide end-to-end antibody and T cell receptor (TCR) discovery libraries. These include a highly diverse synthetic naive single-chain variable fragment and VHH antibody phage display library and combinatorially assembled TCR libraries from gene fragments.

Peter Djali (LUMICKS, UK) introduced the z-Movi® Cell Avidity Analyzer, a unique instrument for the direct

measurement of cell – cell interaction forces. The overall binding strength (or avidity) between T or NK cells and tumor cells is an essential parameter for developing new CARs and monoclonal antibodies. Cell avidity is a measure of the effectiveness of the initial binding and is quantified in live cells. Cell avidity measurement is complicated because it depends on many factors, such as receptor density, different affinities, and the presence of other co-receptors. However, it provides a more complete and physiologically relevant picture of the interactions between cytotoxic lymphocytes and tumor cells. The z-Movi® Cell Avidity Analyzer gives predictive, reproducible, and fast high-throughput results at the single-cell level.

Matti Kimberg (Chief Scientific Officer, Synexa, UK) explained how UK Synexa specializes in the regulatory framework for supporting bioanalysis and applying the guidelines provided by regulators to novel analytical technologies and therapeutic modalities. The objective of Synexa is to help in bringing novel therapeutics to the market in a faster, cheaper, and safer way. Dr Kimberg presented Synexa expertise in cell-based assays for evaluating immunogenicity in gene and cell therapies.

Dr. Emmanuel Donnadieu (Head of the 'Cancer and immune response' group, Cochin Institute, Paris, France) discussed that adoptive transfer of CAR-T cells is well established for B-cell malignancies, but its extension to solid tumors is a challenge. One strategy is based on modulating the tumor microenvironment to improve CAR-T cell infiltration. Before clinical development, new approaches can be tested *in vitro* using preclinical models. This can include human organotypic models. E. Donnadieu's group has developed an imaging platform based on fresh human tumor slices. The preserved tumor microenvironment allows investigating the efficacy of CAR-T cells with fewer limitations than previous models. Modifications of this environment allow assessment of toxicity, including on-target, off-tumor effects. Therefore, this platform offers a new way to evaluate CAR T-cell treatment, which is a major issue in the field of cancer immunotherapy.[14]

Dr. Sascha Abramson (Carisma Therapeutics, USA) discussed how to harness macrophages with the CAR technology (CAR-M) and to use them in solid tumors. Macrophages are abundant in the tumor microenvironment, but they display mainly immunosuppressive functions and the challenge is to redirect them toward a proinflammatory phenotype. Insertion of a CAR can induce this shift by allowing these macrophages to selectively recognize antigens overexpressed by cancer cells. An additional advantage is that macrophages are antigen-presenting cells (APCs) and can present neoantigens to T cells, leading to epitope spreading and adaptive immune system activation. Carisma Therapeutics has developed the cell product CT-0508, in which autologous macrophages express an anti-HER2 CAR. In pre-clinical studies, CT-0508 induced targeted cancer cell phagocytosis while sparing normal cells, decreased tumor burden, and prolonged survival. Carisma Therapeutics is now developing a first-in-human Phase 1 study to evaluate CT-0508 safety, tolerability, cell manufacturing feasibility, trafficking, and preliminary evidence of efficacy in patients with HER2-overexpressing locally advanced/unresectable or metastatic solid tumors.[15]

The session finished with three technical presentations. First, **Katja Schreiter** (Integrated DNA Technologies, Germany) described new technologies to engineer healthier, cleaner, and high-quality DNA fragments for high-throughput applications. Then, **Nicolas Demoures** (Cell&Co, France) discussed about the requirements of a cold chain for most immunotherapy approaches and for EMA approval. Finally, **Linda Tchatchouang** (GenScript, France) described the possible use of CRISPR as non-viral approach for immunotherapies, which should avoid the use of expensive and difficult to produce viral vectors.

Antibodies-based immunotherapies: latest advancements

Prof. Mark Cragg (Southampton University, UK) focused his talk on the engineering of antibodies for immune stimulation. He pointed out that agonistic antibodies against immunostimulatory receptors are a currently untapped source for immunotherapy. In an effort to identify rules for optimizing agonist immunostimulatory antibodies, he discussed the salient properties these antibodies must possess to strongly agonize receptors, as well as potential strategies for designing more effective monoclonal antibody-based therapies. He focused on monoclonal antibodies against CD40 (a receptor essential for adaptive immunity initiation and regulation) and described Fc-dependent and Fc-independent mechanisms involved in the agonistic effect of anti-CD40. He first summarized the essential role of the Fc part and its cross-linking of inhibitory FcγRIIB in anti-CD40 immunostimulatory activity, showing that the isotype choice is an important issue when optimizing therapeutic monoclonal antibodies. He next demonstrated that hinge disulfides in human anti-CD40 antibodies (IgG2 format) modulate receptor signaling by regulating their conformation and flexibility. Importantly, the human IgG2 isotype displays a unique capacity to undergo disulfide shuffling in the hinge region, resulting in a modulation of its ability to stimulate signaling (agonism) in a variety of immune receptors. Indeed, agonistic activity varies as a function of the disulfide pattern: the presence of a disulfide crossover between F(ab) arms favors agonistic activity. This highlights that the properties of the hinge region of human IgG2-A are crucial for IgG2 activity regulation. Thus, engineering the IgG hinge may be considered for optimizing the activity of specific IgG subclasses.[16]

Dr. Federico Bertoglio (Technische Universität Braunschweig, Germany) described the discovery and development of therapeutic phage display-derived anti-spike antibodies through a productive collaboration between academia and industry. Using naive and patient-derived (immune) libraries, they selected potent neutralizing antibodies against the viral entry protein spike. The STE73-2E9 and STE90-C11 antibodies, isolated from a naive and an immune library, respectively, could neutralize SARS-CoV-2 by directly blocking the interaction between its spike protein and the host receptor ACE-2. The STE90-C11 monoclonal antibody (COR-101) has been tested in

a Phase 1b/2 clinical trial (ClinicalTrials.gov ID: NCT04674566).[17] This demonstrates how the collaboration between academia and industry can be productive, effective, and crucial. It also highlights that processes required for the translation of basic research results to the clinic can be effectively and safely accelerated during a pandemic.

Dr. Alain Vicari (Calypso Biotech, Switzerland) discussed the development of CALY-002, a clinical-stage anti-interleukin -15 (IL-15) monoclonal antibody. IL-15 is an attractive target in autoimmune diseases because it controls early inflammation events at the epithelial barrier interface, modulates multiple adaptive and innate immune cell types, and is important for immune disease memory. IL-15 has complex biology and exists in free and receptor-complexed forms. Recent data suggest that both forms might be involved in disease progression, and CALY-002 has the unique capacity to fully neutralize all IL-15 forms. This is due to the specific epitope recognized by CALY-002, at the interface between IL-15 and the IL-15 Rβ chain. CALY-002 could be tested in many autoimmune diseases, but Calypso Biotech chose to focus first on celiac disease and eosinophilic esophagitis, two diseases in which IL-15 inhibition effect was validated in preclinical models, and that are interesting from both medical and regulatory point of views.[18] The first-in-human clinical Phase 1a/b study on CALY-002 has been initiated. The first part in healthy volunteers is now completed and showed good safety and pharmacokinetics profiles, and CALY-002's capacity to modulate IL-15-dependent leukocyte populations. The second part in patients with celiac disease or eosinophilic esophagitis is ongoing. Its primary objective is to determine CALY-002 safety in patients, but it may also allow the determination of whether CALY-002 can limit local inflammation in patients, as proof of its biological mechanism.

Prof. Christian Jorgensen (IRMB-CHRU, Montpellier, France) and **Prof. Guy Serre** (INSERM 1291-Université de Toulouse, France) presented the *in vivo* validation of the therapeutic potential of hybrid Fc/citrullinated peptide-biomolecules in a humanized model of rheumatoid arthritis (RA) (resulting from research performed in the framework of the "Cure RA" project). This project is based on the design and production of hybrid molecules that combine IgG Fc fragments and citrullinated peptides specifically recognized by autoantibodies (anti-citrullinated protein antibodies (ACPA)) present in patients with RA. These innovative hybrid molecules target autoreactive B cells that express membrane ACPA and induce their selective depletion. After promising *in vitro* results, the efficiency of these hybrid molecules was validated in humanized SCID mice harboring ACPA-transduced human B cells. The main effector cell populations, NK cells and macrophages, involved in the specific depletion of autoreactive $ACPA^+$ B cells were investigated. In the presence of effector NK cells and the Fc/alpha citrullinated hybrid, $ACPA^+$ B cells were significantly and specifically depleted, but not $APCA^-$ B cells (control). Similar results were obtained in the presence of macrophages and the Fc/alpha citrullinated hybrid molecule. Conversely when using the hybrid built with the non-citrullinated peptide, $ACPA^+$ B cells were not affected. Overall, this work demonstrated that hybrid molecules including IgG Fc fragments and citrullinated peptides allow the highly efficient and specific elimination of $ACPA^+$ B cells *in vivo*, via two different effector mechanisms. This preclinical validation is the first step toward the clinical development of a specific RA immunotherapy by selective depletion of $ACPA^+$ B cells.

Prof. George Weiner (Iowa University, USA) gave a talk on T cell- and NK cell-mediated ADCC in the context of anti-cancer monoclonal antibody-based therapy. Resistance to such therapy remains a clinical challenge. He reported that IL-2, produced by T cells, maintains NK cell viability and NK cell-mediated ADCC. Lack of T-cell produced IL-2 (i.e., T-cell help) in the tumor microenvironment is a potential mechanism of monoclonal antibody resistance. Therefore, they tested whether bispecific antibodies targeting CD3 and cancer cells (anti-CD3 × anti-lymphoma, myeloma and EGFR) can overcome resistance to anti-cancer antibody-mediated lysis by enhancing T-cell help, and thereby increasing NK cell-mediated ADCC. At low T-cell concentrations, T cells activated by the bispecific antibodies, which were mainly but not exclusively $CD4^+$ cells, were more effective than resting T cells at maintaining NK cell viability, phenotypic changes and ADCC. Short-term exposure to the bispecific antibodies was sufficient to enhance long-term ADCC by NK cells. These findings suggest that T cell activation in the tumor microenvironment mediated by a short systemic treatment with anti-CD3 × anti-cancer antigen bispecifics, administered concomitantly with monospecific anti-cancer monoclonal antibodies, could enhance the efficacy of antibodies that mediate their primary therapeutic effect via NK-mediated ADCC. A clinical trial to test this hypothesis should start shortly.[19]

Analytical & characterization

Dr. Alain Beck (Institut de Recherche Pierre Fabre, Saint-Julien en Genevois, France) gave a talk on multi-level structural characterization of monoclonal antibodies and antibody-based products, such as ADCs, bispecific antibodies, Fc-fusion proteins and immunocytokines. The developability and comparability assessment of current and next-generation complex antibody-based products require state-of-the-art analytical and structural methods.[20] Mass spectrometry (MS) allows the identification and comprehensive structural characterization of proteins with high sensitivity. MS-based approaches play a central role in biopharmaceutical laboratories, complementing and advancing traditional biotherapeutic characterization workflows. A combination of various MS approaches is required to comprehensively characterize monoclonal antibody-based structures. The commonly used bottom-up MS approaches are efficiently complemented by mass measurements at the intact and subunit (middle-up) levels, and by product ion analysis following gas-phase fragmentation of precursor ions performed at the intact (top-down) and subunit (middle-down) levels. A. Beck reported the efficiency of these approaches and the development of novel strategies in the past decade. He also discussed the critical quality attributes (CQAs) of antibody-based products and presented case studies for biologics approved by the Food and Drug Administration (FDA) and EMA, including emicizumab and dulaglutide. Concerning the MS toolbox, he summarized the advantages

of native and ion mobility MS, collision induced unfolding (CIU), cyclic CIU (cCIU), multiplexed top and middle-down MS, multiple fragmentation techniques, including high energy collisional (HCD), electron-transfer (ETD) and UV photodissociation (UVPD), Parallel Accumulation and Serial fragmentation (PASEF, *de novo* sequencing), capillary electrophoresis hyphenated to MS (CE-MS) and quantification of trace-level host cell proteins (HCPs) by MS. Liquid chromatography (LC)-MS approaches are currently unparalleled in terms of specificity, sensitivity and flexibility, and can better keep pace, compared to other analytical technologies, with the innovative R&D developments in the biopharmaceutical sector. The unprecedented level of detail provided by LC-MS-based assays can lead to improvements in biotherapeutic design and production, resulting in more stable, effective, and homogeneous products and ultimately, better patient outcomes. Multi-attribute monitoring with LC-MS is gaining acceptance as a viable, cost-effective alternative to conventional analytical assays. MS-based methods will be increasingly used at all stages of the antibody-based therapeutic development life cycle.

Dr. Dietmar Reusch (Director, Development Analytics Characterization, Roche Diagnostics GmbH, Germany) discussed the key glycosylation features that influence antibody clearance. D. Reusch presented a combination of glycoengineering and glycoform-resolved pharmacokinetic measurements by MS to show the glycoform effects on pharmacokinetic. To this aim, they separately injected four differently glycoengineered monoclonal antibodies in rats and analyzed blood samples at different time-points to follow changes in the glycosylation profiles of each glycoengineered antibody over time. They confirmed the increased clearance of high-mannose and hybrid-type (Man5) glycoforms. Specifically, Man5 showed a 1.8- to 2.6-fold higher clearance than agalactosylated, complex glycans (G0F). Unexpectedly, clearance was even higher for the hybrid-type glycan Man5G0. Conversely, clearance of agalactosylated, monoantennary glycoforms (G0F-N) was only slightly increased compared with the G0F form. D. Reusch concluded that hybrid-type and high-mannose glycoforms should be distinguished in CQA assessments. Interestingly, α2,3-linked sialylation did not affect clearance.[21]

Dr. Udo Roth (Laboratory Head Mass Spectrometry CMC Development/Bioanalytics Sanofi, Germany) described the characterization of Nanobody® and Synthorin™, two new biotherapeutic modalities, by LC-MS. Nanobody® or single-domain VHH fragments and Synthorin™ molecules (recombinant proteins engineered to contain non-natural amino acids at specific sites using an extended genetic code)[22] belong to promising new classes of biologics in the biopharmaceutical field. As they differ substantially from classical monoclonal antibodies, CQAs may change. These CQAs require their in-depth characterization to define a suitable control strategy. In this talk, U. Roth presented the results of the characterization of the posttranslational modifications occurring in these new modalities and discussed their potential of becoming CQAs.

Dr. Géry Van Vyncht (Quality Assistance, Belgium) presented their 2D-LC-MS workflows for the automated analysis of in-process samples and for the characterization of monoclonal antibodies in a good manufacturing practice (GMP)-regulated environment. However, the presence of monoclonal antibodies in a complex mixture during production is a hindrance to their rapid and efficient analysis. Indeed, techniques such as MS, which is commonly used to determine the molecular weight and the presence of modifications, require the use of purified samples, leading to time-consuming sample preparation, while fast characterization is expected. Two-dimensional liquid chromatography (2D-LC in the multiple heart-cutting mode) coupled to MS can be used to analyze monoclonal antibodies after on-line purification or sample preparation, using the second dimension of the system for sample desalting. G. Van Vyncht illustrated this approach using two automated 2D-LC-MS workflows developed at Quality Assistance for: 1) the analysis of monoclonal antibodies in complex mixtures using on-line Protein A purification, in less than 1 h, without prior sample preparation, in a fully automated and GMP-compliant system supported by MS data; and 2) the automated MS characterization of monoclonal antibodies at the subunit level using on-line IdeS digestion and dithiothreitol reduction, with antibody modification quantification at the subunit level.[23]

Dr. Bernard Maillère (CEA Saclay, France) addressed the immunogenicity of therapeutic antibodies. He first recalled that immunogenicity of therapeutic antibodies is characterized by the production of anti-drug antibodies (ADA) by the treated patients. ADAs can alter the antibody pharmacokinetics, therapeutic efficacy, or cause hypersensitivity reactions. Since the description of the first chimeric antibodies, many technologies and methods have been established to humanize antibody sequences and mitigate the immunogenicity risk. Accordingly, many antibodies exhibit a reduced level of immunogenicity. However, several examples of recently approved antibodies show that immunogenicity can still be an issue, including in immuno-oncology. B. Maillère discussed the immunogenicity of recently approved antibodies, the mechanisms leading to ADA production or contributing to their regulation, and strategies that combine functional assays and prediction tools to de-immunize antibodies by removing potential T-cell epitopes present in complementarity-determining regions. He emphasized the importance of the T-cell response, particularly CD4, in triggering ADA production.[24]

Dr. Amy Rosenberg (Senior Director of Immunology and Protein Therapeutics, EpiVax, USA) discussed tolerance induction during treatments based on biologics. Protein-based therapeutics are highly targeted and effective, but their efficacy and safety are strongly influenced by immune responses that can render them ineffective or unsafe. Novel therapeutic modalities, including gene and cell therapies, provide optimal means to deliver protein therapeutics, while avoiding the need of recurrent administration, and to tolerize the immune system to their therapeutic cargo. Reciprocally, protein therapeutics may contribute to ensure the success of gene therapies, including abrogating capsid-specific responses to adeno-associated virus vectored therapeutics, thus allowing repeated administration, and also facilitating cell therapy engraftment. “Setting the stage” for the introduction and effectiveness of antigen-specific tolerizing therapeutics will require

controlling the prevailing immunogenic and inflammatory milieu in many diseases. A. Rosenberg presented these novel approaches to antigen-specific tolerance, in which T regulatory cells are activated and expanded by T regulatory epitopes (Tregitopes), converting APCs and T effector cells to a tolerogenic phenotype.[25] Alternative approaches to immune tolerance involve engineering therapeutic proteins (deimmunized functional therapeutics (DeFTs)), for instance, to produce long-lasting, hyperglycosylated interferon α. Briefly, optimized interferon α variants that contain eight amino acid modifications were obtained using the OptiMatrix *in silico* tool. They are promising candidates to improve interferon α therapy.

Activation of the classical (CP) and lectin pathway (LP) of the complement cascade can contribute to tissue damage and organ dysfunction in antibody-mediated diseases and in ischemia-reperfusion conditions. **Dr. Tim Delahaye** (Scientist, ArgenX, Belgium) described ARGX-117, a humanized inhibitory monoclonal antibody against the complement cascade component C2. By binding to the Sushi-2 domain of C2, ARGX-117 prevents the formation of the C3 proconvertase, the C4bC2 complex, and inhibits both CP and LP activation upstream of C3. As ARGX-117 does not inhibit the alternative complement pathway (AP), it should not affect the protective activity of this pathway against pathogens. ARGX-117 prevents complement-mediated cytotoxicity in *in vitro* models of autoimmune hemolytic anemia and antibody-mediated rejection of organ transplants, and inhibits complement-mediated phagocytosis of IgM-sensitized red blood cells. ARGX-117 exhibits natural pH- and calcium-dependent target binding and is Fc-engineered for increased acidic binding to FcRn and reduced effector functions. In cynomolgus monkeys, ARGX-117 reduced free C2 levels and CP activity in a dose-dependent manner. A two-dose regimen (80 and 20 mg/kg separated by 1 week) strongly reduced CP activity for at least 7 weeks. Taken together, these results suggest that ARGX-117 is a promising new complement inhibitor that is uniquely positioned to target both CP and LP, while leaving AP intact.[26] This mode of action opens new perspectives for treating complement-mediated immune and inflammatory diseases in humans.

In a constantly changing world of biopharmaceutical development and production, **Dr. Antony Munn** (Head of Development Services, Lonza, Slough, UK) described how Lonza looked at its processes in the context of new competition and new biopharmaceutical types, and how they addressed this challenge. He then discussed the philosophies, technologies, and approaches that Lonza took to adhere to its mission of "Enabling a Healthier World" by developing new infrastructure and new monoclonal, bispecific and non-monoclonal antibody biopharmaceuticals.

During her presentation, **Dr. Christelle Dagoneau** (Head of Global Business Development, Just Evotec Biologics, Toulouse, France) described their unique platform that integrates the discovery, design, engineering, development, and manufacture of biologics in very flexible and easily deployable production facilities called J.POD®. C. Dagoneau showed how the intrinsic molecular properties of antibody sequences can result in lead candidates that are difficult to manufacture or keep stable in formulation. This translates into process development and manufacturing challenges that can substantially affect costs and timelines. Just-Evotec Biologics uses its Abacus™ software, an in-house suite of proprietary computational tools, to predict molecules and conditions for development. She also described how J.POD® accommodates perfusion, intensified fed-batch, semicontinuous, and end-to-end continuous biomanufacturing processes at a standard 500 L scale to deliver from few kilograms of antibodies for "first-in-human" studies to metric tons of drug substance (commercial biomanufacturing) for commercial supply.

Dr. Olivier Favre-Bulle (CEO, 3Biotech, Paris, France) emphasized that accelerating the development of a new therapeutic antibody, from discovery to the clinic stage, requires a global strategy and execution plans. There are three key areas that must be considered to succeed in early drug development: efficacy, safety, and manufacturability. The lack of efficacy in patients is one of the main causes of failure in drug development at the clinical phase. For safety, the limits of safe doses, and the specific potential adverse effects of the drug must be assessed to minimize risks in patients and also to the program development. Safety and efficacy are not the only aspects that must be considered for successful drug development: manufacturability also is crucial. However, chemistry, manufacturing and controls (CMC) are among the most underestimated steps of the whole drug development process by start-up companies and small biotechs. O. Favre-Bulle highlighted that it is crucial to demonstrate the quality and consistency of the antibody to be produced, by implementing an agile CMC strategy early in the development process. As an example, he discussed the quality of the cell-line development and characterization.

Dr. Raffaella Balocco Mattavelli (Unit Head, International Nonproprietary Names Program and Classification of Medical Products, World Health Organization, Switzerland) presented the recent changes of the international nonproprietary names (INN) for monoclonal antibodies. Appropriate nomenclature for all pharmaceutical substances is important for clinical development, licensing, prescribing, pharmacovigilance, and identification of counterfeits. Nonproprietary names that are unique and globally recognized for all pharmaceutical substances are assigned by the International Nonproprietary Names Program of the World Health Organization (WHO). In 1991, this program implemented the first nomenclature scheme for monoclonal antibodies. To accompany biotechnological development, this nomenclature scheme has evolved during the years; however, since the scheme was introduced, all pharmacological substances that contain an immunoglobulin (Ig) variable domain have been named using the stem -mab. To date, there are 879 INN with the stem -mab. Due to this high number of names ending in -mab, devising new and distinguishable INN has become a challenge. Therefore, the WHO INN Expert Group decided to revise the system. The revised system was approved and adopted by WHO at the 73rd INN Consultation in October, 2021, and the radical decision was made to discontinue the use of the well-known stem -mab to name new antibody-based drugs. It has been replaced by four new stems: -tug for unmodified Ig (any species or class), -bart for engineered, monospecific antibodies carrying modifications in the constant domains (CH1–4 and hinge), -mig for bi/multi-specific antibodies, and -ment for monospecific Ig

fragments that contain at least one variable domain and no complete Fc.[27] For ADCs, the antibody portion will be named following these new rules and the conjugated drug name will follow as the second word, like in the actual nomenclature.

Driving innovative drugs to clinical success

Dr. Daniel Olive (Head of the Immunity and Cancer laboratory, Marseille Cancer Research Center, CRCM, France) presented the butyrophilin (BTN) family as regulators of the immune response, with a focus on Vγ Vδ2 T cells. Gamma delta T cells (γδ T cells) are T cells characterized by the expression of a TCR that is made of one γ chain and one δ chain. This T-cell type is less common than αβ T cells, but is implicated in several immune responses, and Vγ Vδ2 T cells are the predominant variant in peripheral blood. Unlike the 'classical' αβ T cells, γδ T cells do not seem to require antigen processing and major-histocompatibility-complex (MHC) presentation of peptide epitopes, although some recognize MHC class Ib molecules. γδ T cells may have a prominent role in recognition of lipid antigens. They are of an invariant nature and may be triggered by alarm signals, such as heat shock proteins. γδ T cells are activated by intracellular phosphoantigens (pAg) from malignant or bacterial origins.[28] Their innate pAg-dependent anti-cancer activity can be exploited and amplified by different methods. These include the use of amino-bisphosphonates and also targeting members of the BTN family. In this family of transmembrane proteins, eight members (BTN1A1, BTN2A1/2A2, BTN3A1/3A2/3A3, MOG, and BTNL2) have a structure similar to that of the B7 family that harbors immunoglobulin (Ig)C-IgV extracellular domains. Studies in mice have shown that pAg activation critically depends on cell-to-cell contacts and on BTN3A and BTN2A expression in APCs (tumor or infected cells). BTN3A binds to pAgs intracellularly, while BTN2A is critical for the transport to the membrane and interaction with the γδ TCR. D. Olive described the role of BTN3A and BTN2A in human primary tumors and their regulation, functions, and roles as Vγ Vδ2 T cell biomarkers.

Dr. Stéphanie Cornen (Innate Pharma, France) focused her talk on the clinical development of monalizumab, a potentially first-in-class immune checkpoint inhibitor targeting NKG2A receptors expressed on tumor-infiltrating cytotoxic $CD8^+$ T cells and NK cells. NKG2A is an inhibitory receptor for HLA-E, which is frequently overexpressed in cancer cells of many solid and hematological malignancies. By expressing HLA-E, cancer cells can protect themselves against $NKG2A^+$ immune cell cytotoxic activity. Monalizumab may reestablish a broad anti-tumor response mediated by NK and T cells, and may enhance the cytotoxic potential of other therapeutic antibodies.[29] Monalizumab is currently being assessed in several clinical trials: INTERLINK-1, a randomized global Phase 3 study on the efficacy and safety of monalizumab and cetuximab in patients with recurrent or metastatic head and neck cancer; COAST, a Phase 2 clinical trial in patients with unresectable, stage III non-small cell lung cancer; and NeoCOAST, a Phase 2 trial in patients with resectable, early-stage (stage I [>2 cm] to IIIA) non-small cell lung cancer.

Dr. Thomas Jaquin (Pieris Pharmaceuticals, Germany) presented one of their tumor-targeted 4-1BB agonistic antibody-Anticalin® fusion proteins, also called Mabcalin™ proteins. 4-1BB (or CD137) is an important and well-studied costimulatory immune receptor expressed on and involved in T ($CD4^+$, $CD8^+$ and regulatory T), B and NK cell function.[30] Activation of the 4-1BB pathway results in enhanced proliferation, cytokine production, and cytotoxic activity of these T and NK cells. These characteristics and its expression on tumor-infiltrating lymphocytes suggest that 4-1BB is a promising target for cancer immunotherapy. Like all members of the tumor necrosis factor receptor superfamily, 4-1BB requires higher order clustering for downstream signaling activation. This intrinsic mode of action and dose-limiting toxicities explain the failures of prior clinical trials of anti-4-1BB antibodies, such as urelumab and utomilumab. At Pieris Pharmaceuticals, they circumvent these limitations by combining 4-1BB agonism and antibody-dependent tumor-targeting in bispecific molecules. Several of these molecules have already entered the clinical stage. In cinrebafusp alfa, these 4-1BB-specific Anticalin proteins are fused to the C-terminus of the heavy chains of an anti-HER2 antibody, which results in efficient tumor-targeting and also facilitates 4-1BB cross-linking. T. Jaquin showed that T-cell co-stimulation depends on the format/geometry of the molecule and HER2 expression on tumor cells. He presented data from a safety and efficacy Phase 1 monotherapy study showing that cinrebafusp alfa is safe and increases $CD8^+$ T cell tumor infiltration and serum 4-1BB levels in a dose-dependent manner.

Intellectual property: where do we go from now?

Juliet Redhouse (Mathys & Squire, UK) described how to use patent filing strategies to obtain patent protection for antibody innovations. J. Redhouse presented the types of supporting data that might be needed to obtain patent protection for the antibody product, and explained that patent protection is also available for other aspects of antibody development, including dosage regimens, combination treatments, formulations, manufacturing methods and new therapeutic indications. In her presentation, J. Redhouse indicated what factors must be considered when developing strategies for extending patent protection beyond the antibody, including what data will be needed to support these later innovations.

Interest in how AI can be used to improve drug discovery and development has grown substantially in the past several years. Progress in the generation of data describing antigen binding and developability, computational methodology and AI has enabled the design and generation of new on-demand therapeutic antibodies. **Raphaëlle Gillet** and **Nicolas Bouquin** (Regimbeau, France) discussed how protection of these technologies can be more complex than for regular wet lab inventions, at least because patent protection is overlaid with database IP rights. Some companies may decide to protect their AI methods. AI methods can be protected by patents if they do not fall within any of the exceptions defined by the law. For example, AI methods can be patented at the European Patent Office, as long as they provide a technical solution to

a technical problem. Even if they pass this step, these methods must be new, inventive, enabled, and clearly defined, like any other invention. Although AI patent protection might be interesting for companies in the field, patent protection is absolutely required for antibodies if they are to be developed as marketed products. However, the AI method does not confer any distinctive property to the antibody. Notably, an antibody is not new or inventive simply because AI was involved. All the common patentability requirements (e.g. novelty, inventive step, enablement) must be met by the antibody *per se*. Furthermore, AI technologies rely on access to databases for learning and training. Databases are subject to a specific protection regime, notably in Europe. Particularly, the *sui generis* right prohibits the extraction or re-utilization of any database in which there has been a substantial investment to obtain, verify, or present the data contents. This right may be transferred, assigned, or granted under contractual license. Many issues still have to be solved by contractual negotiation.

The Unitary Patent (UP) system will become operational on 01 June 2023. It will represent the most significant change in European patent law since the introduction of the European Patent (EP) about 50 years ago. This topic was discussed by **Christoph Volpers** (Michalski Huettermann & Partner, Germany). With this new system, in addition to the national patent and the European "bundle" patent, a new UP will be available to provide protection of technical IP. Each patent type has its pros and cons. The second main innovation in the new system will be the establishment of a new Unified Patent Court (UPC) that is expected to harmonize patent litigation procedures and outcomes in Europe. In those EU member states that have ratified the UPC Agreement so far, this new court will be in charge of all infringement proceedings, revocation actions and declarations of non-infringement concerning European "bundle" patents and UPs. A patent proprietor may "opt out" of the UPC competence for a specific European patent during the initial transitional phase or even earlier during the "sunrise period", which will start in March 2023. The UPC decisions will take effect in all UPC member states, whereas in the past, patent litigation was handled by national courts and their decisions took effect only in that country. Hence, requesting an "European patent with unitary effect" (UP), after an EP has been granted, or "opting out" from the UPC competence for a specific EP represents an important strategic decision with legal, financial, and operational consequences to be considered for a successful patent management. Patent proprietors should prepare for these decisions well before the upcoming start of the UP system.

Plenary closing keynotes

Bioassays, also known as potency assays, are a vital component of quality control strategies for biotechnology products,[31] including monoclonal antibodies and bispecific antibodies. Compared with other physicochemical methods, which are compendial or platform methods adaptable to different product types, bioassays are product-specific and require special considerations to meet regulatory requirements. This was the focus of the keynote lecture "*Comprehensive bioassays for neutralizing antibodies against SARS-CoV-2*" by **Dr. Baolin Zhang** (US Food and Drug administration, USA). Dr. Zhang started by giving an update of the current antibody development programs, including anti-SARS-CoV-2 monoclonal antibodies and cocktails authorized for Emergency Use[32] and the emerging bispecific antibodies that bind simultaneously to two distinct sites on the viral spike protein. These products are complex molecules that are associated with multi-faceted mechanisms of action (MoA) and that require bioassays to ensure product quality, stability, lot-to-lot consistency, and comparability when making manufacture changes. B. Zhang discussed the regulatory and technical considerations to develop bioassays for anti-SARS-CoV-2 antibodies.[33] He highlighted four key points: 1) relevance to the drug MoA; the bioassay should ideally correlate with clinical efficacy; 2) ability to capture all functional units of the drug biological activity; 3) suitability to quality control environments; and 4) sensitivity to changes in product quality (e.g., post-translational modifications) that can be used as stability-indicating assays. B. Zhang also shared data from an FDA intramural research project to develop bioassays for the comprehensive assessment of the binding and neutralization activity of antibodies against SARS-CoV-2 variants. In his closing remarks, B. Zhang stressed the importance of discussing with FDA in advance about bioassays and other time-critical elements of development programs for COVID-19.

In the closing keynote, **Dr. Allison August** (Moderna, USA) discussed and illustrated the exciting new possibility of using mRNA delivery to express full-length therapeutic antibodies directly in patients. She illustrated the topics by presenting a Phase 1 trial in patients infected by Chikungunya virus (CHIKV). A nanoparticle-encapsulated messenger RNA encoding the heavy and light chains of a CHIKV-specific monoclonal neutralizing antibody was injected intravenously in patients (one or two doses). Adverse effects increased with the dose, but were still moderate and manageable at the highest dose of 0.6 mg/kg (in one or two injections). Antibodies against CHIKV were detected in the serum with a peak at day 3 (~10 μg/mL) for a single dose, and at day 3 after the second dose (13 μg/mL). At the highest dose, patients maintained a serum level >1 μg/mL for more than 16 weeks. Interestingly, this concentration is theoretically high enough to neutralize CHIKV and should provide patient protection. Altogether, this first-in-human study demonstrated the safety of the approach and its therapeutic potential to replace antibody injection.

Conclusion

The AIS is now a well-established meeting on all the aspects of therapeutic antibodies and adoptive-cell therapies. At the beginning (10 years ago) it was focused on very specific topics, but now it has evolved to a more general meeting that remains still centered on antibodies, as soluble molecules or associated with cells. Because of the extraordinary dynamism of the field, every year the meeting presents exciting new developments in

basic and preclinical research, new analytical methods, therapeutic advances, and regulatory changes.

The next AIS will take place in Tours, France, on June 22–23, 2023, with the same general organization and sessions, and an emphasis on *Accelerating biotherapeutics for patients: Lessons drawn from the clinic*.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the LabEx MAbImprove ["Investissements d'Avenir" program ANR-10-LABX-53-01].

ORCID

Bruno Robert http://orcid.org/0000-0001-5573-6945
Alain Beck http://orcid.org/0000-0002-4725-1777
Pierre Martineau http://orcid.org/0000-0002-7993-7183

Abbreviations

CAR	Chimeric Antigenic Receptor
AIS	Antibody Industrial Symposium
ADC	Antibody-Drug Conjugates
IP	Intellectual Property
TALEN	Transcription Activator-Like Effector Nucleases
FACS	Fluorescence-Activated Cell Sorting
PD-L1	Programmed Death Ligand 1
TEAM	T-Cell Engaging Antibody Molecule
STAb	Secretion of T Cell-Engaging Antibodies
AI	Artificial Intelligence
CODV	Cross-Over-Dual-Variable domain
NK	Natural Killer
EGFR	Epidermal Growth Factor Receptor
NKCE	NK Cell Engagers
ADCC	Antibody-Dependent Cellular Cytotoxicity
CDC	Complement-Dependent Cytotoxicity
AEMPS	Spanish Agency of Medicines and Medical Devices
ATMP	Advanced Therapy Medicinal Product
EU	European Union
EMA	European Medicines Agency
BCMA	B-Cell Maturation Antigen
TCR	T Cell Receptor
CAR-M	CAR Macrophage
APC	Antigen-Presenting Cells
IL-15	Interleukin-15
RA	Rheumatoid Arthritis
ACPA	Anti-Citrullinated Protein Antibodies
MS	Mass Spectrometry
CQA	Critical Quality Attributes
FDA	Food and Drug Administration
CIU	Collision Induced Unfolding
cCIU	cyclic CIU
HCD	High energy Collisional Dissociation
ETD	Electron-Transfer Dissociation
UVPD	UV Photo-Dissociation
PASEF	Parallel Accumulation and Serial Fragmentation
CE-MS	Capillary Electrophoresis hyphenated to MS
HCPs	Host Cell Proteins
LC	Liquid Chromatography
GMP	Good Manufacturing Practice
2D-LC	Two-Dimensional Liquid Chromatography
ADA	Anti-Drug Antibodies
Tregitopes	T regulatory epitopes
DeFTs	Deimmunized Functional Therapeutics
AP	Alternative complement Pathway
CP	complement Classical Pathway
LP	complement Lectin Pathway
CMC	Chemistry, Manufacturing and Controls
INN	International Nonproprietary Names
WHO	World Health Organization
Ig	Immunoglobulin
BTN	Butyrophilin
MHC	Major-Histocompatibility-Complex
pAg	phosphoAntigen
UP	Unitary Patent
EP	European Patent
UPC	Unified Patent Court
MoA	Mechanisms of Action
CHIKV	CHIKungunya Virus

References

1. Parthiban K, Perera RL, Sattar M, Huang Y, Mayle S, Masters E, Griffiths D, Surade S, Leah R, Dyson MR, et al. A comprehensive search of functional sequence space using large mammalian display libraries created by gene editing. MAbs. 2019;11(5):884–98. PMID:31107136. doi:10.1080/19420862.2019.1618673.
2. Dyson MR, Masters E, Pazeraitis D, Perera RL, Syrjanen JL, Surade S, Thorsteinson N, Parthiban K, Jones PC, Sattar M, et al. Beyond affinity: selection of antibody variants with optimal biophysical properties and reduced immunogenicity from mammalian display libraries. MAbs. 2020;12(1):1829335. PMID:33103593. doi:10.1080/19420862.2020.1829335.
3. Leick MB, Silva H, Scarfò I, Larson R, Choi BD, Bouffard AA, Gallagher K, Schmidts A, Bailey SR, Kann MC, et al. Non-cleavable hinge enhances avidity and expansion of CAR-T cells for acute myeloid leukemia. Cancer Cell. 2022;40(5):494–508.e5. PMID:35452603. doi:10.1016/j.ccell.2022.04.001.
4. Haradhvala NJ, Leick MB, Maurer K, Gohil SH, Larson RC, Yao N, Gallagher KME, Katsis K, Frigault MJ, Southard J, et al. Distinct cellular dynamics associated with response to CAR-T therapy for refractory B cell lymphoma. Nat Med. 2022;28(9):1848–59. PMID:36097221. doi:10.1038/s41591-022-01959-0.
5. Jin H, D'Urso V, Neuteboom B, McKenna SD, Schweickhardt R, Gross AW, Fomekong Nanfack Y, Paoletti A, Carter C, Toleikis L, et al. Avelumab internalization by human circulating immune cells is mediated by both Fc gamma receptor and PD-L1 binding. OncoImmunology. 2021;10(1):1958590. PMID:34484871. doi:10.1080/2162402X.2021.1958590.
6. Blanco B, Compte M, Lykkemark S, Sanz L, Alvarez-Vallina L. T cell-redirecting strategies to 'STAb' tumors: beyond CARs and bispecific antibodies. Trends Immunol. 2019;40(3):243–57. PMID:30827461. doi:10.1016/j.it.2019.01.008.
7. Furtmann N, Schneider M, Spindler N, Steinmann B, Li Z, Focken I, Meyer J, Dimova D, Kroll K, Leuschner WD, et al. An end-to-end automated platform process for high-throughput engineering of next-generation multi-specific antibody therapeutics. MAbs. 2021;13(1):1955433. PMID:34382900. doi:10.1080/19420862.2021.1955433.
8. Pekar L, Klausz K, Busch M, Valldorf B, Kolmar H, Wesch D, Oberg H-H, Krohn S, Boje AS, Gehlert CL, et al. Affinity maturation of B7-H6 translates into enhanced NK cell–mediated tumor cell lysis and improved proinflammatory cytokine release of bispecific immunoligands via NKp30 engagement. J Immunol. 2021;206(1):225–36. PMID:33268483. doi:10.4049/jimmunol.2001004.
9. Klewinghaus D, Pekar L, Arras P, Krah S, Valldorf B, Kolmar H, Zielonka S. Grabbing the bull by both horns: bovine ultralong CDR-H3 paratopes enable engineering of "almost natural" common light chain bispecific antibodies suitable for effector cell

redirection. Front Immunol. 2021;12:801368. PMID:35087526. doi:10.3389/fimmu.2021.801368.
10. Klausz K, Pekar L, Boje AS, Gehlert CL, Krohn S, Gupta T, Xiao Y, Krah S, Zaynagetdinov R, Lipinski B, et al. Multifunctional NK cell–engaging antibodies targeting EGFR and NKp30 elicit efficient tumor cell killing and proinflammatory cytokine release. J Immunol. 2022;209(9):1724–35. PMID:36104113. doi:10.4049/jimmunol.2100970.
11. Alteber Z, Kotturi MF, Whelan S, Ganguly S, Weyl E, Pardoll DM, Hunter J, Ophir E. Therapeutic targeting of checkpoint receptors within the DNAM1 Axis. Cancer Discov. 2021;11(5):1040–51. PMID:33687987. doi:10.1158/2159-8290.CD-20-1248.
12. Trias E, Juan M, Urbano-Ispizua A, Calvo G. The hospital exemption pathway for the approval of advanced therapy medicinal products: an underused opportunity? The case of the CAR-T ARI-0001. Bone Marrow Transplant. 2022;57(2):156–59. PMID:35046545. doi:10.1038/s41409-021-01463-y.
13. Gurvich OL, Puttonen KA, Bailey A, Kailaanmäki A, Skirdenko V, Sivonen M, Pietikäinen S, Parker NR, Ylä-Herttuala S, Kekarainen T. Transcriptomics uncovers substantial variability associated with alterations in manufacturing processes of macrophage cell therapy products. Sci Rep. 2020;10(1):14049. PMID:32820219. doi:10.1038/s41598-020-70967-2.
14. Espie D, Donnadieu E. CAR T-cell behavior and function revealed by real-time imaging. Semin Immunopathol. 2023;45(2):229–39. PMID:36688965. doi:10.1007/s00281-023-00983-7.
15. Reiss K, Ueno N, Yuan Y, Johnson M, Dees EC, Chao J, Angelos M, Swaby R, Cushing D, Ronczka A, et al. 633 a phase 1, first in human (FIH) study of autologous macrophages containing an anti-HER2 chimeric antigen receptor (CAR) in participants with HER2 overexpressing solid tumors. J ImmunoTher Cancer. Internet. 2022;10. doi:10.1136/jitc-2022-SITC2022.0633.
16. Yu X, Chan HTC, Fisher H, Penfold CA, Kim J, Inzhelevskaya T, Mockridge CI, French RR, Duriez PJ, Douglas LR, et al. Isotype switching converts Anti-CD40 antagonism to agonism to elicit potent antitumor activity. Cancer Cell. 2020;37(6):850–66.e7. PMID:32442402. doi:10.1016/j.ccell.2020.04.013.
17. Bertoglio F, Fühner V, Ruschig M, Heine PA, Abassi L, Klünemann T, Rand U, Meier D, Langreder N, Steinke S, et al. A SARS-CoV-2 neutralizing antibody selected from COVID-19 patients binds to the ACE2-RBD interface and is tolerant to most known RBD mutations. Cell Rep. 2021;36(4):109433. PMID:34273271. doi:10.1016/j.celrep.2021.109433.
18. Vicari AP, Schoepfer AM, Meresse B, Goffin L, Léger O, Josserand S, Guégan N, Yousefi S, Straumann A, Cerf-Bensussan N, et al. Discovery and characterization of a novel humanized anti-IL-15 antibody and its relevance for the treatment of refractory celiac disease and eosinophilic esophagitis. MAbs. 2017;9(6):927–44. PMID:28581883. doi:10.1080/19420862.2017.1332553.
19. Wang Z, Yin C, Lum LG, Simons A, Weiner GJ. Bispecific antibody-activated T cells enhance NK cell-mediated antibody-dependent cellular cytotoxicity. J Hematol Oncol. 2021;14(1):204. PMID:34886888. doi:10.1186/s13045-021-01216-w.
20. Xu Y, Wang D, Mason B, Rossomando T, Li N, Liu D, Cheung JK, Xu W, Raghava S, Katiyar A, et al. Structure, heterogeneity and developability assessment of therapeutic antibodies. MAbs. 2019;11(2):239–64. PMID:30543482. doi:10.1080/19420862.2018.1553476.
21. Falck D, Thomann M, Lechmann M, Koeleman CAM, Malik S, Jany C, Wuhrer M, Reusch D. Glycoform-resolved pharmacokinetic studies in a rat model employing glycoengineered variants of a therapeutic monoclonal antibody. MAbs. 2021;13(1):1865596. PMID:33382957. doi:10.1080/19420862.2020.1865596.
22. Zhang Y, Ptacin JL, Fischer EC, Aerni HR, Caffaro CE, San Jose K, Feldman AW, Turner CR, Romesberg FE. A semi-synthetic organism that stores and retrieves increased genetic information. Nature. 2017;551(7682):644–47. PMID:29189780. doi:10.1038/nature24659.
23. Largy E, Cantais F, Van Vyncht G, Beck A, Delobel A. Orthogonal liquid chromatography-mass spectrometry methods for the comprehensive characterization of therapeutic glycoproteins, from released glycans to intact protein level. J Chromatogr A. 2017;1498:128–46. PMID:28372839. doi:10.1016/j.chroma.2017.02.072.
24. Meunier S, de Bourayne M, Hamze M, Azam A, Correia E, Menier C, Maillère B. Specificity of the T cell response to protein biopharmaceuticals. Front Immunol. 2020;11:1550. PMID:32793213. doi:10.3389/fimmu.2020.01550.
25. De Groot AS, Rosenberg AS, Miah SMS, Skowron G, Roberts BJ, Lélias S, Terry FE, Martin WD. Identification of a potent regulatory T cell epitope in factor V that modulates CD4+ and CD8+ memory T cell responses. Clin Immunol. 2021;224:108661. PMID:33412295. doi:10.1016/j.clim.2020.108661.
26. Van de Walle I, Silence K, Budding K, Van de Ven L, Dijkxhoorn K, de Zeeuw E, Yildiz C, Gabriels S, Percier J-M, Wildemann J, et al. ARGX-117, a therapeutic complement inhibiting antibody targeting C2. J Allergy Clin Immunol. 2021;147(4):1420–9.e7. PMID:32926878. doi:10.1016/j.jaci.2020.08.028.
27. Guimaraes Koch SS, Thorpe R, Kawasaki N, Lefranc M-P, Malan S, Martin ACR, Mignot G, Plückthun A, Rizzi M, Shubat S, et al. International nonproprietary names for monoclonal antibodies: an evolving nomenclature system. MAbs. 2022;14(1):2075078. PMID:35584276. doi:10.1080/19420862.2022.2075078.
28. Morita CT, Jin C, Sarikonda G, Wang H. Nonpeptide antigens, presentation mechanisms, and immunological memory of human Vγ2Vδ2 T cells: discriminating friend from foe through the recognition of prenyl pyrophosphate antigens. Immunol Rev. 2007;215(1):59–76. PMID:17291279. doi:10.1111/j.1600-065X.2006.00479.x.
29. André P, Denis C, Soulas C, Bourbon-Caillet C, Lopez J, Arnoux T, Bléry M, Bonnafous C, Gauthier L, Morel A, et al. Anti-NKG2A mAb is a checkpoint inhibitor that promotes anti-tumor immunity by unleashing both T and NK cells. Cell. 2018;175(7):1731–43.e13. PMID:30503213. doi:10.1016/j.cell.2018.10.014.
30. Melero I, Johnston JV, Shufford WW, Mittler RS, Chen L. NK1.1 cells express 4-1BB (CDw137) costimulatory molecule and are required for tumor immunity elicited by anti-4-1BB monoclonal antibodies. Cell Immunol. 1998;190(2):167–72. PMID:9878117. doi:10.1006/cimm.1998.1396.
31. The European Medicines Agency. ICH Q6B specifications: test procedures and acceptance criteria for biotechnological/biological products - scientific guideline [Internet]. European Medicines Agency; 2018 [accessed 2023 Jan 12]. https://www.ema.europa.eu/en/ich-q6b-specifications-test-procedures-acceptance-criteria-biotechnological-biological-products
32. Center for Drug Evaluation and Research. Coronavirus treatment acceleration program (CTAP). FDA [Internet]; 2022 [accessed 2023 Jan 12]. https://www.fda.gov/drugs/coronavirus-covid-19-drugs/coronavirus-treatment-acceleration-program-ctap
33. Center for Drug Evaluation and Research. COVID-19: potency assay considerations for monoclonal antibodies and other therapeutic proteins targeting SARS-CoV-2 infectivity [Internet]. U.S. Food and Drug Administration; 2021 [accessed 2023 Jan 12]. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/covid-19-potency-assay-considerations-monoclonal-antibodies-and-other-therapeutic-proteins-targeting