MEETING REPORT

MAbDelivery: Administration routes for antibody therapy
Third LabEx MAblImprove industrial workshop, July 2, 2015 Tours, France

Elsa Bodier-Montagutelli\textsuperscript{a,b,c,*}, Renaud Respaud\textsuperscript{a,b,c,*}, Hervé Watier\textsuperscript{a,d,e}, and Audrey Guillou-Munos\textsuperscript{a,f,g}

\textsuperscript{a}Université François Rabelais, UMR 1100, Tours, France; \textsuperscript{b}INSERM, Centre d’Etude des Pathologies Respiratoires, UMR, Tours, France; \textsuperscript{c}Service de Pharmacie, CHRU de Tours, Tours, France; \textsuperscript{d}Service d’Immunologie, CHRU de Tours, Tours, France; \textsuperscript{e}CNRS, Génétique, Immunothérapie, Chimie et Cancer, UMR, Tours, France; \textsuperscript{f}Groupe IMT, Tours, France; \textsuperscript{g}Bio3 Institute, Tours, France

\textbf{ABSTRACT}

The annual “LabEx MAbImprove Industrial Workshops” are primarily intended to provide a comprehensive view about topics of interest for the pharmaceutical industry to scientists involved in research on therapeutic antibodies. The third workshop in this series, held July 2, 2015 in Tours, was dedicated to the optimization of delivery, namely all processes leading monoclonal antibodies to reach their target site. The commonly used intravenous (IV) route, although advantageous in terms of pharmacokinetics and pharmacodynamics, presents some disadvantages in terms of patients’ convenience, therapeutic target access or treatment cost. Such problems led pharmaceutical companies to consider more straightforward and patient-friendly administration routes, bringing the need for specific formulations adapted to the specific inherent physicochemical challenges. In this context, the workshop provided an overview of these advances and opened discussion on new administration routes and formulation development. In the first session, the opportunities and challenges of 3 main routes of administration (IV, subcutaneous (SC), and pulmonary) were discussed, integrating protein stability issues. The next session was dedicated to medical devices intended for SC and pulmonary administration. The last session focused on specific formulations for monoclonal antibodies, particularly to successfully protect antibodies upon aerosolization, to develop highly concentrated formulations for SC administration, and to use formulation as a mean to overcome the barriers to oral protein delivery. As in the previous editions, this workshop gathered people from the academic and industrial spheres and allowed rich debates and discussions.

\textbf{Abbreviations:} mAb, monoclonal antibody; IV, intravenous; SC, subcutaneous; rHuPH20, recombinant human hyaluronidase; DDC, drug-device combination; pMDI, pressurized metered dose inhaler; DPI, dry powder inhaler; HCF, highly concentrated formulations; Bi-mAb, bispecific monoclonal antibody

\textbf{Introduction}

Since its inauguration in October 2011,\textsuperscript{1} LabEx MAbImprove has organized 3 industrial workshops. After addressing the topics of monoclonal antibody (mAb) biosimilars in 2013\textsuperscript{2} and mAb dosing in 2014, the third workshop was dedicated to mAb delivery,\textsuperscript{3} and was organized by the LabEx MAbImprove Industrial Committee and Polepharma. This initiative is closely linked to the “Biopharmaceuticals” program supported by the French Région Centre-Val de Loire and involved a partnership with the Groupe IMT and ARITT, the regional agency for innovation and technology transfer in the French Région Centre-Val de Loire, which is the leading French region in terms of pharmaceutical production. The industrial workshop “MAbDelivery” was opened by \textbf{Mr Denis Requier}, (Polepharma, Chartres, France). He gave a welcoming speech, starting with a few words about Polepharma. This integrated cluster is the biggest European cluster for pharmaceutical development and production, and thus confers to the Région Centre-Val de Loire a key role in the opportunities of biotherapeutics for the pharmaceutical value chain. Joining and supporting for the first time the LabEx MAbImprove in the organization of this workshop was thus obvious for Polepharma, who aims to be a leader in the global biotech competition.

\textbf{Mme Mélanie Fortier} (representing François Bonneau, President of the Région Centre-Val de Loire) welcomed all attendees and recalled the importance of research and development activity on biotherapeutics in this French Région. She noted that the 2 co-organizers (LabEx MAbImprove and Polepharma) were founded with the same objective: combining fundamental and applied researches to ensure the short- and long-term future of health. She emphasized that this workshop constituted a highly effective link between research and clinical applications, which are much awaited by patients and caregivers. Finally, she renewed the support of the Région Centre-Val de Loire for the development of biopharmaceuticals and expressed high expectations from this fruitful workshop.

Then, \textbf{Dr Sylvain Huille} (Sanoﬁ, Antony, France), as a member of the Industrial and Scientific Committees, highlighted the
opportunity provided by this workshop to gather biopharmaceutical companies and academic laboratories around the topic of biotherapeutics. He stated that mAb delivery, which involves drug formulation for specific routes of administration with the help of dedicated medical devices, is a topic of strategic importance for biopharmaceutical companies. Indeed, the numerous innovations achieved over the past decades have yielded new antibody scaffolds and structures, with increased biologic activities. In parallel, alternative mAb delivery, if developed in a patient-centered approach, should include consideration of all the underlying opportunities and constraints and contribute to fully meeting clinical needs. Dr Huille thus concluded that mAb delivery is a highly innovative field covering various disciplines, and he encouraged open discussions on the main requirements and technology trends for patients and healthcare professionals.

**Session 1: Administration routes**

The first session was designed to present and discuss the benefits, issues, constraints and challenges of mAb administration routes that are currently used. The chair was shared by Dr Roland Beliard (LFB, France) and Dr Laurent Plantier (CHRU of Tours, Tours, France).

Therapeutic biologicals, including mAbs, are commonly intravenously (IV) administered in hospital settings, but other routes such as subcutaneous or aerosol delivery could be preferred for some applications. Because the stability of these drugs is essential, Prof. Alain Astier (Hôpital Henri Mondor, Créteil, France) started the session with a presentation on mAbs stability in hospital clinical practice. Indeed, stability data provided by the manufacturers mainly consider bacterial contamination risk and are often conducted over short periods of time. In practical situations, pharmacists, who do not want to waste these very expensive drugs, need to know if their handling is appropriate, i.e., does not affect the drug stability and thus its biologic activity. After reviewing protein physical and chemical instabilities (with a focus on aggregation) and their main theoretical causes, he detailed the practical situations occurring in hospital pharmacies that may affect protein drugs. These include temperature excursion (in case of cold chain rupture), extended storage time (when administration is not performed or delayed and when dose-bandung is initiated), preparation manipulation (shaking and bubbling, use of metallic needles) and transportation (from pharmacy to ward using modern pneumatic network or between hospitals).

Prof. Astier then shared some results of stability analysis after thermal or mechanical stresses of therapeutic antibodies. In a preamble, he emphasized that analytical methods were chosen following the guidelines for the practical studies of anticancer drugs published by the French and European societies of oncology, with the use of complementary methods to assess stability of biologics. The first study concerns the stability of diluted rituximab that was performed to address the feasibility of dose-bandung to avoid product loss (residues in the vials or bags). Thermic stress was applied to diluted antibody in polyolefin bags (up to 40°C for 6 months) and different physicochemical and biologic stability analysis were performed. The study conclusion was that diluted rituximab is stable up to 6 months at 4°C, allowing in-advance preparation of bags and dose-bandung. Moreover, diluted antibody is also stable at 22°C for 6 months, arguing for the possibility to administer it safely in case of temperature excursion. Similar conclusions could be drawn from stability studies of diluted trastuzumab and of diluted ipilimumab, which is a very expensive treatment. The last study presented was a stability analysis of the mechanical stresses on antibodies transported via pneumatic conveying systems. Prof. Astier emphasized the dramatic effect of air in the bags during transportation. No modification was observed after up to 8 routes (round trip) if the bags are purged of air. In the presence of air, significant modifications, especially aggregation, occur after only 4 circuits for rituximab and one for cetuximab (an aggregation-prone antibody). This presentation was quite reassuring in indicating that therapeutic antibodies are often more stable than what manufacturers indicate in their recommendations. This also confers greater flexibility to healthcare professionals’ practice, to adapt to daily constraints. One question from the audience noted the lack of standardization for the analysis of mechanical stress. Prof. Astier argued that it is generally assumed that if no modification is observed after a strong stress (stirring in vials, shaking), stability is guaranteed with lower stresses. In particular, if antibodies are resistant to the mechanical energy in pneumatic conveying systems (after purging the air to avoid air-liquid interface) they will be resistant to a transfer between 2 hospitals.

Thereafter, Dr Nathalie Heuzé-Vourch (CEPR INSERM U1100, Tours, France) reported on her laboratory work on administration of mAbs by aerosol. She first reminded participants that inhalation can be used to deliver drugs with either a systemic action (by taking advantage of the passive diffusion via the large air-blood interface in the lungs), or a local action for respiratory diseases like chronic obstructive pulmonary disease or asthma. She illustrated these points with some examples of either small molecules or biologics on the market. The small number of mAbs approved to treat respiratory diseases are administered by intravenous injection, but several products are in late-stage clinical trials and, among them, one is meant to be delivered by aerosol (ALX-0171, a trimeric anti-respiratory syncytial virus nanobody). The scientific rationale to deliver antibody-based therapeutics through the airways is based on 2 facts: for respiratory diseases, most mAbs operate into the lungs, but these large molecules hardly diffuse passively from the blood vessels into the targeted compartment (for example, there are 500 to 10,000 times less mAb in the lungs than in the systemic circulation). To demonstrate that mAbs delivery through the airways is both feasible and relevant, Dr Heuzé-Vourch’s team developed 2 animal models of lung cancer to test aerosol delivery of cetuximab (an approved anti-epidermal growth factor receptor antibody), and of an anti-murine vascular endothelial growth factor antibody. The results of both studies indicated that airway-delivered mAbs reach their target antigen in the tumor and are pharmacologically efficient in limiting tumor growth. Moreover, pharmacokinetics of the different mAbs delivered through the airways in mice and non-human-primates showed a limited lungs-to-bloodstream passage and an accumulation of the antibodies in the lungs. This scientific talk convinced the audience that mAb delivery through the airways is therapeutically relevant. The next questions Dr Heuzé-Vourch plans to address are the
effectiveness of the aerosol delivery of antibodies in pathophysiological conditions (indeed, patients with respiratory diseases do not breathe the same as healthy patients), stability of the antibodies during administration (in particular, specific formulations must be designed to limit aggregation induced by the air-liquid interface) and safety of this administration route. Finally, a question from the audience emphasized the heterogeneity of the tissues contained in the lungs and asked about the ability of the antibodies to cross the mucus barrier to reach the bronchial epithelia. Dr Heuzé-Vourch’s answer was based on the experiments performed in non-human primates, where an antibody fraction was found in the bloodstream, arguing for the capacity of these molecules to cross the mucus.

In the last presentation of the session, Dr Claudia Mueller (F. Hoffmann-La Roche Ltd, Basel, Switzerland) discussed a novel approach for subcutaneous (SC) administration of high dose mAbs using co-formulations containing mAbs and recombinant hyaluronidase (rHuPH20). SC administration represents a convenient alternative for patients, offering several advantages compared with IV infusion, which so far is the typical administration route for mAbs. Whereas IV infusion usually takes several hours for preparation and administration, SC injection is expected to simplify and shorten administration time, resulting in a reduced treatment burden for patients and improved resource utilization at the treatment facility. However, SC applications have limitations regarding the overall volumes to be administered. Because mAbs are usually prescribed in relatively high doses, a decrease in the injection volume administered SC compared with IV is required. Several different approaches exist, with temporary enlargement of the interstitial space at the injection site using rHuPH20 being one of them.

After this introduction, Dr Mueller described the chosen formulation development strategy, which involved a combination of both increasing the mAb concentration and the dosing volume. Viscosity and stability were discussed in terms of aggregation due to high mAb concentration, biologic activity depending on the chosen excipients, and absence of interactions between the 2 recombinant proteins (e.g., potential structural modifications, aggregation). Finally, challenges during the manufacturing process were addressed, e.g., compounding operations and potential sensitivity of the co-formulation toward decontaminating agents during fill and finish. Following the lecture, a participant asked about the time needed to inject a 10 mL dose and the advantage compared with IV. It was brought forward that the injection, being performed by nurses, takes ~5 minutes compared with an IV infusion, which usually takes several hours. Considering that the drug is ready to use, thus requiring considerably less preparation time, a gain of resources and convenience for both the patient as well as the treatment facility is obtained. A discussion on the status of rHuPH20 in the available drug product followed. rHuPH20 is an enzyme and may be considered a drug substance within the coformulation. Dr Mueller confirmed that regulatory bodies, among them European Agencies, validated the excipient status for rHuPH20 within the respective coformulations with mAb(s). Additionally, it was specified that substantial pre-clinical and clinical trials were performed with the co-formulation(s) evaluating the safety and efficacy of both the mAb(s) and the excipient rHuPH20, resulting in the successful approval of 2 products (rituximab and trastuzumab) within the European Union and other countries.

**Session 2: Administration devices**

The next session focused on the devices (marketed or under development) designed for the administration of mAbs via the alternative routes discussed previously. This session was chaired by Dr Nathalie Heuzé-Vourch (CEPR INSERM U1100, Tours, France) and Dr Jan Jezek (Arecor, Cambridge, UK).

To start the session, Mr Didier Pertuy (Sanofi, Gentilly, France) presented the current landscape and challenges in the field of devices for SC injection of biopharmaceuticals. Indeed, a fast transition occurred toward mAbs-based therapies, which became a dominant segment of the pharmaceutical market. For these therapeutics, the SC route is increasingly considered as an alternative to the IV, given its numerous advantages (e.g., user-convenience, better therapy adherence or economic impacts). In response to this increasing demand, many technologies dedicated to SC administration are proposed, differing by their complexity and maturity (e.g., pre-filled syringes, pen injectors, auto-injectors, infusion pumps). Based on a common architecture, each of these platforms has specific characteristics (e.g., injection volume, injection time). Overall, the market of SC administration devices is dominated by pre-filled syringes, pens and auto-injectors, although large volume devices are becoming available. For these platforms, the main expected improvements concern primary containers to better fit highly concentrated mAbs, device connectivity, patient-centricity and costs to facilitate access.

The second part of Mr Pertuy’s talk emphasized the challenges and opportunities inherent to these SC self-injectable drug-device combinations (DDCs), with a special focus on mAbs. Overall, an ideal delivery platform should be sufficiently generic to be cost-effective, but also customizable to fit patients’ symptomatology and convenience. Besides, in the particular case of mAbs, compatibility between the drug molecular design and the injection system (so called device-ability) is a critical parameter for the development of a DDC. The concept of device-ability covers 2 main problems: the ability of the drug product to be injected by the device (i.e., the solution’s behaviors, which are related to molecule characteristics) and the potential interactions between the solution and the device’s components (i.e., interfacial and leachable–induced interactions). The first point is mainly driven by mAbs’ behavior in high concentration solutions. Indeed, protein-protein interactions often lead to aggregation and increased viscosity, potently compromising injectability. The viscosity of mAbs solutions relies on several molecular structure-sequence related factors (e.g., electrostatic interactions, surface charge distribution, dipole moment, solvent-exposed hydrophobic patches), which can be investigated for candidate mAbs with the help of relevant tools. Considering drug-device interactions, mAbs display heterogeneous sensitivities to interfacial or leachable–induced interactions with primary container components, such as silicone oil and rubber stoppers. These parameters must be anticipated when developing SC-administered mAbs. To conclude, Mr Pertuy highlighted that designing device-able
mAbs molecular structure and sequence through targeted protein engineering is a strategic requirement for the benefit of the patient’s injection experience. Protein engineering may also allow the design of long-lasting mAbs, increasing their half-life, reducing dosing frequency to further improve patients’ treatment adherence.

The next talk was given by Dr Laurent Vecellio (Aerodrug, Tours, France), who discussed nebulization devices that allow pulmonary delivery through aerosolization. Nebulizers are designed to convert several milliliters of bulk liquid into aerosol droplets suitable for patient inhalation. Three categories co-exist on the market: jet nebulizers (working with a gas source), ultrasonic nebulizers (operating with a piezoelectric transducer) and mesh nebulizers (creating droplets through a mesh). Among therapeutic aerosol-generating systems, nebulizers differ from pressurized metered dose inhalers (pMDIs) and dry powder inhalers (DPIs), in which the drug and the device are combined and require active cooperation from the patient (e.g., synchronization between device actuation and breathing). In practice, nebulizers bear the great advantage of being suitable for any clinical situation. Several other features render nebulizers interesting for pharmaceutical development: they are approved, ready to use and suitable for a large range of liquid formulations (no dry powder formulation step required). A quick review of the operating mode of each category allowed Dr Vecellio to emphasize their respective advantages and drawbacks. Overall, nebulizers share a common limitation, namely a partial lung delivery of the drug loaded in the device, which is dependent on several parameters and can be optimized by engineering the aerosol’s particle size between 0.5 and 5μm.

After this general introduction, Dr Vecellio provided a summary of the literature on the compatibility of nebulizers with biologic drugs. The first presented study demonstrated that nebulizers display heterogeneous thermal behaviors, resulting in either temperature increases or decreases during nebulization. Several biopharmaceuticals being thermolabile, such temperature variations may alter their structural or functional integrity. The same authors also followed drug concentration in the devices’ reservoir: liquid recirculation and evaporation occurring inside jet and ultrasonic nebulizers leads to an increase in drug concentration over time, which was not observed with mesh nebulizers. Such modifications in drug compositions may also modify the viscosity of the therapeutic liquid, potently impairing aerosol generation, particle size or lung deposition. Nebulization may also modify the properties of the aerosolized drug. For instance, some authors demonstrated a partial loss of enzymatic activity for single-chain urokinase plasminogen activator after ultrasonic nebulization. Regarding antibodies, the results of a comparative study seem to indicate that mesh nebulizers would be the most suitable to aerosolize cetuximab, generating lower nebulization-induced aggregation rates than other nebulizers. Although interesting, such results cannot be extrapolated to all marketed mesh nebulizers. Indeed, because each mesh nebulizer has specific features, specific mAbs-compatibility assays are needed. Furthermore, the effect of meshes aging should be further investigated, as discordant results were published about their robustness. Dr Vecellio concluded that mesh nebulizers seem best-suited for mAbs delivery, for they allow a highly efficient lung delivery up to 80%, reducing the drug mass loaded in the nebulizer, and consequently the treatment cost. Although there are numerous advantages, a few drawbacks leave room for optimization. For instance, potent nebulization-induced drug damage might be avoided if considered during drug development. Dr Vecellio then took a question from the audience about the likelihood of a transition toward DPIs for mAbs. He answered that liquid formulation was a first-intention strategy for pharmaceutical companies. Nevertheless, such a transition is feasible, but development of a dry powder formulation and a specific device are required.

### Session 3: Specific formulations for mAbs

The last session of this workshop was dedicated to specific formulations for mAbs, linked to the administration routes and devices previously discussed. The chair was shared between Dr Sylvain Huille (Sanofi, Antony, France) and Dr Audrey Munos (Groupe IMT, Tours, France).

This session began with a general talk about innovative approaches to the formulation of therapeutic antibodies, given by Dr Jan Jezek (Arecor, Cambridge, UK). As an introduction, Dr Jezek reviewed the challenges of formulating high concentration liquid biologics, which are often associated with altered quality attributes (aggregation, viscosity and physicochemical stability). In this context, formulation is a powerful tool to control stability using combinations of excipients (e.g., buffers, tonicity modifiers, stabilizers, surfactants) to optimize critical features. Though already performed by pharmaceutical companies, biotherapeutics formulation design remains subject to substantial patenting activity, regarding for instance protein viscosity or novel antibody formats. Thus, in Dr Jezek’s view, there is still room for innovation in this field. Indeed, several general approaches may lead to substantial improvements in biopharmaceuticals’ stability, such as modifying protein characteristics by selecting formulation conditions (e.g., pH), removing excipients that cause unfavorable interactions, introducing competition via specific excipients (to prevent aggregation, for example) or scavenging species causing covalent or non-covalent changes. These strategies were illustrated with a few examples. In the first case study, the Arestat™ formulation developed by Arecor successfully prevented aggregation of a high concentration therapeutic protein. In this composition, benzoate was used to lower ionic strength and cover protein hydrophobic patches, hence reducing hydrophobic aggregation at 40°C. This protective effect may probably be driven by an interaction between the amphiphile and the antibody’s hydrophobic parts. In the second example, the activity of a protein was measured at constant pH, controlled either by conventional buffers (pKa within 1 unit of the pH), or by displaced buffers wherein the pKa of the buffer is >1 unit away from the pH. These experiments showed that using displaced buffers successfully locks the pH while preventing the continuous proton exchange between buffers and proteins that favors aggregation and activity loss. Then, Dr Jezek presented another strategy, which consists of using novel excipients to stabilize proteins. He gave the example of the MolecularGuard™ technology, a tool based on oligoethyleneimines and currently approved for the oral route. This technology achieves control of many
degradation pathways (e.g., aggregation and fragmentation), as well as viscosity. Applying this strategy to marketed antibodies (bevacizumab and rituximab) prevented their aggregation and the formation of visible particles under stress conditions for several weeks.

The last section of this presentation highlighted that a high concentration mAbs formulation strategy is often driven by control of charge-charge interactions. To achieve this, ionic strength is routinely used as a key parameter. However, it was shown that preferential increase of ionic strength pertaining either to cations or anions, achieved by multi-cationic or multi-anionic species, can have a surprisingly positive effect on protein stability. Dr Jezek concluded that formulation is a powerful tool for controlling stability when increasing protein concentration. He emphasized that several physicochemical features of excipients must be considered in the formulation design because subtle (often non-obvious) changes in formulation can have a significant effect on stability. A participant asked whether the presented formulations could potently modify antibodies’ binding to their Fc receptors. Dr Jezek answered that biologic activity and binding are tested by Areocor's collaborators; so far, such alterations have not been observed.

The next talk, given by Dr Renaud Respaud (University François-Rabelais and CHRU of Tours, Tours, France), provided insight into antibodies formulation for aerosol administration. He started by addressing the challenges of inhalation therapy applied to antibodies. As discussed previously by Dr Heuzé-Vourc'h, this route is of great interest for direct lung delivery of antibodies, but presents technical hurdles. Indeed, the aerosolization device and the generated particle size directly condition lung deposition. Regarding the drugs themselves, several parameters must be controlled to produce an efficient aerosol: formulation, concentration, pH and viscosity. In Dr Respaud’s opinion, drug loss along the aerosolizing process is a restraining factor and results in a gap between the dose charged in the device and the actual dose reaching the lung. All these parameters must be considered during the development of an antibody, in a specific formulation, associated with a dedicated device; the resulting combination should allow the right amount of drug and aerosol characteristics to successfully reach the target site within the lungs.

Dr Respaud then discussed the strategy to develop a formulation for inhaled antibodies. The first step is to select the format of the aerosol (solid or liquid). Solid aerosols delivered through DPIs bear some advantages, especially for patients’ convenience. They may require, however, a longer drug-and-device development and can deliver only limited amounts of active drugs. Among liquid aerosolization devices, MDIs are rather unsuitable with mAbs due to interactions with the propellant gas. Finally, nebulizers seem suitable for antibodies liquid aerosolization, even though they may induce deleterious stresses toward antibodies through heating, shearing and the generation of a substantial air-liquid interface. In this prospective, mesh nebulizers seem to induce lower stresses, and might thus be the most adapted for antibody nebulization. Finally, a protective formulation should be developed to protect antibodies against physical and chemical degradations, especially aggregation. To this end, several parameters can be adjusted, such as the antibody concentration, the buffer, or the use of specific excipients. Dr Respaud and his team followed this strategy to contribute in the development of a nebulized anti-ricin mAb. This was summarized in the last part of his talk, focusing on the effect of formulation on the antibody’s stability. Their results indicate that high antibody concentrations could play a protective role against aggregation upon nebulization. Besides, they confirmed that mesh nebulizers were not equal in terms of antibodies preservation, with an influence of the device on the proportion of large aggregates. They also demonstrated that surfactants successfully protected antibodies from aggregation during nebulization. The results of these experiments, which were obtained with polyclonal antibodies due to the limited supply of the mAb, were validated with the therapeutic monoclonal immunoglobulin. To conclude, Dr Respaud highlighted the paramount importance of formulation to stabilize antibodies during nebulization. He also reminded participants that specific optimization is required for each “drug and device” couple. After a few questions from the audience, participants debated about the quite surprising protective effect of high antibody concentrations against aggregation. Dr Respaud suggested that there may be a variable spatial distribution of antibodies within the aerosol droplet, in function of their concentration. Further studies are needed to confirm or refute this model.

Dr Sophie Carayon (Sanofi, Vitry-sur-Seine, France) then discussed on the challenges related to highly concentrated formulations (HCFs) of biopharmaceuticals. Indeed, the current trend toward HCFs is driven by an increase in demand for the SC route, owing to its numerous assets. In making HCFs for SC products, stability is a major challenge and particularly with regards to aggregation, which results from complex mechanisms, originating from a molecular crowding effect and a reduction of intermolecular distances. Increasing concentrations also result in higher viscosities, which have implications in manufacturing and drug delivery, and thus may limit the development of antibodies HCFs. Dr Carayon illustrated these challenges through 2 case studies. The first one, about a bispecific antibody (Bi-mAb), exemplified the aggregation induced by protein concentration. In the liquid form, the SC Bi-mAb was very prone to aggregation with increasing concentrations, likely due to higher probabilities of bi-molecular collisions. At high concentrations, these increased aggregation levels were associated with steeper viscosity increases upon storage. Finally, syringeability studies investigated the drug’s suitability for SC injection and the acceptable range of viscosity, compatible with a SC injection. The second case study, about an IgG4 antibody, focused on the effect of viscosity on SC delivery. As part of the development, it was showed that viscosity could be modified by small concentration or temperature variations, which might potentially alter the delivery time and the dose accuracy. Hence, viscosity variations must be considered during process development and delivery system definition. Dr Carayon concluded that the major challenges while developing HCFs are aggregation and viscosity increase, which are interlinked and concentration-dependent phenomena. The successful development of HCFs and the increase in SC injection volumes should offer IV-to-SC conversion opportunities to many antibodies depending on the indications, aiming at improving patients’ compliance. This presentation raised
several questions from the audience, focused on HCFs manufacturing. Dr Carayon explained the need to carry on HCF development very closely with the development and manufacturing teams to anticipate scale-up. It was also pointed out that analytical methods may require specific development and the use of alternative techniques adapted to high concentrations.

Prof. Randy Mrsny (University of Bath, UK) ended the session and the workshop scientific program with a presentation on a strategy to facilitate the oral delivery of biopharmaceuticals. This approach is based on biologic principles used by pathological microorganisms to deliver exotoxins through the intestinal epithelial cells via a transcytosis pathway that hijacks endogenous cell receptors. With physical and chemical methods now available to protect biopharmaceuticals as they pass through the stomach, the huge surface area of the small intestine now represents a great opportunity for oral drug delivery. Prof. Mrsny described the discovery and mechanistic dissection of the natural transport of certain microbial exotoxin proteins capable of transporting across intestinal epithelium to target cells involved in antigen presentation found in the lamina propria. Transcytosis of these exotoxins involves receptor-mediated endocytosis and intracellular vesicular trafficking involving a ganglioside second receptor that is engaged by endosomal acidification. In vitro assays were performed with a growth hormone-carrier chimera to validate apical to basolateral transport. Recovery of intact protein, free from its cargo, in the basolateral compartment confirmed the capacity of the system to deliver it across a polarized monolayer of Caco-2 cells. This test also demonstrated uni-directional transport by this carrier-cargo strategy. In vivo studies performed with Rhesus macaques showed that the exotoxin-based carrier could readily deliver growth hormone into mesenteric veins after injection of the growth hormone-carrier chimera into specific segments of the small intestine (experiments performed under surgery).

Prof. Mrsny also discussed the flexible nature of the carrier applications from peptides to nanoparticles and presented an in vitro model of siRNA delivery through a Caco-2 cells monolayer. He concluded by emphasizing the fact that, although formulation is still a big challenge to protect the biopharmaceuticals from stomach digestion, these in vitro and in vivo examples are new tools to explore the potential for proteins oral delivery based on basic biologic principles. Questions from the audience allowed Prof. Mrsny to give his point of view on the prospect of oral formulations applied to biopharmaceuticals, through 2 examples. For insulin, he argued that oral delivery seems rather unrealistic because of the need to get a precise dose at the right timing; conversely, he announced that one mAb was in queue for assays in his laboratory, making the perspective of mAbs tablets quite reasonable in the near future.

Conclusion

This industrial workshop was concluded by Prof. Hervé Watier (CNRS UMR 7292, Université François-Rabelais of Tours, France), coordinator of the LabEx MAbImprove and of the regional program ARD 2020 Biopharmaceuticals. He expressed his satisfaction that the workshop fulfilled its objectives: as a crossroads between different actors of the biopharmaceuticals dynamics, including small companies and startups, it aroused fruitful discussions between industrial and academic experts on common problematics. He invited the participants to meet again at the next edition in Montpellier, which will focus on new antibody formats, and ended by thanking the speakers, the session moderators, the attendees, Polepharma and the LabEx team for their participation to the meeting.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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