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MEETING REPORT

Therapeutic monoclonal antibodies for respiratory diseases: Current challenges and perspectives, March 31 – April 1, 2016, Tours, France

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ABSTRACT

Monoclonal antibody (mAb) therapeutics have tremendous potential to benefit patients with lung diseases, for which there remains substantial unmet medical need. To capture the current state of mAb research and development in the area of respiratory diseases, the Research Center of Respiratory Diseases (CEPR-INSEM U1100), the Laboratory of Excellence “MABImprove,” the GDR 3260 “Antibodies and therapeutic targeting,” and the Grant Research program ARD2020 “Biotherapeutics” invited speakers from industry, academic and government organizations to present their recent research results at the Therapeutic Monoclonal Antibodies for Respiratory Diseases: Current challenges and perspectives congress held March 31 – April 1, 2016 in Tours, France.

On March 31, 2016, the meeting Monoclonal Antibodies for Respiratory Diseases (TMARD, http://tmard.sciencesconf.org/): Current challenges and perspectives was opened by Hervé Watier (Co-Director of LabEx MABImprove), Nathalie Heuze-Vourc'h (President of TMARD scientific committee), Patrice Diot (Dean of Tours School of Medicine) and Pierre Commandeur (Vice-President of Center-Val de Loire region), who welcomed participants and thanked the organizers and both institutional and industrial sponsors.

The first speaker, Janice M. Reichert (The Antibody Society; Reichert Biotechnology Consulting LLC), discussed monoclonal antibody (mAb) therapeutics in development for respiratory disorders, as well as neurological, infectious, cardiovascular / hemostasis diseases. While antibody therapeutics for cancer and common immune-mediated disorders, e.g., rheumatoid arthritis, psoriasis, Crohn’s disease, are discussed frequently, development of mAbs for these other therapeutic areas are not often the focus of attention. To provide context, Dr. Reichert...
noted that global antibody therapeutics research and development by the biopharmaceutical industry has undergone remarkable expansion in the past ~5 years. Resulting from a substantial dedication of effort and resources, over 100 novel antibodies entered first clinical studies in 2015, and the overall clinical pipeline now includes ~480 antibodies. Importantly for patients, these molecules are progressing through the phases of clinical testing, and being approved for marketing. To date, over 50 antibody therapeutics for a variety of diseases are in Phase 3 studies.1

A record number, i.e., 9 new products, were granted first marketing approvals in the United States or European Union in 2015, and the evidence suggests that a similar number may be approved in 2016. As examples, Dr. Reichert provided details for obiltoxaximab, ixekizumab and reslizumab, 3 mAB products already approved by the Food and Drug Administration in 2016. Obiltoxaximab (Anthim®), targeting the protective antigen of Bacillus anthracis exotoxin, is indicated in adult and pediatric patients for treatment of inhalational anthrax due to Bacillus anthracis in combination with appropriate antibacterial drugs, and for prophylaxis of inhalational anthrax when alternative therapies are not available or appropriate. Ixekizumab (Taltz®), targeting interleukin IL17A, is indicated for treatment of adults with moderate-to-severe plaque psoriasis, whereas reslizumab (Cinqair®), targeting IL5, is indicated for severe asthma in adults.

Dr. Reichert then focused on antibodies developed for respiratory, neurological, infectious or cardiovascular / hemostasis diseases, which, excluding cancer and common immune-mediated disorders, are the therapeutic areas that include the most mAbs in development. Neurological disorders represent the largest area (27 mAbs; 11 in Phase 2/3 or Phase 3 studies), followed by cardiovascular / hemostasis (25 mAbs; 6 in Phase 3 studies), infectious disease (24 mAbs; 3 in Phase 3 studies) and respiratory disease (22 mAbs, 4 in Phase 3 studies). Most (~90%) mAbs in development for these therapeutic areas are canonical IgG1, IgG2, IgG4 or IgM that may have been Fc- or glyco-engineered. In contrast, relatively few, i.e., less than 20 mAbs, have non-canonical formats, e.g., bispecific, antibody fragments (domain, nanobody, single-chain variable fragment (scFv), Fab). In the respiratory disorder area, Dr. Reichert discussed 4 mAbs that are undergoing evaluation in Phase 3 studies for asthmatic patients: anti-IL5 receptor benralizumab, anti-IL4 receptor α dupilumab, anti-IL13 lebrikizumab, and anti-IL13 tralokinumab. Benralizumab is also undergoing evaluation in Phase 3 studies of patients with chronic obstructive pulmonary disease (COPD). Thus, Dr. Reichert noted that this latter is an “antibody to watch” in 2016, because its results for 4 Phase 3 studies of asthma patients are expected in 2016, and, if they are positive, marketing application submissions may occur later in the year.

In concluding her talk, Dr. Reichert emphasized that the recent increase in the number of mAbs in clinical studies is expected to drive a trend toward first approvals for ~6–8 new mAbs per year (or more), including mAbs for respiratory disorders. She cautioned that the sustainability of this trend depends on the verification of expected increases in potency of engineered antibodies and bispecific antibodies, and the validity of the novel targets for mAbs in development. However, if the recent past reflects the near future, unmet medical need should be reduced and patient choices for antibody therapeutics should increase in the next ~8 years, which is the average time for mAb clinical development.

Session 1: Anti-infectious monoclonal antibodies

Matthew Sleeman (MedImmune) opened the session dedicated to anti-infectious mAbs with a talk entitled ‘Targeting Pathogens’. Increasingly, mAbs targeting different cytokines, including IL13 and IL5, are being considered as therapeutic options for the treatment of severe respiratory conditions such as asthma, idiopathic pulmonary fibrosis (IPF) and COPD. While many of these anti-cytokine approaches demonstrated promise, the majority of hospitalizations in these diseases are caused by common pathogens triggering exacerbations of their conditions. Therefore, therapies directly targeting pathogens may provide significant benefit. He showed first that, due to antigenic diversity, direct targeting of pathogens has been challenging and requires a detailed understanding of the stable proteins on a pathogen surface that can be accessed by mAbs. One such example is the antibody palivizumab (Synagis®), which binds to the key target fusion-protein of respiratory syncytial virus (RSV). It has been approved for the prevention of infections in premature infants. In addition to this, anti-RSV vaccines have been generated that, if successful, could provide longer term protection for at risk individuals. MAb therapies, such as palivizumab, are unfortunately the exception rather than the rule in the prevention or treatment of infectious disease. Thereafter, Dr. Sleeman discussed an alternative approach, which is the design of antibodies to the host co-receptor to prevent viral entry and infection. To illustrate his talk, he presented the human rhinovirus (HRV), which is responsible for the common cold and virally-driven respiratory exacerbations in asthma and COPD. HRV is made up of 3 distinct clades: HRV-A, HRV-B and HRV-C consisting of greater than 167 distinct serotypes. With such diversity, the ability to design a mAb that would neutralize all serotypes is extremely challenging; a different option could be to target one of the co-receptors: intercellular adhesion molecule 1 (ICAM-1), low-density lipoprotein receptor (LDLR) or cadherin-related family member 3 (CDHR3). Using mouse models of HRV infection, Dr. Sleeman showed that ICAM-1 was elevated on the bronchial epithelium of the lung, and that an ICAM1 neutralizing antibody (14C11) could prevent HRV driven lung inflammation, viral infection and cytokine production whether the antibody was administered directly to the airways or was given systemically.2 In addition, he also showed published data that soluble-ICAM1 given to healthy human volunteers pre-inoculation with HRV significantly reduced daily symptoms scores compared with placebo.3 While these data support the hypothesis of using the co-receptors as a target to prevent viral infections, significant questions remain, especially in the context of a respiratory exacerbations, such as which viral serotypes triggers the exacerbations, could non-HRV viruses, bacteria or fungal pathogens be the cause of some exacerbations or would one need to target all 3 co-receptors for maximal impact? Finally, Dr. Sleeman considered targeting the downstream pro-inflammatory molecules produced as a consequence of pathogen invasion in the lungs. One of these key targets gaining substantial interest is the cytokine
IL33, which is highly expressed in the epithelium of the lung and rapidly released upon lung infection with, for example, influenza-A, RSV or HRV. Using a mouse model of COPD, he also went on to show that chronic cigarette smoke (CS) exposure caused an accumulation of IL33 in the epithelium, and that this was rapidly released following an influenza infection, causing chronic inflammation. Furthermore, he showed that this response was significantly blunted in mice deficient in either IL33 or interleukin 1 receptor-like 1 (ST2). The association with this cytokine and respiratory exacerbations has also been shown in samples from severe asthmatics. In conclusion, targeting respiratory pathogens with mAbs is a distinct possibility and has been clearly demonstrated with molecules such as palivizumab; however, significant but not insurmountable challenges remain. Firstly, isolating neutralizing antibodies to many pathogens is complex and challenging; secondly, targeting co-receptors is a distinct possibility, but there could be significant redundancy where a pathogen may use different or multiple co-receptors; or thirdly targeting downstream pathways of infection could provide benefit, but may require a personalized healthcare approach to identify the key pathways that predominate in any one individual.

Thomas Secher (INSERM U1220-IRSD) presented results on panobacumab, a human IgM against Pseudomonas aeruginosa serotype O11 lipopolysaccharides. Panobacumab is able to reduce P. aeruginosa burden in lungs by enhancing neutrophil recruitment and reducing the host-derived production of pro-inflammatory mediators, and thereby reduces lung injury in a murine model of lung infection, whatever the immune status. Regarding its additional effects when given in association with meropenem, panobacumab may be combined with standard antibiotic therapy. These encouraging preclinical data have been recently confirmed in a short-scale Phase 2 clinical trial in which the full treatment of panobacumab was shown to induce a complete resolution in patients presenting with nosocomial P. aeruginosa pneumonia compared to untreated individuals.

Session 2: Anti-cancer monoclonal antibodies

Karen L. Reckamp (City of Hope Comprehensive Cancer Center) started the second session of the congress by reminding attendees how clinical trials using mAbs in lung cancer have recently improved patient outcomes. She began her talk with a discussion of the anti-vascular endothelial growth factor (VEGF) bevacizumab (Avastin®), which was approved for treatment of non-small cell lung cancer (NSCLC) a decade ago. The initial Phase 3 trial, which compared carboplatin and paclitaxel with and without bevacizumab in patients with advanced NSCLC with non-squamous histology, demonstrated a statistically significant improvement in objective response rate (ORR) and progression-free survival (PFS) with the addition of bevacizumab. It was also the first study to report a median overall survival (OS) greater than 12 months in such a population. A subsequent 3-arm Phase 3 trial investigated cisplatin and gemcitabine with placebo or bevacizumab at 2 dose levels in patients with similar NSCLC; patients assigned to the bevacizumab arms had statistically superior ORR and PFS.

Dr. Reckamp then showed results of the Phase 3 trial in which docetaxel with placebo or ramucirumab (Cyramza®), a mAb against the extra-cellular domain of VEGF-receptor 2, was administered to patients who had experienced disease progression after platinum-based therapy. Statistically significant higher ORR, longer PFS and longer OS were observed with ramucirumab. In all, predictive biomarkers for anti-angiogenesis antibodies have not been identified. Afterwards, Dr. Reckamp reported the multiple clinical trials that have evaluated anti-epidermal growth factor receptor (EGFR) mAbs in combination with chemotherapy to enhance the efficacy of cytotoxic therapy. For instance, a Phase 3 trial investigated cisplatin and gemcitabine with and without necitumumab (Portrazza®), a mAb against the extra-cellular domain of the EGFR in patients with advanced NSCLC with squamous histology. Those who received necitumumab had a similar response rate, but a statistically significant longer PFS and OS, while a similar trial in patients with advanced non-squamous NSCLC failed to demonstrate improved efficacy.

Thereafter, Dr. Reckamp showed how anti-programmed death 1 (PD-1) mAbs, like nivolumab (Opdivo®), have improved survival as monotherapy for patients with NSCLC as second-line therapy compared to docetaxel. A Phase 3 study of nivolumab versus docetaxel in squamous cell histology showed an improvement in OS of 9.2 months vs. 6 months, respectively. Nonetheless, in these subjects, PD-1 ligand (PD-L1) expression did not appear to be prognostic or predictive of patient outcome at any level. In addition, the Phase 3 trial in patients with previously treated non-squamous histology showed that median OS was significantly improved at 12.2 months for nivolumab and 9.4 months for docetaxel. In contrast with the previous trial, in this study PD-L1 expression was predictive of clinical benefit, although the OS was also similar in both arms when PD-L1 was not expressed. Then, Dr. Reckamp provided details on pembrolizumab (Keytruda®), a highly selective humanized IgG4-kappa mAb against PD-1: a single arm trial enrolled 495 NSCLC patients and correlated PD-L1 expression with response to treatment. In a Phase 2/3 randomized trial assessing pembrolizumab compared to docetaxel in patients with previously treated advanced NSCLC who were PD-L1 positive, the median OS was significantly longer for both doses of pembrolizumab. The OS was substantially longer in those who had at least 50% PD-L1 expression in tumor cells. Atezolizumab is another mAb that prevents the binding of PD-L1 to PD-1 and CD80, in addition to preserving the interaction between PD-L2 and PD-1. It demonstrated efficacy when compared to docetaxel in NSCLC in a randomized Phase 2 study. To note, activation of the immune system to produce antitumor responses leads to distinct toxicities related to immune stimulation. Dr. Reckamp underlined the numerous immune-related adverse events in clinical trials and practice, which are greater with cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) inhibition compared to anti-PD-1 and anti-PD-L1 agents, although all these mAbs may result in life-threatening toxicities. She concluded that antibody-based therapy for lung cancer is well-established, and new treatments with antibodies have demonstrated improved efficacy, confirming the success of
this modality. Antibody-drug conjugates (ADC) are emerging agents in clinical trials that may lead to new therapeutic options in the future.

Rita De Santis (Sigma Tau SpA) drew the attention of the audience with her talk about AvidinOX – conjugated biotinylated-antibody to treat cancer. AvidinOX is an oxidized version of avidin that forms Schiff’s bases with tissue proteins, thus constituting an artificial stable receptor for biotinylated therapeutics. Biotinylated anti-cetuximab and anti-panitumumab antibodies, which both target EGFR, showed anti-tumor efficacy at a dose lower than 1/25,000 the intravenous (IV) effective dose, when delivered after AvidinOX through the airways, in an experimental model of lung cancer in severe combined immunodeficiency (SCID) mice. She explained the effect was due to the inability of EGFR to dimerize, and subsequent inhibition of signaling. She also emphasized the excellent tolerability of AvidinOX in animals. Dr. De Santis concluded that AvidinOX offers a great opportunity to treat lung tumors using aerosol delivery.

Session 3: Monoclonal antibodies in asthma

Opening the third session, Bernhard Ryffel (INEM - UMR7355; CNRS) reviewed clinical asthma and recent progress with therapeutic antibodies for severe and steroid resistant asthma, highlighting bi- and multi-specific antibodies. Novel mechanisms of allergic asthma focusing on Th2 and Th17 polarized immune responses were discussed, together with recent discoveries that pyrin domain containing 3 (NLRP3) may act as a Th2 transcription factor enhancing Th2 dependent asthma in mice. By contrast, the NLRP3 inflammasome complex is required for IL1/IL17-dependent asthma. The IL1 family cytokine IL33 is involved in the Th2 response, since antibody neutralization reduces asthma, while the NLRP3 inflammasome is a negative regulator dampening allergic asthma. In addition, RORγt-dependent production of IL17A/F and IL22 by ILC3 and T-cells drives severe neutrophilic asthma, representing the interplay of microbiota, nutrition, infection and the immune response, new preventive/therapeutic options are likely to emerge. Selected food, probiotics, recombinant bacilli, and microbial metabolites such as short chain fatty acids may have anti-inflammatory effects and could be used as complementary prevention or therapy. Reduced production of tryptophan metabolites by microbiota from caspase activation and recruitment domains 9 (CARD9)-deficient mice and patients with Crohn’s disease diminish aryl hydrocarbon receptor (AhR) activation and increase inflammation. Therefore, the administration of AhR agonists inducing an AhR-dependent protective IL22 response may be beneficial in asthma. Dr. Ryffel also underlined the use of germfree and mono-colonization with protective bacterial species, which may provide new insights on the protective effect of Clostridia controlling inflammatory Bacteroidetes species. However, investigation by bacterial depletion with antibiotics is problematic due to selection of resistant strains.

Dr. Ryffel then reminded the attendees that environmental factors, including air pollution, chemicals, tobacco smoke, ozone and respiratory infections, are other factors influencing the allergic respiratory responses. Experimental studies in mice exposed to ozone develop severe, neutrophilic, steroid resistant airway hyper-reactivity and inflammation representing a major therapeutic challenge. Preliminary data using ozone exposure suggest that this type of severe asthma is sensitive to M3 muscarinic antagonists, but IL17 targeting might also be considered. In addition to classical immune mediators, the targeting of neural reflexes, central nervous system mediators/neuropeptides could represent another exciting and promising approach. The preclinical investigation of novel human targeted therapeutics is required prior to use in patients. Human mAbs, but also single chain antibodies, dominant negative inhibitors, nanofibres, fusion proteins and lipocalins targeting specific human proteins may be tested in non-human primates, if there is sufficient cross-reactivity. The recent development of humanized immune system mice and human knock-in mice, in which mouse protein is replaced by the human analog of, for example, tumor necrosis factor (TNF), CD20, CD64, B-cell activating factor (BAFF), or neonatal Fc receptor (FcRn), are available and may be helpful. These novel humanized mice will enable in vivo investigations of efficacy, pharmacokinetics and, importantly, novel routes of administration such as inhalation. In conclusion, Dr. Ryffel added that basic research leading to new therapeutics that neutralize key-mediators is emerging, which may improve the control of allergic asthma and reduce steroids.

Harshad P. Patil (Université Catholique de Louvain) began his talk by reminding attendees of the deleterious role of IL17 in asthma. He showed the benefit of an anti-IL17 Fab’ antibody associated with high molecular-weight polyethylene glycol (PEG, 20–40 KDa) and delivered locally into the lungs to achieve local targeted activity against inflammation. The PEGylated anti-IL17 Fab’ substantially reduced neutrophils recruitment, lactate dehydrogenase (LDH) and TNF in bronchial-alveolar lavages (BAL) of asthmatic mice. Moreover, weekly pulmonary delivery of 20 µg PEGylated anti-IL17 Fab’ was as effective as 10 times-higher doses of non-PEGylated anti-IL17 Fab’ given by subcutaneous route. Dr. Patil also revealed that PEGylation prolonged residency of anti-IL17 Fab’ in the luminal side of the lungs, through different mechanisms like enhanced proteolytic resistance, attachment to the mucus and escape from alveolar macrophages. These findings are promising for the future development of anti-asthma strategies.

Session 4: Antibody format

Opening session 4, Jörg P. Adamczewski (Sanofi R&D) gave an overview on bi- and multi-specific antibodies from a drug developer’s perspective. He raised 2 key questions that were discussed on the basis of current examples from multiple therapeutic areas: 1) how to exploit the biological effects of a multi-specific antibody to achieve a therapeutic effect, and 2) what antibody format to choose for a desired outcome. Bispecific antibodies have 2 biological effects: they bind to 2 targets simultaneously and bring them into spatial proximity. Therefore, which of these mechanisms is exploited to achieve the desired therapeutic effect defines the 2 basic classes of such antibodies. However, even if the spatial action is not the aim, it needs to be considered as a potential source of undesired biological outcomes, especially if neither target is a soluble protein.
Dr. Adamczewski reminded attendees that multi-targeting antibodies typically block 2 or more receptors or ligands to inhibit parallel pathways leading to converging biological or pathophysiological processes. However, they can also be used to target an escape pathway at the same time as the principal target, or to achieve deep inhibition by blocking the same pathway at 2 points. As specific example, SAR156597 is a tetravalent bispecific antibody that targets the 2 Th2 cytokines IL4 and IL13. Both cytokines have been implicated in pathophysiology of IPF, a progressive fibrotic disease of the lungs in which 3 key cell types play a possible role in the disease pathogenesis, airway epithelial cells, lung fibroblasts and lung macrophages, making them an attractive target for parallel pathway blockade. He pointed out that SAR156597 is currently the only multi-specific antibody in clinical development for non-infectious respiratory disease, with a Phase 2 proof-of-concept trial in IPF (clinicaltrials.gov/ct2/show/NCT02345070) ongoing.

Dr. Adamczewski then highlighted the interest in using bispecific antibodies to bring 2 targets into physical proximity. Spatial use is the most common approach for anti-cancer immune cell engagers, in which the antibody activates an immune effector cell, typically a cytotoxic T-cell targeted on CD3, and homes it to a surface antigen on a tumor cell, resulting in cell death. The approval of blinatumomab (Blincyto®), targeting both CD3 and the B-lineage antigen CD19, for relapsed/refractory acute lymphoblastic leukemia demonstrated the power of such T-cell engagers.29 An equivalent approach can be used to force the association of 2 proteins, as illustrated by emicizumab, which induces binding of factor Xa to factor X in the absence of sufficient factor VIII, thus restoring blood coagulation. A Phase 3 trial in hemophilia A patients is ongoing (clinicaltrials.gov/ct2/show/NCT02622321). The speaker explained that optimization for different biological properties, but also parallel historical developments, led to over 50 bispecific formats, allowing fine-tuning for the desired biological effect, e.g., pharmacokinetics (PK), including tissue penetration, antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependant cytotoxicity (CDC). Stable symmetric, IgG-like formats such as the dual-variable-domain (DVD) format class or tetravalent bispecific tandem immunoglobulin (TBTi) including SAR156597, combine longer PK, the option of effector functions and greater ease of process development over smaller constructs based on antibody fragments, which may have superior tissue penetration. Dr. Adamczewski indicated that Sanofi’s third-generation crossover dual variable IgG-like proteins (CODV) bispecific format allows greater versatility and maintains full binding specificity of both parental antibody sequences.30 He concluded that multi-specific antibodies are thus a versatile tool to achieve powerful therapeutic effects that currently seems underutilized in respiratory diseases, and may provide additional opportunities for patient benefit in the future.

Next, Jean-François Gestin (Inserm U892 – CNRS 6299) gave an overview of the radiolabelled companion diagnostic tests that he introduced as non-invasive “imaging biopsy-like” tools. For instance, he explained that radiolabelled vector 160-[18F]Fluoro-17β-estradiol ([18F]FES) has proven to be a valuable tracer for the studies of the estrogen receptor (ER) status of primary and metastatic breast cancer. Dr. Gestin then focused on other impressive examples related to radiolabelled companion diagnostic tests, like anti-human epidermal growth factor receptor 2 (HER2) antibody ([68Zr-trastuzumab or [64Cu-DOTA-trastuzumab) to determine the HER2 status in primary breast tumor, but also PK and accessibility to adapt the treatment in case of relapse and metastasis.31,32 He concluded that radiolabeled companion diagnostics, like radiolabeled mAbs, are supporting tools to aid therapeutic decisions, ensuring effective selection of patient eligible for treatment and giving individuals more effective and safer treatments. They may be of interest for lung cancer patients.

Session 5: Inhaled antibodies

Theresa Sweeney (Nektar Therapeutics) opened the session focused on use of the pulmonary route to deliver mAbs for respiratory diseases. She noted that mAb therapy is well accepted for the treatment of cancer and auto-immune disease, but few mAbs are approved for the treatment of respiratory disease and none are delivered by the pulmonary route. In theory, inhalation delivery offers the advantage over intravenous delivery of a high dose delivered directly to the target organ, i.e., lungs, while limiting systemic exposure and potential side effects. Yet until recently, the delivery efficiency of inhalation devices and the added cost of therapy have limited the development of inhaled biologics for pulmonary disease. Dr. Sweeney reviewed the history of inhaled mAbs delivery by describing the experience with anti-IgE (omalizumab, Xolair®) for the treatment of asthma. She described the rationale for inhalation delivery of anti-IgE and the data that supported the targeting of lung IgE over systemic IgE.33 Anti-IgE was not successful in ameliorating the broncho-constrictive response in mild asthmatics when delivered by inhalation despite a high lung dose, suggesting that both lung and systemic IgE neutralization was required for efficacy. Full-length mAbs have low bioavailability,35 and smaller forms could offer advantages because they may better penetrate tissues to reach target receptors. Dr. Sweeney suggested that recent advances in the pathogenesis of asthma and COPD may provide better understanding of therapeutic targets, and that the development of new technologies may offer improvements over full-length mAbs. Lower molecular weight and more potent forms of antibodies are being evaluated for topical delivery, including an anti-IL13 Fab for the treatment of asthma36 and a highly potent nanobodyTM to treat RSV infection.37 Dr. Sweeney concluded that improved knowledge of therapeutic targets, antibody fragments, potent molecules, and more efficient delivery systems are important for the success of inhalation delivery for the treatment of pulmonary disease.

Next, Rita Vanbever (Université catholique de Louvain) unraveled the fate of pulmonary-delivered mAbs. She noted that a major drawback of inhalation is the short residence time of antibodies in the lungs as they are mostly cleared from the lungs within one to 2 d.38,39 In contrast, plasma half-lives of full-length antibodies after injection reach 3 weeks and more.38 Accordingly, most antibodies developed for the treatment of respiratory diseases are delivered by injection, except for an inhaled single-domain antibody (Nanobody®) specific for RSV fusion protein. Because injection presents limitations, including high delivered doses, low antibody penetration in the lungs and
potential systemic side effects, she explained that inhalation of antibody therapeutics deserves further investigation. Based on that, Dr. Vanbever presented her results on antibody formats that would allow the longest retention within the lungs. Compared with full-length antibodies, engineered antibody fragments present advantages, such as enhanced tissue penetration, binding to cryptic epitopes, multi-specific actions and economical production in bacteria. A F(ab)₂ and a Fab' antibody fragments were shown to be as quickly cleared from the lungs as a full-length antibody. Therefore, full-length antibodies do not provide any apparent lung pharmacokinetic advantage over these antibody fragments. Interestingly, she showed that conjugation of antibody fragments to one large molecular weight PEG chain sustained the residence time of the proteins within the lungs. High levels of PEGylated antibody fragments persisted in the lungs for more than 2 d post-delivery. All antibody constructs were principally located within the airway lumen rather than the lung parenchyma. In addition, PEG increased pulmonary retention of antibody fragments through muco-adhesion and escape from alveolar macrophages, rather than by increased hydrodynamic size or improved enzymatic stability. The deeper the deposition site of the antibody constructs within the lungs, the longer the molecules were retained. A two-armed 40 kDa PEG better prolonged residence time than a linear 20 kDa PEG, although the effect of PEG molecular weight was slight. Dr. Vanbever concluded by showing that the increased pulmonary residency of antibody fragments through PEGylation was confirmed in 3 small animal species.

Finally, Renaud Respaud (CEPR-INSERM U1100) showed evidence to support that delivery of full-length mAbs through the airways is feasible and relevant in the treatment of respiratory diseases. When they are delivered through the pulmonary route, mAbs achieved a therapeutic response, in some experimental conditions, greater than when they were delivered systemically. Although the fate of mAbs within the lungs remained unclear after airway delivery, they passed slowly and poorly into the systemic circulation. Dr. Respaud also highlighted the importance of mAb stability during aerosolization, and showed that mesh nebulizers prevented better mAbs degradation and concentration. Addition of surfactant was critical to maintain mAb molecular integrity and pharmacological activity during vibrating-mesh nebulization.

**Concluding remarks**

Patrice Diot (Dean of Tours School of Medicine; CEPR-INSERM U1100) concluded the first day of the TMARD symposium by reiterating the basics and challenges related to respiratory medicine. He started by summarizing the challenges in medicine, from the beginning of its history to the current times, i.e., 1) to take into account the epidemiology of the diseases, and to establish priorities to set up public health programs; 2) to understand their pathophysiology, which has eventually allowed over time to simplify the concepts and to make bridges between various types of disorders; 3) to establish the accurate diagnosis; 4) to prescribe the efficient treatment; and 5) eventually to establish and communicate the prognosis, at the level of individuals and of communities. He underlined that respiratory medicine is special because it mainly concerns diseases which, in terms of epidemiology, are at the 2 extremes, either very frequent, or on the opposite very rare or even orphan, and which, in terms of treatment, can often not be cured nowadays. As examples of frequent, severe and non-curable respiratory diseases worldwide, COPD is projected to be the third leading cause of death by 2020, while lung cancer is currently the first cause of death by cancer, and 16% of children are suffering from asthma. In contrast, cystic fibrosis and IPF seem rarer, extremely severe, and non-curable diseases.

In terms of their pathophysiology, Pr. Diot said that: “respiratory diseases, as diseases of any other system in fact, are very simple, as they basically correspond to genetic, infectious, inflammatory or cancer disorders.” He pointed out that the concept of the accuracy of a treatment, in medicine in general, is a mixture of its efficiency, side effects, convenience, and price. There have been several periods in the history of medicine with regards to the development of therapeutics: the chemical period, with for example the major advances represented by the discovery of aspirin, penicillin, or streptomycin; then the robotic advent which allowed major advances in surgery; and thereafter the digital period. More recently, personalized therapies, like those based on mAbs, have arisen, especially in respiratory diseases. Pr. Diot concluded that several challenges with regards to the proliferation of mAbs in therapeutics, e.g., the target(s), the physico-chemical characteristics, the route of administration, the safety and the price, and acknowledged that all these aspects have been addressed during the various sessions of the symposium.

**Session 6: Animal models in respiratory diseases**

On the second day, Caroline Owen (Brigham and Women’s Hospital; Harvard Medical School) started by outlining the healthcare burden associated with COPD. She pointed out that, despite over 50 y of research on COPD, we still lack any disease-modifying medical therapies. She described animal models of COPD that have been used to test hypotheses on pathogenesis or the efficacy of novel therapies for this disease: the current gold standard model for COPD is to expose mice to CS for 6 months. Dr. Owen described the advantages of CS-exposed mice as a model system, including the fact that: 1) they develop some features of the human disease (pulmonary inflammation, small airway fibrosis and airspace enlargement); 2) their genome can be manipulated to delete or over-express single genes; 3) they breed rapidly; 4) they are relatively inexpensive to house and study; and 5) tools to study their pathways are generally commercially-available. Dr. Owen then enumerated some disadvantages of CS-exposed mice as a model system for COPD, including the major differences in the anatomy and immunology between humans and mice, the fact that mice develop minimal COPD-like airway disease, they do not develop mucus hyper-secretion like in chronic bronchitis, and the fact that longitudinal blood and lung sampling is challenging or not feasible in mice. Dr. Owen also highlighted that therapies that had efficacy in limiting the progression of COPD-like lung disease in CS-exposed mice were subsequently shown not to have efficacy in limiting disease progression in human COPD patients in randomized clinical trials.
Dr. Owen then discussed the anatomical and immunological relevance of non-human primates (NHP), and thus how they could have potential as a new animal model of COPD. Thus, she went on to describe a novel NHP larger animal model of COPD-like airway disease that she and her colleagues have recently characterized. After presenting the methodology, she showed that NHPs exposed to CS for 12 weeks did not lose weight, but developed robust COPD-like airway disease, including airway epithelial mucus cell metaplasia, submucosal gland hypertrophy and hyperplasia, had higher airway leukocyte counts, i.e., macrophages, polymorphonuclear (PMN) leukocytes, and lymphocytes, small airway fibrosis, increases in peri-bronchial lymphoid aggregates, and robust reductions in airway Club cell protein-16 (CCL16) immunostaining similar to that occurring in human COPD airways. Although CS-exposed NHPs did not develop emphysema or increases in lung compliance by 12 weeks, they exhibited changes that contribute to emphysema development including increases in: 1) BAL fluid levels of matrix metalloproteinase-9 (MMP-9), interleukin-8 and CCL2; 2) alveolar macrophage MMP-12 levels; 3) parenchymal macrophage, PMN, and lymphoid aggregate counts; 4) lung oxidative stress levels; and 5) alveolar septal cell apoptosis. CS-exposed NHPs also had a strong trend toward reduction in forced expiratory volume in 0.1 second, which may reflect the small airway disease that develops after 12 weeks of CS exposure. Dr. Owen concluded that CS-exposed NHPs have considerable potential as a model of airway disease phenotypes occurring in COPD patients, especially chronic bronchitis and small airway fibrosis. Unlike mice, NHPs can safely undergo longitudinal sampling in both blood and lung compartments, which could be useful for validating novel biomarkers for COPD, and performing PK and pharmacodynamics (PD) studies of novel therapeutics for COPD-like airway disease.

Next, Frédéric Ducancel (iMETI; DRF; CEA; U1184; IDMIT) presented the IDMIT facility for “Infectious Disease Models and Innovative Therapies” (IDMIT, http://www.idmit.center.fr) dedicated to preclinical studies using NHP models. He emphasized that these latter are under development in the field of respiratory medicine to test different prophylactic and/or therapeutic candidates for human influenza, whooping-cough, tuberculosis and anthrax. For instance, a model of flu infection is being carried out in collaboration with the Baylor Institute for Immunology Research and Tulane University to study vaccine proteins engineered to specifically target skin and mucosal dendritic cells. The goals are to characterize the early molecular and cellular mechanisms at the skin level following vaccination by in vivo imaging to evaluate immune-induced to vaccine-candidates, and to compare/establish the protective efficacy of vaccine versus challenging with various flu virus strains.

**Session 7: Relevance of animal models**

Clive Page (King’s College of London) gave an engaging lecture, picking up on Pr. Diot’s points and enlightening attendees on the strengths and limitations of animal models to support drug development for respiratory diseases and predict translation to humans. First, he mentioned that the mouse is increasingly being utilized as a model to investigate the pathogenesis of asthma and COPD, and to help in the search for novel treatments. However, there is growing concern about how predictable murine models are in selecting new treatments because there has been a catalog of failures of potential new drugs based on work in the mouse. He explained that a major problem is that much of our understanding of allergic asthma has heavily relied on allergic models in the mouse but there is very little consistency between laboratories concerning the protocols used for sensitization and challenge. The superficial attractiveness of the mouse is the availability of tools and reagents, particularly to investigate the immunological basis of allergic inflammation. However, as stated before by Caroline Owen through the example of COPD, the lung physiology of the mouse is quite different to that of man, and due to their size they are not the most convenient species to reliably measure lung function, particularly using the same animal as its own control to investigate longitudinal changes in lung function or bronchial responsiveness. Furthermore, most models of allergic asthma have been acute and have evaluated drugs on allergen-induced eosinophilia rather than evaluating chronic changes or measurements of lung function.

Pr. Page then underlined that one of the major phenotypic characteristic of asthma is bronchial hyperresponsiveness, which is not normalized by current therapies, even following treatment with glucocorticosteroids for a decade. This suggests that mechanisms other than airways inflammation contribute to this phenomenon. Accordingly, animal models that allow longitudinal measurement of lung function in the same animal investigated chronically are required to understand this process in more details. Furthermore, such a model must exhibit the spectrum of airway responsiveness to both direct and indirect acting provocation stimuli as seen in patients with clinical asthma. Then, Pr. Page showed how his group used rabbits and guinea-pigs, rather than just relying on mice, over several decades to investigate the mechanisms of bronchial hyperresponsiveness. He first pointed the value of the rabbit in this context as it is of a size that allows minimally invasive lung function recordings to be made in the same animals over time. More recently, Pr. Page’s group used the guinea pig to aid in the development of a novel class of inhaled bifunctional drug for the treatment of asthma and COPD, the dual phosphodiesterase (PDE) 3/4 inhibitor RPL554, where animal studies predicted the clinical efficacy of this drug in man. RPL554 is now in Phase 2b clinical trials. He concluded on the importance of choosing the right species for drug development, in particular for mAbs.

Next, Stéphanie Blanc (Cynbiose) presented a cynomolgus macaque model of mild infection with human RSV (hRSV), a common respiratory infection in premature infants and children, associated with a high mortality when developing with other chronic diseases. She showed that age and repeated infections affected virological, clinical and immunological parameters. Even in infant macaques, intranasal hRSV infection induced both local and systemic immune responses to efficiently control the virus. Then, Dr. Blanc illustrated that this model was pharmacologically validated using a reference topical treatment in humans based on palivizumab (Synagis®) administered intranasally, allowing a significant reduction in
Antoine Guillot (CEPR-INSEMr U1100) described a novel method to determine lung PK. First, he reminded the audience that PK studies are required to characterize the kinetics of tissue/fluid deposition, transformation and clearance of an inhaled drug for pulmonary disease. Classically, PK parameters are estimated by monitoring drug concentrations in the systemic circulation, then computed in mathematical compartmental models to predict the behavior of both local and systemically acting drugs. Dr. Guillot emphasized that mAbs do not diffuse passively into organ/tissue compartments; thus, indirect estimation of lung concentrations by modeling from plasma drug profiles is limited and sometimes biased. He provided details on a new method to quantify the time-course exposure of inhaled mAb by direct sampling in the lung parenchyma, using lung microdialysis. Lung microdialysis was already established and validated, but not for large molecules. In vitro, the recovery of mAbs with a 1,000 KDA cut-off semi-permeable membrane allowed 34% drug recovery and sampling rate every 180 min with a flow rate of 0.3 μL/min. In vivo, lung microdialysis was set up in NHPs, a relevant animal model for both biotherapeutics and aerosols therapy to attempt the dynamic quantification of mAbs in the interstitial lung space. He explained that a microdialysis probe was implanted in the lung by thoracic surgery immediately after delivery of mAb aerosol in conscious NHPs, and animals were thereafter maintained under prolonged anesthesia and mechanical ventilation for at least 55 hours. Microdialysate and blood samples were collected at time intervals for the determination of mAbs and endogenous/control markers to control the permeability of the probe and determine in vivo mAb recovery. He concluded that the conditions are now established for lung microdialysis of inhaled mAbs targeting soluble-antigens, although this technique remains challenging.

Invited lecture on imaging modalities in animals to explore the lungs

To complete the “animal models” sessions, Alain Le Pape and Stéphane Lerondel (Center for Small Animal Imaging, Phenomin-TAAM CNRS) gave an overview of imaging technology to explore the lungs. They described the imaging modalities available for lung exploration: X-ray computed tomography (CT), radioisotopic single photon emission CT (SPECT) or positron emission tomography (PET) imaging and magnetic resonance imaging (MRI), which directly result from medical imaging. Thanks to technological advances, the detectors had been improved for both resolution and sensitivity, making it possible to use these devices in rats and mice to perform lung explorations with sub-millimetric resolution. During the last decade, development of optical approaches such as bioluminescence and near-infrared fluorescence (NIRF) imaging have revolutionized the use of molecular imaging in mice for upstream translational research. Imaging can provide anatomical or functional information, sometimes at a molecular level, that can be combined by multimodality approaches. Pr. Le Pape and Dr. Lerondel discussed the opportunities and applications for lung in vivo imaging, from mice to primates and men, giving examples from lung cancer, asthma, emphysema and illustrating aerosol-based therapies delivery. They highlighted the advantages and limitations of the different techniques depending on their physical basis and the size of the specimen submitted to examination. In particular, they provided details on: 1) the requirement for synchronization of images during acquisition to prevent blurring in imaging at millimetric resolution due to respiratory movements; 2) the dosimetry delivered to the tumor for CT and nuclear bimodalities SPECT/CT and PET/CT for onco-pharmacology studies in mice models to avoid bias; 3) the limitations for accurate quantitative imaging in bioluminescence when the tumor becomes hypoxic; and 4) the potential of NIRF imaging with a variety of probes to explore biomarkers for cancer, inflammation and infection.

Drs. Le Pape and Lerondel also described the advanced imaging strategies to assess interaction of mAbs or bioactive molecules with their targets. First, they explained that direct quantitation of the amount of a conjugated molecules concentrated into a lesion is not an accurate quantitation of the specific recognition/interaction because the expanded space of diffusion associated with inflammation, compartments inside the lesion and its environment, enhanced permeability and retention (EPR) effect, Fc interactions with mAbs and many other parameters may contribute to non-specific uptake. Quantitation of interaction requires that imaging data be corrected using those obtained with a representative but non-active molecule, like mutant protein or isotype mAb for example. They also presented some results of high resolution CT and Krypton 81m ventilation scintigraphy imaging in animal models of asthma and emphysema. They went on to explain the rationale for the choice of the imaging modalities (tomosintigraphy vs. NIRF imaging) as illustrated with a biodistribution study of cetuximab in a mouse model of lung cancer. Finally, the potential of imaging for translational research was illustrated with aerosolized therapies, e.g., cetuximab or gemcitabine in lung cancer, papillomavirus vaccine, which provided key information on efficacy, biodistribution and safety assessments in mice, rats, primates and humans.

Conclusion

Up to 2015, a limited number of mAbs for respiratory disease were in the market, but several molecules were recently approved and numerous are in late phase development. This demonstrates the potential of mAbs to benefit patients with respiratory diseases that still represent unmet medical need. This meeting aimed at giving an overview of the molecules approved or in development for major respiratory diseases, discussing the format and route of administration to improve efficacy and safety of those drugs. As mentioned by Pr. Diot, one should keep in mind that the accuracy of a treatment is a subtle balance between its efficiency, side effects, convenience, and price. Major respiratory diseases are still non-curable, with drugs addressing mainly the symptoms or limiting disease progression rather than reversing it. This symposium also raised concerns regarding relevant animal models for preclinical studies. Although animal models are critical in the development of mAbs, the choice of the species depends on the scientific question to be addressed, and the model that might mimic human disease best.
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