IUPHAR Fibrosis Symposium, 20/11/2020.

NC-IUPHAR, now chaired by Steve Alexander, has organized high-level scientific meetings for receptor classifications over three decades, and these meetings have validated the molecular basis for modern pharmacology. The meetings are traditionally held over a weekend in Paris, and have been made possible by educational grants from Servier. We therefore wished to develop these meetings, which are normally held under Chatham house rules, allowing confidential presentations, but to expand the scientific part to have a one day symposium to progress certain fields thought to be crucial for pharmacology. The slides are available. We may therefore expand these symposia in future to make them publicly available as webinars.

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Fibrosis is associated with pathological wound-healing, leading to permanent scar tissue. It is associated with ageing, although alcohol-induced cirrhosis or viral-induced fibrosis occurs frequently in middle age. The extracellular matrix components (ECM), such as the collagen and glycosaminoglycans are produced in excess, by fibroblasts, leading to fibrotic scarring, which interferes with physiological function. The imbalance between would healing and scar formation leads to chronic fibrosis. Furthermore, when normal tissue works continually against scar tissue as in pulmonary and cardiac fibrosis, and in some athletic injuries, this may lead to extension of the fibrosis, progressively extending the injury. Thus, breathing in fibrotic lungs increases TGFb expression in a feed-forward process. Although chronic inflammation may be associated with fibrosis and favor a fibrotic environment, it is not causative, without other injury. TGFb/STAT3 signaling is a major molecular driver, and TGFb ligands accumulate in ECM. Fibrosis in lung and liver is associated with elevated risk of carcinoma and a mechanism linking lung carcinoma with TGFb signaling has just been described.

As an example, liver disease is associated with viral hepatitis (e.g. from HBV and HCV), alcoholic liver disease, and non-alcoholic fatty liver disease (e.g. from obesity/metabolic syndrome), as well as inherited metabolic disorders. PPARg is a crucial player in metabolism. IVA337 is a pan-PPAR agonist, active in fibrosis models, while increasing the expression of β-oxidation-related and fatty acid desaturation-related genes, supporting its use in NASH. PPARg negatively regulates myofibroblast differentiation generating hepatic myofibroblasts, promoting liver fibrogenesis. PPARγ is also a negative regulator of this process epigenetically via MeCP2, EZH2, and miR132.

Idiopathic Pulmonary Fibrosis (IPF) is a highly progressive, age-related disorder associated with shortness of breath and cough, incidence starting from age 50, but with highly variable progression, and an average survival of three years from diagnosis. It is a disease of the alveolar unit: epithelial cell pneumocyte type II, these cells may also show senescence with senescence-associated secretory phenotype (SASP) affecting multiple cells. The alveolar surface is composed of alveolar type I (extremely thin cells, 95% of surface area) and type II (spherical) cells which constitute 60% of alveolar epithelial cells and 10–15% of all lung cells: they have crucial roles in lung regeneration, surfactant production, but are targets for many viruses

including SARS-CoV-2. In IPF, TGFβ1 initiates profibrotic signaling via the SMAD pathway and the small heat shock protein B5 (HSPB5). Tripartite motif-containing 33 (TRIM33) negatively regulates TGF-β/SMAD signaling and fibrosis. As IPF is highly age-related, its mortality has exceeded multiple myeloma and bladder cancer. Familial pulmonary fibrosis (FPF) has been linked to the genes encoding the lung surfactant proteins C and A2 (*SFTPC* and *SFTPA2*) causing increased endoplasmic reticulum stress in type II alveolar epithelial cells. Viruses also increase ER stress in cells in creating viral envelopes. IPF has been associated with mutations in genes encoding components of the telomerase complex (such as *TERT* and *TERC*), with consequently shortened telomere lengths, and exhausting of lung stem cells. A common polymorphism in the promotor region of the *MUC5B* gene affecting mucin5B expression, is associated with a 6-fold increased incidence in fibrosis. whereas *MUC5AC* expression is in proximal, cartilaginous airways. Nintedanib and pirfenidone are approved for IPF, albeit with rather obscure mechanisms at least for pirfenidone. Animal models of lung fibrosis are difficult – the classic bleomycin mouse model has shown 300 positive compounds with only two clinically active to date. Paired-related homeobox proteins (PRRX1) is upregulated in some fibroblasts and antisense oligonucleotides (ASOs) are being studied.

Kidney fibrosis develops from any form of kidney damage progressively replacing kidney tissue with fibrotic tissue. Polycystic kidney disease is associated with a ciliopathy, affecting 12M individuals worldwide.

Arthrofibrosis, such as found after knee replacement, is also a significant medical problem.

Neutrophils accumulate at early stages of wound healing crucially modifying wound repair and fibrosis. Formyl peptide receptor 1 (FPR1) is expressed on neutrophils and FPR1-deficient (fpr1-/-) mice were protected from bleomycin-induced pulmonary fibrosis but developed renal and hepatic fibrosis normally. FPR1 and FPR1 ligands are required for effective neutrophil recruitment to the damaged lung. Failure to recruit neutrophils or depletion of neutrophils protects from pulmonary fibrosis.

TGFb1 targets:

- Quiescent fibroblast to become matrix-secreting fibroblasts
- Deletion of the NF-κB subunit c-Rel limits fibrosis in several tissues. c-Rel regulates expression of a pro-fibrogenic secretome and the c-Rel-Pfkfb3 axis is critical.
- induction of NADPH oxidases, such as dual oxidase (DUOX1/2). DUOX enzymes, as extracellular H_2O_2-generating systems, are involved in extracellular matrix formation and in wound healing; the TGFβ1 pathway amplifies the DUOX1-derived H_2O_2 in a positive feedback
- Via multiple receptors, SMAD-2/3 signaling, to produce collagens, plasminogen activator inhibitor-1, connective tissue growth factor, and matrix metalloproteases. However, SMAD1/5 signaling is antifibrotic.

The glycome in the glyocalyx is also critical to establishing the ECM, along with fibroblasts and other specialized cells. Sphingolipids such as ceramide are, with cholesterol, major components of lipid rafts, with glycosphingolipids projecting into the glycocalyx. The balance

between ceramides, sphingomyelin, glucosylceramide, and sphingosine is critically modified in many pathological conditions, including fibrosis. Glucosylceramides, and gangliosides form glycans in the glycocalyx. Sialic acids, which tip many glycans in the glycocalyx, and SIGLECS increase structural diversity immensely and recent human evolution has been accompanied by major changes in sialic acid availabilities. The sugar linkages, together with lipid chain length, generate immense complexity, to which molecular biology techniques are blind, except for the enzyme mutations leading to multiple rare diseases, with severe phenotypes. This complexity is exploited by many viruses, including SARS-CoV-2. The human lung glycome has been assessed and specific targets for viruses defined in the human glycome project. Inhibiting ceramide synthesis is beneficial in cystic fibrosis, where sphingosine and glucosylceramide may be beneficial. Sphingosine, on the apical surface of lung membranes is a potent antibacterial substance contributing to host defense but disappears in cystic fibrosis.

Using metabolomics, sphingolipid metabolism has been shown to participate in the pathophysiology of amyotrophic lateral sclerosis (ALS), and ceramide, glucosylceramide, and ganglioside levels are dysregulated in the CNS and at the skeletal neuromuscular junctions of both animal models and patients. Glucosylceramide is the main precursor of complex glycosphingolipids and is degraded by lysosomal (GBA1) or non-lysosomal (GBA2) glucocerebrosidase. GBA2, but not GBA1, activity is markedly increased in the spinal cord, of $SOD1^{G86R}$ mice, even before disease onset, whereas GBA1 mutations are an important cause of Parkinson's disease. These enzymes are critical to many steps in viral infection, and ceramide is a major contributor to fibrosis in lung, muscle, liver, and kidney. Metabolomics, together with modulation of key enzymes, deconvolute complexity and yield new therapeutic approaches.

New Strategies for Future Therapies

Rebecca Ritchie (MIPS, Monash) described current challenges in the treatment of heart failure. Heart failure, which may occur in 1 in 5 people in their lifetime with no effective "cure". Fibrosis is a major issue. Treatment of heart failure remains the same, regardless of the type of heart failure present in the patient, their gender, or whether the patient has diabetes and/or other comorbidities. These therapies are largely based on clinical trials in patients where left ventricular ejection fraction (EF) is reduced, known as HFrEF. Diabetes increases heart failure risk >2.5-fold, independent of concomitant comorbidities, and even more so in females. There is, however, significant heterogeneity across patients with LV dysfunction and diabetes, with comorbidities commonly incorporating obesity, dyslipidaemia and hypertension. This heterogeneity also encompasses the nature of the impairments in LV function, whether at the level of cardiac relaxation and compliance ('diastolic dysfunction') or impaired cardiac contractility ('systolic dysfunction'). Thus, there are multiple, distinct phenotypic patient clusters described, each exhibiting different degrees of LV systolic and diastolic dysfunction. Further, heart failure with preserved EF, HFpEF, is now more common than HFrEF (especially in women). HFpEF is a heart failure diagnosis in patients with heart failure symptoms yet no impairment in LVEF is observed. Increased HFpEF prevalence is likely secondary to increased prevalence of diabetes, and an ageing population, in addition again to obesity and/or

hypertension. These disconnects are often evident in clinical trials (where females are often under-represented), as well as in preclinical studies and the different gender, heart failure phenotype and concomitant comorbidities likely impact the efficacy of pharmacotherapies for tackling cardiomyopathy, and cardiac fibrosis.

Anthony Davenport described how mining the 100,000 Genomes Project could validate new receptor targets in cardiovascular disease. The apelin receptor is a class A GPCR that regulates the cardiovascular system by beneficially increasing cardiac output and causing vasodilatation, in health and disease, through binding of two endogenous peptide ligands, apelin and Elabela/toddler (ELA). Loss of the apelin signalling pathway may contribute to pathophysiological conditions such as pulmonary arterial hypertension. Natural genetic variation in GPCRs may be a cause of individual differences in responses to medications. A single amino acid change in the apelin receptor in the fish *Danio* results in a loss of function mutation, called *Grinch*, where the cardiovascular system fails to develop but similar mutations have not been identified in humans. We identified 11 rare (< 1 in 10,000) apelin receptor variants in human patients with cardiovascular diseases and bleeding disorders in the National Institute for Health Research BRIDGE component of the Genomics England 100,000 Genomes Project. We assessed these receptor variants in ligand binding and a high content screening system, using validated novel fluorescent apelin receptor ligands to determine pharmacological consequences of mutations on receptor binding and internalisation, and to identify any differences in the pharmacology of the two endogenous ligands at these variant receptors. We identified variants where binding of apelin receptor ligands was either abolished (three) or significantly reduced. Further characterization of these variants in human embryonic stem cell derived cardiomyocytes will determine the significance of changes to the apelin signalling pathway in cardiovascular disease.

Francesca Levi-Schaffer, The Hebrew University of Jerusalem, Israel, described new strategies for unmet clinical needs in chronic allergic inflammatory diseases. Allergic inflammation (AI) is a reaction occurring in asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, etc. AI is triggered by the cross-linking of IgE and FcεRI on the surface of mast cells, the main effector cells of allergy, which orchestrate the early phase of AI. Subsequently, the late phase takes place, in which other cells, notably the eosinophils, are recruited. The late phase is usually followed by resolution, which is orchestrated by specialized pro-resolving lipid mediators in concert with inhibitory receptor activation. However, most of the time allergic diseases can become chronic, which is still an unmet clinical need. Over the years, several approaches have been developed to treat allergy, but these strategies mostly help to reduce the inflammation and ease the symptomatology, without leading to full healing. Therefore, there is a need for better and more sensitive biomarkers and therapeutic targets. Her group has proposed the inhibitory receptors CD300a and Siglec-7, and the specialized pro-resolving lipid mediators LXA_4-B_4 and RvD1 as novel approaches to diagnose and possibly treat chronic allergic inflammation.

Thierry Wurch (Ipsen) presented a high-level overview on novel bispecific immunotherapies aiming to activate T lymphocytes in cancer patients. After an introduction summarizing the main bispecific antibody formats having reached clinical development stage, three main mechanisms of action (MoA) were detailed: T cell redirection, NK cell redirection and

bispecific immune checkpoint modulators. T cell engagers aim to trigger direct TCR activation by cross-linking its CD3 epsilon component via binding to a tumor antigen. Strength of T cell activation is correlated with the expression level of the tumor antigen. The prototypical example of such MoA is blinatumomab, a bispecific CD19xCD3 molecule approved in 2014 for the treatment of patients with relapsed/refractory acute lymphoblastic leukemia. About fifty T cell engagers are currently in clinical development, based on various bispecific formats and targeting different tumor antigens both in solid tumors and hematologic malignances. The second MoA described is comparable to T cell engagers, but the immune effector cell type corresponds to NK cells. The selected immune activating receptor can be either FcgRIIIa (CD16a) or NKp46. The most advanced asset applying such MoA is AFM13 developed by Affimed. It corresponds to a bispecific antibody targeting CD30 and CD16a. It is currently in Phase 2 clinical development in various T cell lymphomas. The third MoA described corresponds to next-generation immune checkpoint modulators. Several types of bispecific antibodies were developed during the last decade with the aim to by-pass resistance/relapse to PD1-targeted therapies, or to limit the toxicity observed with antibodies targeting co-stimulatory T cell receptors such as 4-1BB. Dozens of such molecules were designed, but most of them have not yet reached clinical development. A bispecific Her2x4-1BB molecule developed by Pieris and currently in clinical phase 1 showed indeed a good safety profile, and early signs of clinical efficacy.

Further reading on Fibrosis:

1. Wilson CL, Mann DA, Borthwick LA. Epigenetic reprogramming in liver fibrosis and cancer.
   Adv Drug Deliv Rev. 2017 Nov 1;121:124-132.
2. Wallace MC, Friedman SL, Mann DA. Emerging and disease-specific mechanisms of hepatic stellate cell activation.
   Semin Liver Dis. 2015 May;35(2):107-18. doi: 10.1055/s-0035-1550060.
3. Tsukui T, Sun KH, Wetter JB, Wilson-Kanamori JR, Hazelwood LA, Henderson NC, Adams TS, Schupp JC, Poli SD, Rosas IO, Kaminski N, Matthay MA, Wolters PJ, Sheppard D. Collagen-producing lung cell atlas identifies multiple subsets with distinct localization and relevance to fibrosis. Nat Commun. 2020 Apr 21;11(1):1920.
4. Spagnolo P, Distler O, Ryerson CJ, Tzouvelekis A, Lee JS, Bonella F, Bouros D, Hoffmann-Vold AM, Crestani B, Matteson EL. Mechanisms of progressive fibrosis in connective tissue disease (CTD)-associated interstitial lung diseases (ILDs).
   Ann Rheum Dis. 2020 Oct 9:annrheumdis-2020-217230.
5. Norman J. Fibrosis and progression of autosomal dominant polycystic kidney disease (ADPKD).
   Biochim Biophys Acta. 2011 Oct;1812(10):1327-36. .
6. Juge PA, Crestani B, Dieudé P. Recent advances in rheumatoid arthritis-associated interstitial lung disease.
   Curr Opin Pulm Med. 2020 Sep;26(5):477-486.
7. Henderson NC, Rieder F, Wynn TA. Fibrosis: from mechanisms to medicines.
   Nature. 2020 Nov;587(7835):555-566.
8. Hartupee J, Mann DL. Role of inflammatory cells in fibroblast activation.
   J Mol Cell Cardiol. 2016 Apr;93:143-8.

9. Froidure A, Marchal-Duval E, Homps-Legrand M, Ghanem M, Justet A, Crestani B, Mailleux A. Chaotic activation of developmental signalling pathways drives idiopathic pulmonary fibrosis.
Eur Respir Rev. 2020 Nov 18;29(158):190140.
10. Borie R, Le Guen P, Ghanem M, Taillé C, Dupin C, Dieudé P, Kannengiesser C, Crestani B. The genetics of interstitial lung diseases.
Eur Respir Rev. 2019 Sep 25;28(153):190053.

Summary by Michael Spedding, Steve Alexander and the speakers.