

New Strategy

Early identification of efficacy is critical to improving pharma attrition rates. Many drugs fail because their efficiency varies widely across the patient population, even when the drug might benefit a subpopulation

David Bunton at ReproCELL Europe, Marian McNeil at Stratified Medicine Scotland and Pamela Brankin at Aridhia

To move away from 'all-comers' trials, the early identification of genetic factors influencing effectiveness in patient subpopulations is key. A recent increase in the use of predictive human tissue models led to the idea for a nationwide multidisciplinary collaboration that combined assays in fresh, diseased human tissues studied *ex vivo*, with whole exome sequencing. This allowed genomic markers to be recognised, distinguishing patients identified by the human tissue assays as 'good' or 'poor' responders.

This Scottish pharmacogenomics project has made great strides in the understanding of chronic obstructive pulmonary disease (COPD) and inflammatory bowel diseases (IBD) by using whole exome sequencing, bioinformatics and fresh human tissue assays to relate drug response to genotype. The study findings underpin a new framework for patient stratification in clinical trials that combines *in vitro* and molecular informatics to improve pharma success rates and accelerate the availability of effective medications.

The most common cause of drug failure is poor efficacy at Phase 1 or 3, which is, in part, attributed to clinical trials of entire patient populations, including both 'responders' and 'non-responders' (1,2). Precision medicine can improve the prediction of clinical efficacy by selecting only those patient subpopulations likely to gain clear benefit for trials, but such forecasts require

methods to identify patient subpopulations at an early stage, ideally during preclinical testing (2-4). Human tissue data generated in early discovery via a wide range of models – including stem cells, fresh human tissues and pathological specimens – is increasingly being used in preclinical testing (5-7). Tissue samples collected from the target patient population allow researchers to directly test the effect of new drugs in systems that closely reflect the *in vivo* situation, providing an early prediction of efficacy. The responses of tissues from different patients when studied *in vitro* can vary considerably, reflecting the clinical reality of responders and non-responders.

A Collaborative Project

In the first project of its kind, a collaboration of several organisations combined and shared their data from fresh tissue assays with genomics and demonstrated a new model for the early preclinical prediction of efficacy and patient stratification.

Tissue from two chronic inflammatory diseases – COPD and IBD – was used to investigate inter-patient variability in drug efficacy using *ex vivo* organocultures of fresh intact samples. In the presence of standard of care drugs, the reduction in inflammatory cytokines was used as a measure of drug efficacy. The individual patient reactions were matched against genotype and microRNA

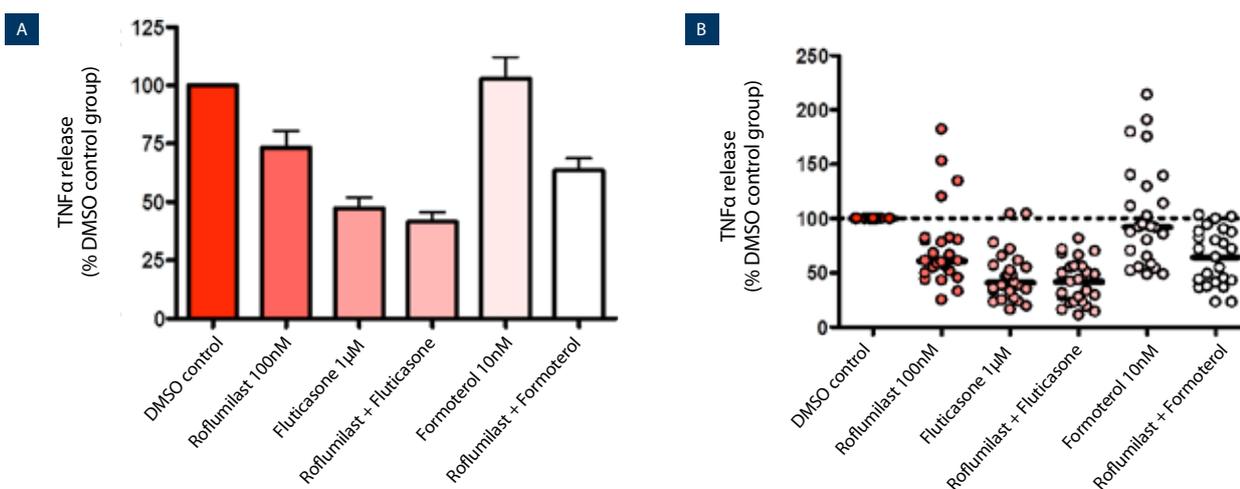


Figure 1: Graphs showing the effects of test articles on tumour necrosis factor (TNFα) release from Lipopolysaccharide-stimulated human lung parenchyma biopsies. n = 25 donors, all diagnosed with COPD. For each donor, two replicates, each containing two biopsies, were included in each treatment group. Data is displayed as a percentage of the corresponding dimethyl sulfoxide (DMSO) control group. (A) Bar graph depicting mean ± SEM TNFα release. (B) Scatter graph depicting the changes in TNFα release in each patient (dots) and median responses (thick black line)

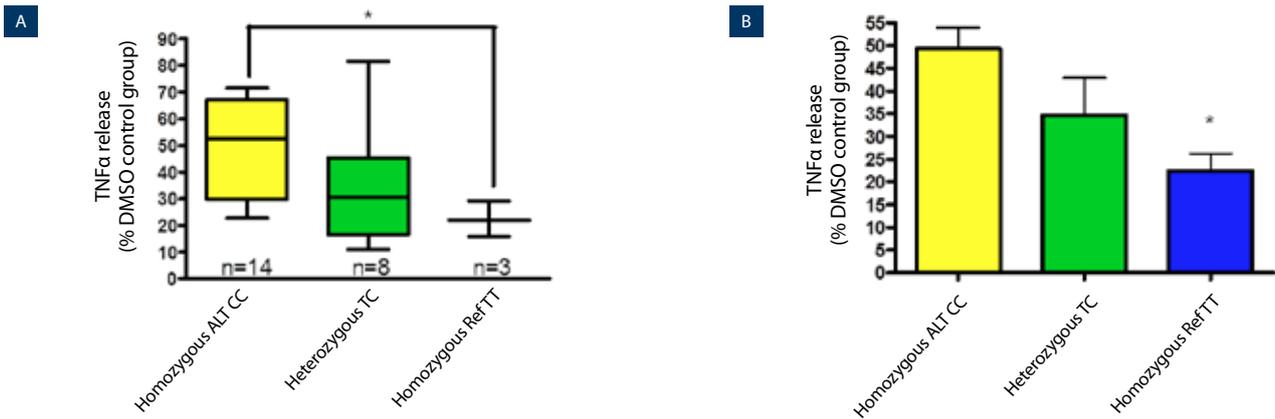


Figure 2: Graphs showing the relationship between SNP rs2249695 genotype and TNFα release from stimulated human lung parenchyma biopsies following roflumilast and fluticasone co-treatment. n = 25 donors, all diagnosed with COPD. For each donor, two replicates, each containing two biopsies, were included in each treatment group. Data is displayed as a percentage of the corresponding DMSO control group. Asterisks indicate significant differences (P < 0.05 for one, P < 0.01 for two and P < 0.001 for three). (A) Box and whiskers graph depicting TNFα release. The 25th and 75th percentiles of each group are represented by the box with the minimum and maximum values shown by bars, the line within each box denotes the median value. (B) Bar graph depicting mean ± SEM TNFα release

profiles in an attempt to identify predictors of responsiveness. A snapshot of the data from the COPD study highlights the utility of the method. Lung parenchyma was collected from 25 COPD patients undergoing therapeutic resections for cancer. Small biopsies of parenchyma were prepared and then cultured for 24 hours in the presence of typical COPD standard of care treatments. The effectiveness of the drugs was measured by the reduction in the release of cytokines (see Figures 1A and 1B, page 74).

There was an obvious spread of efficacy across treatment groups and between patients. One organisation's analysis identified a possible bimodal response in the effect of roflumilast in combination with formoterol. Following whole exome sequencing conducted at one of the participating centres, the collaboration used bioinformatics to test for associations between genotype and drug effect. Raw data were accessed and analysed using one organisation's informatics platform in conjunction with another's proprietary software. Within the COPD cohort, 30 single-nucleotide polymorphisms (SNPs) corresponding to 23 genes were found to be tentatively associated with response to roflumilast plus fluticasone. Several of these genes have already been identified as correlating with COPD. The data suggested that genetic variation in the cytochrome p450 enzyme (CYP2E1) gene, namely SNP (rs2249695), may partly explain the observed difference in drug response.

Biopsies from donors who had at least one copy of the reference allele for this SNP generally responded better to roflumilast and fluticasone co-treatment. As shown in Figure 2, mean TNFα release was inhibited by 77.6% (homozygous reference haplotype [TT]) and by 50.74% (homozygous alternative haplotype [CC]). Levels of inhibition between these two haplotypes were found to be significantly different with a P value of 0.02 (unpaired, two-tailed t-test). The TT has previously been associated with low CYP2E1 expression.

It is possible that CYP2E1 expression is capable of influencing treatment responses. It induces reactive oxygen species

production that may, in turn, inhibit reductions of TNFα release through various treatments (8,9). All three patients in the TT group were high responders to roflumilast plus fluticasone, 5 out of 8 patients in the heterozygous reference haplotype (TC) group were high responders, whereas 10 out of 14 patients in the CC group were low responders. An IBD study data visualisation shows differing levels of response to a novel treatment between the ulcerative colitis (UC) and Crohn's disease cohorts (see Figure 3). An identical analysis approach to the COPD study was conducted to explain differing response levels. A congruence analysis was performed to determine any overlap between the exome association analysis and microRNA (miRNA) profiles in relation to response. Three genes (ROBO 1, ROCK2 and TNFSF-10) were found to overlap between both platforms. This overlap is not significantly higher than would be expected by chance, however, ROCK2 and TNFSF-10 have previously been shown to be associated with IBD (13,14).

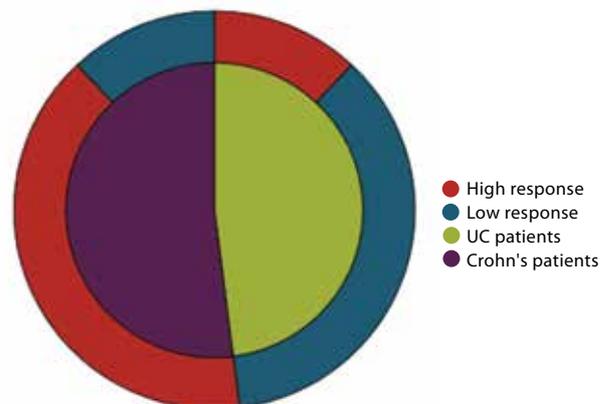


Figure 3: Data visualisation showing the relationship between IBD phenotype and response to 10 nM Doramapimod (BIRB-796). Response was defined as high or low by dichotomising the data of 10 nM BIRB-796 measured by IL1 release using the average level. This generated two groups of samples (12 low responders and 13 high responders). The chart was generated via mini-app data visualisation software

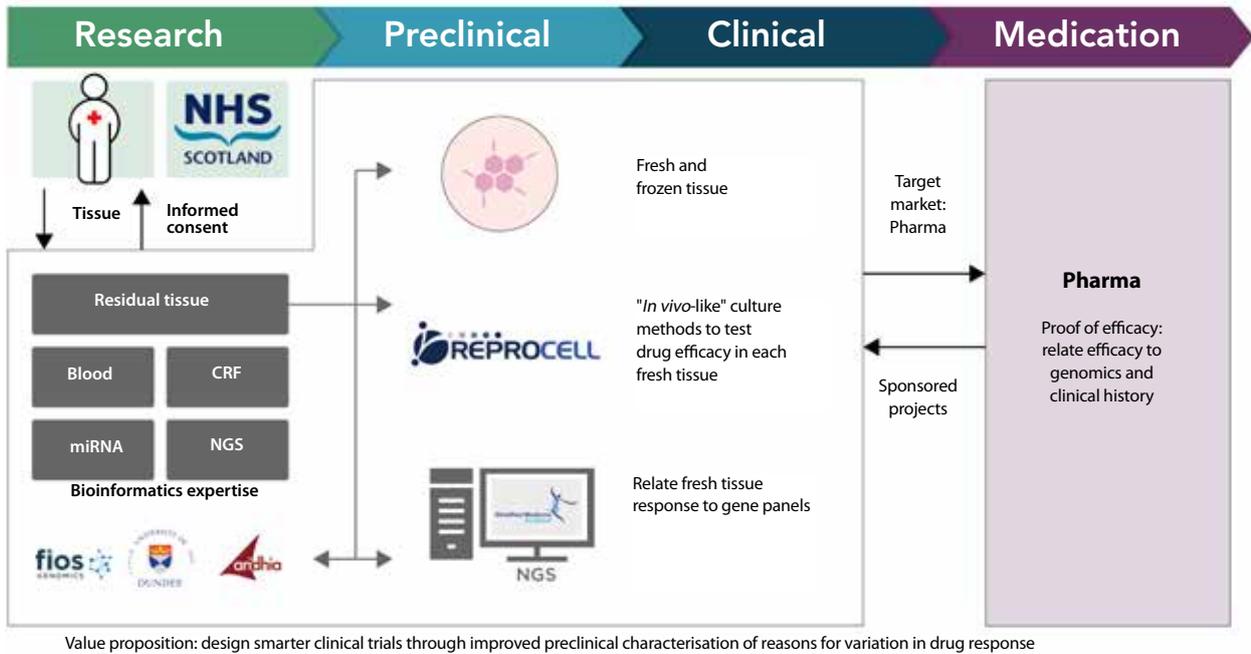


Figure 4: A model for the early identification of patient subpopulations by combining human, fresh tissue assays with genomics

Full of Potential

The authors acknowledge that, while a very high volume of functional and genomics data was generated, the total number of patients was low for a genomics study. Therefore, the scientific conclusions remain tentative, but serve to demonstrate the potential offerings of a larger study. This collaboration showed that testing drug effectiveness in fresh disease-relevant tissue samples and relating these responses to genomic data allows drug developers to link genotypes to patient variation in drug responses at a much earlier stage than previously possible (see Figure 4). As pharma increasingly utilises phenotypically-relevant models early in drug discovery, this approach shows the potential to design smarter clinical trials with heightened probability of success in patient subpopulations.

References

1. Paul SM *et al*, How to improve R&D productivity: The pharmaceutical industry's grand challenge, *Nat Rev Drug Discov* 9(3): pp203-14, 2010
2. Trusheim MR *et al*, Quantifying factors for the success of stratified medicine, *Nat Rev Drug Discov* 10(11): pp817-33, 2011
3. Biankin AV *et al*, Patient-centric trials for therapeutic development in precision oncology, *Nature* 526(7573): pp361-70, 2015
4. Cardon LR and Harris T, Precision medicine, genomics and drug discovery, *Hum Mol Genet* 25(R2): R166-72, 2016
5. LaVallee T, Found in Translation, *European Pharmaceutical Review* 6: pp43-5, 2012
6. Ashgar W *et al*, Engineering cancer microenvironments for *in vitro* 3-D tumor models, *Master Today* 18(10): pp539-53, 2015
7. Zaroni M *et al*, 3D tumor spheroid models for *in vitro* therapeutic screening: A systematic approach to enhance the biological relevance of data obtained, *Sci Rep* 6: 19103, 2016
8. Linhart K *et al*, The role of reactive oxygen species (ROS) and cytochrome P-450 2E1 in the generation of carcinogenic etheno-DNA adducts, *Redox Biol* 3: pp56-62, 2014

9. Arif E *et al*, Association of CYP2E1 and NAT2 gene polymorphisms with chronic obstructive pulmonary disease, *Clin Chim Acta* 382(1-2): pp37-42, 2007

About the authors



David Bunton is Chief Executive Officer of ReproCELL Europe. He is an experienced and respected human tissue pharmacologist and co-founder of Biopta, which merged with Reinnervate Ltd in July 2016 to form ReproCELL Europe.
Email: david.bunton@reprocell-europe.com



Marian McNeil is Business Development Manager at Stratified Medicine Scotland – Innovation Centre. She has recently gained a master's degree in stratified medicine and pharmacological innovation from the University of Glasgow, Scotland, and has extensive experience in the medical marketing interface for oncology pipeline products.
Email: marian.mcneil@glasgow.ac.uk



Pamela Brankin joined Aridhia in 2011, taking on the role of Head of Marketing and Communications in 2015. She holds a BA Hons in English and French from the University of Dundee, UK, a postgraduate diploma in information technology from the University of St Andrews, UK, and an advanced diploma in professional French from the Chambre de Commerce et d'Industrie de Paris, France.
Email: pamela.brankin@aridhia.com