PI profile

## Deborah Gill

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| [] | **Professor Deborah Gill****Titles**: Professor of Gene Medicine / Co-Director of the Gene Medicine Group / Head of NDCLS **Location**: John Radcliffe Hospital, (NDCLS)**Department**: Radcliffe Department of Medicine (RDM)**Group:** Gene Medicine Group**Webpage**: <https://www.rdm.ox.ac.uk/people/deborah-gill>**Email**: Deborah.gill@ndcls.ox.ac.uk |

### GMS themes:

* From genes to clinic
* Application of genomics in the clinic (therapeutics)

### Research Overview:

The manipulation of nucleic acids for therapeutic purposes (gene therapy, gene editing, etc) is generating success in the clinic. A key challenge, however, is to understand and control the delivery and expression of genes in target tissues. My research focuses on the development of genetic treatments for respiratory conditions with a large unmet clinical need. We have substantial expertise in the development of novel non-viral and viral gene delivery systems; we developed a CpG-free plasmid DNA/cationic liposome formulation for the treatment of cystic fibrosis (CF) lung disease that successfully completed a Phase IIb clinical study in 136 CF patients, which showed for the first time that gene therapy could halt the inexorable decline of lung function that leads ultimately to the premature death of CF patients. In the viral vector space, we are developing novel, lung-targeted lentiviral and AAV vectors for a range of lung disorders and applications.

In particular, there is an urgent need for treatments for childhood Interstitial Lung Diseases (ILD), affecting the interstitium - the tissue in and around the alveoli (air sacs) of the lungs. In adults, ILD often occurs after a lung injury triggers an abnormal healing response causing thickened, fibrotic alveolar tissue, leading to poor lung function. In children, ILD is more typically caused by genetic conditions, several of which can be lethal at birth. There are very few interventions/treatments available for these rare genetic diseases, with life expectancy typically measured in weeks or months. For example, genes involved in lung surfactant production are essential for establishing a healthy lung in newborns and studies in transgenic mouse models have provided proof of principle for therapeutic gene therapy/gene editing approaches. We are therefore developing a genetic treatment for a range of childhood ILDs, including deficiencies in surfactant protein B, surfactant protein C, and adenosine triphosphate-binding cassette transporter A3 (ABCA3).

Project areas: Gene editing, gene therapy, transgenic mouse models, human lung culture models.

Please contact directly for further information.

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Project proposal

Title: Strategies to facilitate targeted high-efficiency gene editing for treatment of lung diseases

Supervisors: Professor Deborah Gill & Dr Altar Munis

Wet/dry lab mix (approx): 75% wet lab, 25% dry lab

### Description:

### The clustered regularly interspersed palindromic repeats (CRISPR) system, a powerful genome editing tool, has now been used ubiquitously in medical research including gene therapy. Several proof-of-concept studies have demonstrated the feasibility of *in vivo* gene editing to correct disease-causing mutations using both Homology Directed Repair (HDR) and Homology-Independent Targeted Integration (HITI) strategies. A successful therapeutic gene editing strategy to tackle lung disorders will require the targeted and highly efficient repair action of the CRISPR-Cas9 system adapted for specific cell types; for example, targeting alveolar type II pneumocytes in the case of interstitial lung diseases, and also ionocytes and ciliated cells for cystic fibrosis. We have developed a novel transgenic reporter mouse model, which ubiquitously expresses the fluorescent TdTomato transgene, to evaluate genome editor tools and delivery methods *in vivo*. We aim to use this model to explore, validate, and demonstrate the utility of *in vivo* genome editing strategies for lung disorders*.*

The rotation project, which can serve as a starting point for subsequent DPhil study, focuses on gene editing using a range of delivery vectors, specifically comparing recombinant Lentiviral and AAV vectors and also vectors encoding cell-specific promoters. Gene editing strategies will first be tested *in vitro* using primary human lung airway cultures and surfactant air-liquid interface (SALI) cultures modelling human lung parenchyma. Off-targets and editing efficiencies will be assessed via droplet-digital PCR (ddPCR) and Nanopore MinION-based third generation next-generation sequencing (NGS) method AFIS-Seq. Utilising *in vitro*data to optimise experimental design (e.g. gRNA and donor design, Cas9 to donor ratios, etc), targeted, cell-specific, gene editing experiments can be performed in the reporter TdTomato mouse model. In parallel, a (partly) humanised mouse model can be generated for surfactant B deficiency (a lethal rare genetic disorder affecting the lung parenchyma) and those candidates with the ‘best’ combination of vector and cell-specific promoter will be used to demonstrate proof-of-concept.  We also have a conditional surfactant protein B knockout mouse model, which can be utilised as proof-of-principle if needed. Demonstrating efficient gene editing of specific and rare disease-causing mutations will support the use of personalised medicine to treat rare lung diseases where no other treatment options exist.

### Training Opportunities: The proposed project will involve: human cell culture including cell manipulation via vector transfection & transduction; recombinant virus (lentiviral & AAV) vector design, production & titration; general recombinant DNA techniques including plasmid DNA manipulation, DNA & RNA purification, cloning, etc; gene quantification by PCR and droplet digital PCR; flow cytometry; confocal and immunofluorescence microscopy; immunoblotting; CRISPR‐Cas9 genome engineering; Nanopore MinION based, third-generation next generation sequencing & relevant bioinformatics pipeline involving Python3 and R coding/programming; use and design of transgenic mouse models of human disease, including handling, processing, and analysing mouse blood and tissue samples, and training for a Home Office Personal Licence. The student will also receive training in scientific writing, oral presentations and public engagement activities. The project would suit someone who would like to focus on wet lab techniques and contribute to the overall translation of a genetic therapy to the clinic.

### Background reading:

Munis, A. M., Hyde, S. C., & Gill, D. R. (**2020**). A human surfactant B deficiency air-liquid interface cell culture model suitable for gene therapy applications. *Molecular therapy. Methods & clinical development*, *20*, 237–246. <https://doi.org/10.1016/j.omtm.2020.11.013>

van Haasteren, J., Munis, A. M., Gill, D. R., & Hyde, S. C. (**2021**). Genome-wide integration site detection using Cas9 enriched amplification-free long-range sequencing. *Nucleic acids research*, *49*(3), e16. <https://doi.org/10.1093/nar/gkaa1152>

Miura, H., Imafuku, J., Kurosaki, A., Sato, M., Ma, Y., Zhang, G., Mizutani, A., Kamimura, K., Gurumurthy, C. B., Liu, D., & Ohtsuka, M. (**2021**). Novel reporter mouse models useful for evaluating *in vivo* gene editing and for optimization of methods of delivering genome editing tools. *Molecular therapy. Nucleic acids*, *24*, 325–336. <https://doi.org/10.1016/j.omtn.2021.03.003>

Hu, J., Bourne, R. A., McGrath, B. C., Lin, A., Pei, Z., & Cavener, D. R. (**2021**). Co-opting regulation bypass repair as a gene correction strategy for monogenic diseases. *Molecular therapy: the journal of the American Society of Gene Therapy*, S1525-0016(21)00204-5. Advance online publication. <https://doi.org/10.1016/j.ymthe.2021.04.017>

Kelly, J. J., Saee-Marand, M., Nyström, N. N., Evans, M. M., Chen, Y., Martinez, F. M., Hamilton, A. M., & Ronald, J. A. (**2021**). Safe harbor-targeted CRISPR-Cas9 homology-independent targeted integration for multimodality reporter gene-based cell tracking. *Science advances*, *7*(4), eabc3791. <https://doi.org/10.1126/sciadv.abc3791>