Gene therapy aims to correct disease-causing changes (known as mutations) in the genetic code, process unofficially referred to as “genomic surgery”, or deliver an additional copy of the mutated gene to the affected cells - “genetic prosthetics”. Gene therapy is rightly being heralded as the most exciting frontier in modern medicine with stunning therapeutic successes being realised NOW! Modern genomic and cellular technologies are beginning to deliver a rapidly increasing number of unprecedented therapeutic triumphs that are changing the face of modern medicine. After almost three decades of research, knowledge generation and capability building, a spectrum of diseases, previously thought to be incurable, have cures within technological reach at last. Infants who would otherwise have succumbed to spinal muscular atrophy (SMA) are now developing normally with the very real prospect of living full and healthy lives. Individuals with vision impairments owing to retinal disease are seeing more clearly. Those living with haemophilia are being freed from dependency on life-long factor replacement therapy. And this is just the beginning. This grant proposal seeks to facilitate and accelerate these ground-breaking advances by developing the next generation of gene delivery tools.

Gene delivery vehicles (vectors) derived from Adeno-Associated Virus type 2 (AAV2) are the technological foundation underpinning these recent clinical successes in human clinical trials, most notably for diseases of the central nervous system, eye and liver. Advances in *AAV capsid technology* have been the cornerstone of this progress. Development in this area is essential to bringing many more diseases, theoretically amenable to gene therapy, within technological reach. The liver, in particular, is a key target for the development of more efficient AAV vector delivery, given its functional complexity and the existence of >100 genetic and acquired liver diseases.

Despite the early exciting clinical successes there are number of obstacles that still need to be overcome before the benefits of AAV-based gene therapy can be enjoyed by the millions of paediatric and adult patients, and their families, suffering from debilitating genetic diseases. Notably, even the most advanced vectors currently available are unable to target the human liver and deliver the genetic payload with a clinically relevant efficiency. Furthermore, humans have evolved a highly sophisticated defense mechanisms (the immune system) that protect us from viral infections. While vital to our survival, our natural anti-viral defenses cannot distinguish disease causing viruses from therapeutic vectors. Lastly, to ensure highest impact on the society, the developed and optimized clinical vectors must be able to be produced in large quantities, which will decrease the cost of each therapeutic application.

This project builds directly on our recent revolutionary discovery that natural AAV viruses recovered from human liver samples can function as highly efficient gene therapy delivery tools. In addition to their functional superiority over currently utilized clinical vectors, the vectors derived from natural variants may be able to evade the recognition by human defense mechanism, making them ideal candidates for clinical development. Finally, our data shows also that the natural variants are compatible with current vector manufacturing technologies enabling large-scale manufacturing.

In this project we will screen primary human liver samples for the presence of natural AAV variants. All identified variants will be converted into vectors and will undergo detailed analysis that will allow us to rate them based on their ability to target primary human hepatocytes, ability to evade human immune system and the easy of clinical manufacturing at scale. Candidates will also undergo additional rounds of engineering on primary human liver cells with the aim to further enhance their function and patentability. Final candidates will be tested for their ability to cure a genetic disorder in patient derived liver cells implanted into a mouse model of human liver.

Upon completion of the proposed studies, we anticipate to have successfully developed a powerful set of novel AAV vectors ready for clinical development in academic and/or commercial settings. The manufacturability and sero-reactivity data collected will further enhance the commercial value of our novel gene therapy tools and will enhance translational development from bench to the bed site. Finally, the knowledge generated will allows us to better understand fundamental biology of AAV and cell biology of human liver.