This response was submitted to the Call for Evidence held by the Nuffield Council on Bioethics on *Genome editing* between 27 November 2015 and 1 February 2016. The views expressed are solely those of the respondent(s) and not those of the Council.

Nuffield Council on Bioethics

Genome Editing: open call for evidence

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Biomedical research and human applications

1. Scope
2. Current state of the art and progress
3. Technical limitation
4. Efficacy and safety
5. Timescale
6. Ethical concerns
7. Impact/industrial development

1 - Biomedical research is increasingly reliant on the use of large animals, notably the pig. Cutting edge research is dependent on genetic engineering/genome editing for the purpose of creating suitable surrogates for the study of genetic diseases (*1-4*) and regenerative medicine (*5*), or for supplying an unlimited source of cells, tissues, organs and scaffolds that can be used effectively and safely for transplantation to cure human diseases (*6*). We would like to bring together the contribution of two large EU-funded research consortia, namely Xenoislet (http://xenoislet.eu/) and TransLink (http://www.translinkproject.com), involved in the development of the clinical application of xenotransplantation for the purpose of improving human health.

2 - The progress in the field of xenotransplantation has changed gear since nuclease-based techniques have been implemented for editing of the pig genome (*7-10*). The number of transgenic animals with inactivated genes and/or integrated novel transgenes has dramatically increased thus facilitating the development and completion of pre-clinical studies (*3-5, 11-15*). Genome editing technology has significantly evolved since the initial reports (*6*). Accordingly, recent work has demonstrated remarkable survival rates, reaching up to almost 3 years post transplantation using transgenic pigs for heterotopic cardiac xenografts in baboons (*16*). Restrictions on the use of animals in research (including primates), however, may unduly delay the translation to clinical use of this technology and the potentially beneficial products that could result in both the fields of xenotransplantation and regenerative medicine. For instance, the generation of transgenic porcine islets with improved efficacy specifically designed in the context of the Xenoislet project is expected to greatly advance progress toward clinical islet xenotransplantation. Similarly, genetic engineering of pigs in the context of the TransLink project will lead to the generation of animal-derived cardiac valves more compatible with man.

3 - The use of nucleases, especially the CRISPR/Cas9, has greatly simplified the editing of the genome in species where a high-resolution map of the genome is available. The genome sequence currently available for the pig, however, is still not sufficiently accurate and this is currently the main limitation to be able to target any gene present in the genome of this species. As a consequence, at present a considerable amount of time is required to validate or to clone a specific gene that has to be targeted.

4 – The development of engineered pig lines suitable for xenotransplantation requires the knock out / knock in of multiple transgenes. Multi-transgenic pigs have already been generated by classical means of homologous recombination and/or random integration. With the use of programmable nucleases, multiple recombination events (ins/del or homologous directed repair) can be obtained in one single step. Targeted integration can ensure the optimal performance of any transgene integrated therefore increasing the efficacy of the expected function. The principal safety issue surrounding xenotransplantation has for many years been the potential for transmission of zoonotic pathogens. The Porcine Endogenous Retrovirus (PERV) has been the main focus of this concern since PERV is present as integrated elements in the pig genome. Recent work using CRISPR/Cas9 technology has convincingly demonstrated that multiple copies of PERV can be targeted simultaneously to obtain PERV free cells (*17*). However, it is yet to be demonstrated that this technology can give rise to healthy animals. Genome editing in live pigs is required to test the safety and efficacy of this technology for xenotransplantation products. Although potential off target effects are often indicated as possible complications, animals generated after genome editing do not appear to be different from those generated by conventional technologies. Therefore, there are no fundamental reasons why genetically modified pigs utilising gene editing pose *a priori* different risks compared with those engineered by conventional transgenesis. However, risks should be carefully assessed case by case depending on the modified/added genes.

5 – In the last five years, with the advent of programmable nucleases more recombinant pigs have been generated than in the previous 25 years combined by conventional genetic engineering. It is reasonable to assume that, in the next 5 years, due to genome editing further considerable advancements will be made. This is expected to rapidly impact on clinical applications that entail the use of cells, tissues or scaffolds and, within 10 years, on the clinical application of solid organ xenotransplantation (heart, kidney, liver).

6 – To date, the multi-transgenic pigs generated by conventional means for xenotransplantation are healthy and can reproduce. Unexpected phenotypes can potentially emerge, however, after repeated rounds of multiple genetic engineering as vital functions could be affected in addition to side-effects due to inadequate transgene function.

With genome editing, the same concerns may arise and investigators are expected to strive to ensure the highest levels of care and welfare are provided to the animals. Unfortunately, to progress to the clinic this technology will unavoidably still require validation in primates. However it is estimated that genome editing will significantly reduce the overall numbers of primates needed due to the expectation that genome editing will permit simultaneous insertion of multiple transgenes and enable considerable reduction in the use of primates in accordance with the 3R tenet.

Ethical requirements are high on the list of importance of scientific societies, policy makers and international agencies such as the WHO and are heavily embedded in the regulatory frameworks dealing with xenotransplantation (*18-20*). Indeed ethical considerations are an integrated part of any xenotransplantation practice and our research consortia include individuals who have been and still are involved in these important issues and are contributing to the development and harmonisation of guideline policies (*21*).

It is paramount that human trials should only be considered in the presence of demonstrated preclinical efficacy and safety and in the context of a relevant xenotransplantation regulatory framework. It is also worth reminding that the increased availability of organs through xenotransplantation would contribute to preventing the illegal trade of human organs, ensuring health for everyone and precluding the exploitation of vulnerable individuals.

7 – To take any new form of therapy from ‘bench to bedside’ requires significant investments from large pharmaceutical industries. However, earlier phases often rely on the fundamental contribution of small start-up companies that develop academic intellectual property into pre-clinical and initial clinical trials. Our EU-funded research initiatives has enabled the generation of two start-ups (in France and Belgium) that are reliant on genetically modified pigs to develop their IP. Therefore, any restrictions on genome editing would impede progress. In addition, it is worth commenting on the fact that the existence of broadly granted patents can often pose serious obstacles to progress towards the development of novel therapies.

With respect to impact for xenotransplantation, genome editing has revitalised the field giving new options for safer and more effective products from transgenic pigs (http://www.nature.com/news/new-life-for-pig-to-human-transplants-1.18768). This is expected to significantly drive new funding for research in this scientific discipline and reduce the time for development of this promising therapeutic approach.

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Other activities

COST Action
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