PROJECT LICENCE NARRATIVE

Title of project (section 1)

The consequences of renal transplantation in the pig.

(NB This project licence does not authorise cross-species transplantation of solid organs.

Personal details (sections 2 to10)

This section contains personal information relating the licensee against the headings set out on the form of application.

Place details (sections 11 to13)

States the designated establishment at which the licensed research is to be carried out and the relevant personal details of one deputy Project Licence Holder.

The deputy project licence holder signed a declaration *"I have read and understood the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, and the authorities requested in this application. I agree to abide by the terms and conditions of the Act and of any licence authorities that may be issued."*.

Permissible purpose (section 14)

Control of disease, ill health or abnormality is assigned as the primary permissible purpose of this programme of work.

Referral to Animal Procedures Committee (section 15)

The licence does not indicate that it involves tobacco or tobacco products, the specific training of practising surgeons in microsurgery, non-human primates in substantial procedures, or wild-caught non-human primates.

Duration of project (section 16)

The project licence was granted in February 2005 and is valid for five years.

Background, objectives and potential benefits (section 17)

Referring to information in the public domain and unpublished findings, the background to the programme of work is set out. The specific objectives to be pursued and the potential benefits of the programme of work are recorded.

A short overview establishes that the general area of interest is to seek an improved understanding of and characterise, manipulate and seek to overcome, the biological responses to transplanted organs that lead to rejection, including the response to solid-organ xenotransplants. This programme of work does not authorise solid-organ transplantation between species. There is reference to manipulation of gene structure and function; of genotype and of phenotype.

The background section reviews the contributions the research group has already made to the understanding of transplantation biology and its expertise with the animal models it is proposed to use. Based on published and unpublished material the current understanding of aspects of the biology of hyperacute rejection and related responses is discussed in detail highlighting the role played by endothelial cells in transplanted organs. This is followed by consideration of immunomodulation as a result of cellular adaptation and its relevance to a significant proportion of potential transplant recipients, and the clinical benefits that might be realised if a safe and effective means could be derived from exploitation of these phenomena to overcome hyperacute rejection. This leads to a discussion of the relevance of a specific phenotype[1] seemingly associated with such phenomena, a summary of the relevant clinical and in vitro supporting evidence, and the scientific basis for a hypothesis on the relationship between anti-graft antibodies and the relevant biological phenomena, and the case that this should now be evaluated in a pig model with a view to producing insights relevant to clinical practice.

The licence then outlines the component parts of the plan of work. Plans for the development of a non-invasive means of identifying endothelial activation markers as an alternative to biopsy procedures to characterise and manage the host response are set out. The case for a small study to establish the pharmacodynamics (as opposed to the pharmacokinetics) of a key immunosuppressant is provided. The use of skin grafts to assess degrees of immunological adaptation is discussed. Additional text considers the potential role of circulating white blood cells in the host reaction to transplanted organs, and the means to track the distribution of such cells in transplant models is discussed.

The background section concludes with a consideration of a potentially efficient means of producing genetically altered animals[2].

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[1] In this context the term phenotype is used not to indicate the general conformation of the animals, rather it refers to patterns of gene expression.

[2] NB: If that approach proved successful in a small proof of concept study the licence document indicates this would result in a new and separate project licence application to allow the breeding of such animals relevant to the study of xenotransplantation of solid organs.

Six objectives are listed.

1. To evaluate a proposed strategy to prevent hyperacute rejection.
2. To develop a model of pre-transplantation immunological adaptation.
3. To develop a non-invasive model to visualise endothelial cell activation in transplanted allograft.
4. To develop a non-invasive method for visualising renal blood flow in transplanted organs.
5. To assess the contribution of white blood cells in graft rejection.
6. To evaluate a method of producing genetically altered animals.

The potential benefits are set out in the context of providing insights relevant to advancing clinical allotransplantation (transplants involving only one species), including the possible application of three monitoring methods, and, in addition, of potential relevance to xenotransplanation (cross-species transplants).

The sources of funding are declared.

A detailed bibliography is supplied in support of the key scientific arguments detailed in the narrative.

Plan of work (section 18)

This section confirms the licence does not involve the use of non-human primates, the release of animals to the wild, the production of monoclonal antibodies by the ascites method, lethality testing for regulatory purposes, testing for skin corrosivity, testing for skin phototoxicity for regulatory purposes, or the testing of cosmetic products or ingredients.

The licence acknowledges that animals undergoing related procedures on another project licence will be re-assigned to this programme of work.

The plan of work is mapped onto the six specific objectives listed above. In some cases only after some of the objectives have been achieved can the others be addressed. All of the work is to be undertaken using pigs.

1. A strategy to prevent hyperacute rejection is outlined using pig renal and arterial allotransplantation models. This is linked to protocols 19b1, 19b2 and 19b3.The strain of pig to be used is given, and optimisation of the housing and care conditions, anaesthetic and surgical practice, is mentioned. The ability to sensitise animals, and for sensitised animals to reject transplanted material, already having been demonstrated in other studies, novel gene constructs will be used to modify cell-membrane bound molecules on the epithelium of transplanted material. Only if the arterial transplant model confirms a positive effect will the construct be

evaluated in the renal transplant model once an appropriate ex vivo or in vitro method of introducing the construct into the kidney have been devised. In control animals unmodified kidneys will be transplanted. Rejection will be monitored. If any construct has the requisite effect consideration will given to establishing genetically altered animals expressing the construct and a separate project licence sought.

2. To develop a model of pre-transplantation immunological adaptation. The available in vitro, in vivo and clinical evidence is reviewed and the basis for the working hypothesis reprised. Protecting cells from immunological damage will be induced by the injection of specified materials (working from low doses to higher doses), and organs examined thereafter by immunohistology (microscopic inspection revealing how the immune system has interacted with the tissue). If an appropriate pattern of biomarker expression and response can be established, then kidneys from animals expressing adaptation would be implanted into sensitised animals and aspects of rejection studied. This is linked to protocol 19b4 and 19b5.

3. To develop a non-invasive model to visualise endothelial cell activation in transplanted allografts. The available in vitro and in vivo evidence to support the working hypothesis is reviewed. If suitable reagents can be generated the proposal is to develop a non-invasive method to study selective gene expression in normal and transplanted kidneys supplemented, in some cases, by immunohistology. This is linked to protocol 19b6.

4. To complete an ongoing study to develop and evaluate a non-invasive method for measuring blood flow in transplanted kidneys based on a safe technology already used for other clinical applications. It is proposed to use a small number of animals to non-invasively measure renal blood flow and the effects of a named immunosuppressant. This is linked to protocol 19b7.

5. To assess the contribution of white blood cells to graft rejection. The available in vitro evidence is reviewed and the proposal is to optimise and validate these findings in vivo. This is linked to protocol 19b8.

6. To evaluate a potentially efficient method of producing genetically altered animals. A pilot study in pigs to demonstrate proof of concept is outlined, premised on findings in another species. This is linked to protocol 19b9.

The nature and proposed management of various foreseeable surgical complications is outlined, and the basis of the statistical evaluations underpinning the study design, group sizes and data analyses is provided.

A further bibliography lists publications relevant to understanding the rationale for the programme of work.

Section 18a offers a scientific justification for the use of the pig models, and briefly reprises the attempts being made within the programme of work to minimise animal numbers and the degree of invasiveness.

Sections 18c and 18d were not completed.

Protocols (section 19)

Section 19a lists nine protocols, all using pigs, with the maximum number to be used each year estimated at 217. Eight of the protocols are assigned a moderate severity limit, and one a mild severity limit.

19b 1, assigned a 'moderate' severity limit, authorises the harvesting of tissues and organs (under anaesthesia and with and without recovery from anaesthesia) and a number of options are outlined. Blood samples may be taken on one or more occasion (including from cannulated animals). The blood sampling regimens are defined. Under general anaesthesia with recovery small split skin grafts or specified blood vessels might be removed. Other organs might be removed under general anaesthesia without recovery. In some animals genetic constructs would have been previously introduced (intra-operatively – with in some cases the tissues being removed during a second non-recovery procedure) into named blood vessels to be expressed by the endothelium (the cells of the blood vessel in contact with the blood). In some studies contrast agents would be injected to facilitate non-invasive monitoring. The nature of the peri- and post-operative management and care, as developed in consultation with the named veterinary surgeon (this is the case for each protocol), are outlined, the adverse effects and endpoints to be applied are specified. A separate schematic representation of the treatment options is provided.

19b2, assigned a 'moderate' severity limit, authorises the taking of blood to establish the phenotype of the animal and, subsequently from sensitised animals, further samples from which immunoglobulin will be extracted (the blood sampling regimen is defined). There is authority for in some cases establishing of vascular access (by placing a vascular cannula under general anaesthesia and with recovery) to permit sensitisation to be induced by injecting specified materials. Alternatively animals may be sensitised by heterotopic transplantation[3] of a kidney[4]. Non-sensitised animals may serve as controls and passively sensitised animals also studied. The immune response of such animals will be monitored

[3] Here, and at other appropriate points in the protocols, details of the anaesthetic, surgical and post-operative management of the surgically prepared animals are provided.

[4] In all studies involving the transplantation of kidneys the intention is to study the host reaction to the kidney, not to study the performance of the transplanted kidney. Unless otherwise stated, animals will retain one of their own kidneys, and renal failure is not therefore expected.

(including by needle biopsy) and suitable responders used as a source of antibodies for further studies. Tissues might then be implanted, some of which would have had the expression of surface molecules manipulated and the immune response monitored. Initially this will take the form of transplanted blood vessels, but ultimately transplanted kidneys might be studied and this will include the talking of needle biopsies and the non-invasive monitoring of renal blood flow. Most animals would not require immunosuppressants. One variant of the procedure was intended to model hyperacute rejection. The animals will be humanely killed at the end of the protocol. The nature of the peri- and post-operative maintenance and care are outlined and the adverse effects and endpoints to be applied specified. A separate schematic representation of the treatment options is provided.

19b3 , assigned a 'moderate' severity limit, authorises the taking a a blood sample to establish the phenotype of the animal and the placement under general anaesthesia and with recovery of vascular cannulae, followed by sensitisation as per 19b2. Under sedation, needle biopsies will be taken from animals with renal transplants and non-invasive measurements or renal blood flow taken. Alternatively animals will be sensitised by transplantation of a kidney; the immune response of such animals will be monitored and suitable responders used as a source of antibodies for further studies. Tissues may then be implanted, some of which will have had the expression of surface molecules manipulated, and the immune response will be monitored. Initially this will take the form of transplanted blood vessels, but ultimately transplanted kidneys may be studied and this would include the talking of needle biopsies and the non-invasive monitoring of renal blood flow. Most animals will not require or receive immunosuppressants. One variant of the procedure is intended to model hyperacute rejection The animals will be humanely killed at the end of the protocol. The nature of the peri- and post-operative maintenance and care are outlined and the adverse effects and endpoints to be applied were specified. Any animal with renal failure will be killed. 'A separate schematic representation of the treatment options is provided.

19b4, assigned a 'moderate' severity limit authorises the taking of blood to determine the phenotype of the animal and the placement of a vascular cannula under general anaesthesia and with recovery to permit the administration of defined test materials to alter the immune response and gene expression and produce the desired immunological adaptation. Thereafter the kidneys will be removed under general anaesthesia and without recovery for further study. The nature of the peri- and post-operative care are outlined, the adverse effects and endpoints to be applied are specified. A separate schematic representation of the treatment options is provided.

19b5, assigned a 'moderate' severity limit, allows tissue from animals that show the desired immunological adaptation generated under procedure 19b4 to be

transplanted[5] to recipient animals that have been actively or passively sensitised. It authorises the taking of blood to determine the phenotype of the animal and the transplantation of a kidney produced under 19b4 into recipient animals pre-treated to show a specific immunological profile (by the administration of substances or transplantation of a donor kidney). Needle biopsies may be taken from the transplanted kidneys and blood flow evaluated non-invasively. A specific immunosuppressive agent may or may not be administered The animals are killed at the end of the procedure. The nature of the peri- and post-operative maintenance and care are outlined and the adverse effects and endpoints to be applied were specified. A separate schematic representation of the treatment options is provided.

19b 6 is assigned a 'moderate' severity limit A blood sample will be taken to determine the animal's phenotype, and a venous cannula placed under general anaesthesia and with recovery. A unilateral kidney transplant will be performed. Most animals will be immunosuppressed with a specified immunosuppressive agent and subsequent needle biopsies may be taken, and renal blood flow monitored non-invasively. Diagnostic imaging of the transplanted kidney using a gamma camera may be undertaken – and antibodies and/or contrast agents administered during the course of this. At the end of the procedure the animals will be killed and tissue retained for analysis. The nature of the peri- and post-operative maintenance and care are outlined and the adverse effects and endpoints to be applied are specified. A separate schematic representation of the treatment options is provided.

19b7 is assigned a 'moderate' severity limit and is designed to characterise the vasoconstrictor properties of a defined test material. A blood sample will be withdrawn to determine phenotype, and a venous cannula inserted. A specified immunosuppressant will be administered through the cannula, and blood samples withdrawn to measure the blood levels of the drug. Renal blood flow will be measured non-invasively. The animals are to be killed at the end of the procedure. The expected adverse effects and endpoints to be applied specified. Any animal with renal failure will be killed. A separate schematic representation of the treatment options is provided.

19b8 is assigned a 'mild' severity limit. A single blood sample will be withdrawn to determine the phenotype of the animal. Thereafter under general anaesthesia and without recovery vascular cannulae will be inserted and open surgery performed to mobilise specified blood vessels which will then be temporarily clamped to induce controlled renal ischaemia. Human or pig white blood cells labelled with fluorescent dyes will be administered and their distribution determined by fluorescence photography and ex vivo study of tissues. The period of anaesthesia will not exceed 12 hours. Reassurances are given that an adequate depth of general anaesthesia will be maintained at all times.

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[5] In some animals this will involve a second renal transplant – replacing the originally transplanted organ with a donor kidney that is from a normal animal or which has the desired form of immunomodulation.

19b9 is assigned a 'moderate' severity limit. Blood samples will be withdrawn to determine the phenotype of the animals and the response to treatment. A chemical agent will be administered to eliminate existing sperm cells and this will be confirmed by testicular biopsy under general anaesthesia. When this is achieved then, during open surgery under general anaesthesia and with recovery, test materials (to promote the incorporation of a defined DNA sequence) will be injected into the testis. Up to 14 days later under general anaesthesia and without recovery the testes will be removed for further study. The nature of the peri- and post-operative management and care are outlined, the adverse effects and endpoints to be applied are specified. A separate schematic representation of the treatment options is provided.

Overall severity band (section 20)

The licensee has assigned the project a moderate severity band.

Declaration by applicant (section 21)

The applicant has made the required declaration that the feasibility of achieving the purpose of the project by means not involving regulated procedures on animals protected under the Animals (Scientific Procedures) Act 1986 has been considered; that no such alternatives would achieve the objectives of this project; that all reasonable efforts have been made to minimise the suffering likely to be caused and the number of animals to be used; that the advice of the named veterinary surgeon and named animal care and welfare officer has been sought; that he/she has read the Home Office *Guidance on the Operation of the Animals (Scientific Procedures) Act 1986*, understands the terms and conditions under which he/she may hold a project licence and undertake responsibility for the management of the project as set out in the *Guidance*; and that he/she is responsible for the supervision, conduct and competence of the deputy project licence holder(s) and the personal licence holder(s) working under the authority of the licence.

Declaration by the holder of the certificate of designation (section 22)

The Certificate of Designation holder has confirmed that the application completed the establishment's local ethical review process and accepted responsibility for ensuring that suitable facilities will be available in accordance with *the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures* and his/her responsibilities as set out in the Home Office *Guidance on the Operation of the Animals (Scientific Procedures) Act 1986.*

Licence conditions

In addition to the standard project licence conditions permission was granted to transfer animals from another project licence.

Animals Scientific Procedures Division
Home Office