Version Date	

Applicant to complete version date

PPL No. /

Home Office

APPLICATION FOR A PROJECT LICENCE

UNDER THE ANIMALS (SCIENTIFIC PROCEDURES) ACT 1986

PROJECT TITLE Section 1 (<50 characters including spaces)

Tumour neovascularisation

A. PROJECT LICENCE HOLDER

Under ASPA 5(2), project licences are granted to the person who has overall responsibility for the programme of work specified in the licence

a. Title (e.g. Professor, Dr, Mr)	Professor
b. Surname	Black
c. Forename(s)	John
d. Qualifications	BSc MSc PhD
e. Position or appointment	Professor of Tumour Biology

If you have previously been known by another name, give that name:

a. Surname	
b. Forenames	

CONTACT DETAILS

a. Address for correspondence *This will normally be the address of the establishment where you are working and must be within the UK* Post Code	Dept Oncology University of X University Walk X WX1 1YZ
b. Telephone number and extension	xxxxx xxxxxx
c. Mobile phone number (optional)	xxxxx xxxxxx
d. Fax number	xxxxx xxxxxx
e. E-mail address	John.Black@X.ac.uk

DATE OF BIRTH *(dd, mm, yyyy)*:	01/04/1956

Your relevant knowledge, skills and experience

Give brief details of your knowledge, skills and experience. Indicate your position within your organisation which makes you a suitable person to take responsibility for this programme of work.
Qualifications BA (Natural Sciences), University of Y (1977); MSc (Pharmacology), University of Z (1978); PhD (Tumour Biology), University of Z (1982) Experience Cruelty to Animals Act 1876 licence holder since 1978; PIL holder since 1987; PPL holder since 1989 Competent in experimental design and data analysis generally, and in tumour biology (including tumour vascularisation) in particular. 187 full scientific papers on tumour biology published since 1978: see publications file attached.

Unless you hold/have held a project licence within the last 5 years, list the relevant modular training (Modules 1, 2, 5) you have completed successfully within the last five years, with dates and enclose copies of the certificates with your application.
Modular Training Modules 1-4 passed (2000); Module 5 passed (2000)

Funding, expertise and other resources

What resources do you have for this project? What expertise, staffing, facilities, equipment and funding are available to you? Has the proposed work been peer-reviewed? If so, by whom?
Technical expertise acquired through 31 years relevant experience. The research team consists of myself as principal investigator, two post-doctoral scientists, one PhD student and one laboratory technician. Laboratory space is made available by the University of X. I possess the required tumour biology equipment, and have access to shared facilities for the production and maintenance of genetically altered animals. Funding has been obtained from Research Council A and Charity B after peer review.

Personal licences

Provide the number of your current or previously held ASPA personal licence.
PIL XX/XXXX

Project licences

Provide the number(s) and expiry date(s) of your current or previously held (in the last 5 years) ASPA project licence(s).
PPL AA/AAAA expires DD/MM/YYYY

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Continuation of work

If you are seeking authority in this application to continue work under one or more current ASPA project licences, provide the number of the relevant expiring project licence(s) and expiry date(s).
PPL AA/AAAA expires DD/MM/YYYY

Duration of project

Under ASPA 5(7), the maximum allowable duration of a project licence is five years

Specify the duration of licence you require if less than five years

HOME OFFICE LIAISON CONTACT (if you have one; this must be someone at your establishment)

a. Name	Jane White
b. Telephone number and extension	xxxxx xxxxxx
c. Fax number	xxxxx xxxxxx
d. E-mail address	Jane.White@X.ac.uk

In your absence, who may we contact if we have any questions about the management of your project?

a. Name	Dr Philip Green
b. Position held	Lecturer
c. Telephone number and extension	xxxxx xxxxxx
d. E-mail address	Philip.Green@X.ac.uk

B. PLACE(S)

Under ASPA 5(1), a project licence must specify a place or places (so-called 'availabilities') where the regulated procedures will be carried out.

Primary availability

a. PCD number:	XX/XXXX
b. Name of designated establishment:	University of X

Additional availability (if any)

If you intend carrying out regulated work at more than one additional designated establishment paste in a copy of this section for each establishment. **You should note that the relevant parts of this application must complete the ethical review process at each additional establishment and that the Certificate Holder at each of these must complete a declaration in Part F (3) of this application.**

a. PCD number:	
b. Name of designated establishment:	

Why do you need this additional availability? Please indicate whether you intend to move animals between establishments during the course of a series of regulated procedures and if so describe the reasons for such transfers.

Who will be responsible for supervising the work at this additional establishment?	
a. Title (e.g. Professor, Dr, Mr, Ms)	
b. Surname	
c. Forename(s)	
d. Address for correspondence *This will normally be the address of the establishment where the supervisor is working and must be within the UK* Post Code	
e. Telephone number and extension	
f. Mobile phone number (optional)	
g. Fax number	
h. E-mail address	

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Places other than a designated establishment (PODEs) (if any)

List any place(s) that is not a designated establishment and where you intend to carry out regulated procedures

Why do you need to undertake regulated work at this PODE?

C. SCIENTIFIC BACKGROUND Section 17

Once the licence is granted, you should only need to amend Part C if you are significantly changing your project's purpose.

The total response to this Part must not exceed **2000 words**

Background

- For research projects: What is the current position in your area of work and how will this project help to advance knowledge or meet a clinical need?
- For testing or screening projects: What are the relevant statutory requirements or regulatory guidelines?
- For service or production projects: What are the likely demands for the service or product in the lifetime of the licence?
- Where applicable, summarise relevant progress under any previous project licence.

A key event in tumour progression is the deregulation of angiogenesis. The main objective is to determine whether combinations of inhibitors of vascular endothelial growth factor (VEGF) and chemotherapy are more effective than monotherapy in preventing tumour vascularisation and tumour growth.

Angiogenesis

Angiogenesis is a fundamental process by which new blood vessels are formed and capillary networks developed. Normally, for example in development or wound repair, angiogenesis is highly regulated: it may be turned on for a few days, and then be turned off completely.

Capillary blood vessels consist of endothelial cells lining the luminal surface and pericytes embedded in a basement membrane. Specific angiogenic molecules (eg vascular endothelial growth factor (VEGF), fibroblast growth factor, platelet derived endothelial growth factor) can act in a paracrine or autocrine manner to initiate and/or sustain the angiogenic process, and specific inhibitory molecules (eg angiostatin, endostatin, thrombospondin, α-interferon, tumstatin) can stop it. A common trigger for angiogenesis is hypoxia, which stabilises the transcription factor hypoxia-inducible factor 1 (HIF-1), resulting in increased transcription of hypoxia-responsive genes such as VEGF.

VEGF

Vascular endothelial growth factor (VEGF) is actually a sub-family of growth factors of the platelet-derived growth factor super-family. The most important member is VEGF-A (often simply termed VEGF): other members are VEGF-B, VEGF-C, VEGF-D and placenta growth factor (PlGF). VEGF-A is now well confirmed as the primary and the most potent inducer of angiogenesis. It is primarily produced by parenchymal cells and tissue-infiltrating cells such as macrophages, and acts in a paracrine manner on endothelial cells to activate cellular signalling pathways, by binding to receptor tyrosine kinases VEGF-R1, R2 and R3, as well as the neuropilins. It then promotes several events required for the formation of new blood vessels, such as endothelial cell survival, proliferation, migration and vascular permeability.

VEGF receptors

VEGFRs are cell surface tyrosine kinases, expressed mainly but not exclusively on endothelial cells. Vascular endothelial cells primarily express VEGFR-1 and VEGFR-2 (whereas lymphatic endothelial cells express VEGFR-2 and VEGFR-3). VEGF-A binds to VEGFR-1 and VEGFR-2. The VEGFR-1 has some independent signalling role but is also thought to modulate VEGFR-2 signalling, and act as a decoy receptor, sequestering VEGF from VEGFR-2 binding (which may be important during vasculogenesis in

the embryo). VEGFR-2 appears to mediate almost all of the known cellular responses to VEGF. VEGF-C and VEGF-D, but not VEGF-A, are ligands for a third receptor (VEGFR-3), which mediates lymphangiogenesis.

Neuropilin-1 and neuropilin-2 enhance VEGF stimulated signal transduction by the VEGFR-2 receptor. Recent evidence has shown that manipulating neuropilin function can regulate tumour growth and metastasis through effects on vascular biology in the case of neuropilin-1 and lymphatic biology in the case of neuropilin-2.

VEGF receptor signal transduction

All members of the VEGF family stimulate cellular responses by binding to tyrosine kinase receptors (the VEGFRs) on the cell surface, causing them to dimerise and become activated through transphosphorylation, although to different sites, times and extents. Kinase activation and autophosphorylation of tyrosine residues leads to the activation of signal transduction molecules phospholipase C-γ (PLC-γ), PI3, Akt, Ras, Src and MAPK. Activation of PLC leads to the release of Ca^{2+} from internal stores and activation of PKC. This in turn stimulates the RAF/MEK/ERK pathway leading to cell proliferation. Ca^{2+} mobilisation and PKC activation are thought to be key signalling events in VEGF-A induced vascular permeability via activation of endothelial nitric oxide synthase activity.

<u>VEGF and angiogenesis</u>

VEGF expression can be triggered during the early stages of neoplastic transformation by environmental stimuli (e.g. hypoxia, low pH) or by genetic mutations (eg in Ras, p53 or HER2/ErB2).

VEGF is a potent mitogen for micro- and macro-vascular endothelial cells derived from arteries, veins and lymphatics. It induces angiogenesis via a direct effect on endothelial cells, and its binding to VEGFR-2 has been shown to elicit an efficient endothelial cell response in multiple studies, both in-vitro and in-vivo. Key in vitro studies have demonstrated that VEGF can induce confluent microvascular endothelial cells to invade collagen gels forming capillary-like structures, indicative of angiogenesis. This VEGF driven process induces the degradation of the extracellular matrix through the secretion and activation of enzymes such as plasminogen activator and the MMP interstitial collagenase. However, although VEGF is able to induce the development of neo-vasculature, the blood vessels formed have multiple structural and functional abnormalities, many of which are a consequence of the actions of VEGF (eg high vascular permeability – VEGF is also known as VPF or vascular permeability factor). In brief, tumour blood vessels have irregular lumens, lack effective mural cell support, are highly disorganised and permeable. All of these abnormalities lead to further abnormalities in the tumour environment that aid disease progression.

In order to support continued growth, the tumour's vascular system persistently remodels itself. Hence angiogenesis is a process which continues until the tumour is removed or killed, or the host dies.

<u>The Angiogenic Switch</u>

During the development of a tumour, there can be a prolonged period (weeks/months in mice, years in humans) during which the tumour is not angiogenic, and is restricted in growth to a few mm^3 (eg in-situ carcinoma). The ability of a tumour to progress from a non-angiogenic to an angiogenic phenotype is central to the progression of cancer. It is only when sufficient cells within the tumour have switched to an angiogenic phenotype, and when the relatively short-lived pro-angiogenic proteins exceed the local concentration of anti-angiogenic proteins, that pathological angiogenesis may occur - termed the "angiogenic switch".

The tumour microenvironment is a complex disorganized mixture of tumour cells, extracellular matrix molecules, endothelial cells, fibroblasts and immune cells. The switch to the angiogenic phenotype is

driven by:

- increased expression by tumour cells of angiogenic proteins such as VEGF and bFGF
- increased expression of angiogenic proteins by stromal cells (eg stromal fibroblasts), a process induced by the tumour itself
- increased expression of VEGF by tumour associated macrophages (TAMs)
- decreased expression of endogenous angiogenesis inhibitors (eg thrombospondin-1, TSP1) by tumour cells and by stromal fibroblasts
- in some tumours, recruitment of bone-marrow derived endothelial precursors

The expansion of the tumour mass is associated with the recruitment of endothelial cells, which occurs after the tumour cells and stroma undergo a switch from the non-angiogenic dormant phenotype to the angiogenic phenotype.

Angiogenesis Inhibitors for Cancer

There are several approved anti-cancer therapies with recognised antiangiogenic properties. These agents, which interrupt critical cell signalling pathways involved in tumour angiogenesis and growth, include:

- monoclonal antibodies directed against specific pro-angiogenic growth factors and/or their receptors, eg bevacizumab, a humanized monoclonal antibody that binds biologically active forms of vascular endothelial growth factor (VEGF) and prevents its interaction with VEGF receptors (VEGFR-1 and VEGFR-2), thereby inhibiting endothelial cell proliferation and angiogenesis
- small molecule tyrosine kinase inhibitors (TKIs) of multiple pro-angiogenic growth factor receptors, eg erlotinib, a small molecule TK inhibitor of EGFR
- inhibitors of mTOR (mammalian target of rapamycin) represent a third, smaller category of antiangiogenic therapies, eg temsirolimus, a small molecule inhibitor of mTOR, part of the PI3 kinase/AKT pathway involved in tumour cell proliferation and angiogenesis.

Other approved anti-cancer agents may indirectly inhibit angiogenesis through mechanisms that are not completely understood. Bortezomib is a proteasome inhibitor that disrupts signalling of the cancer cell, leading to cell death and tumour regression. It may have indirect antiangiogenic properties, although the mechanisms are unclear. Thalidomide possesses immunomodulatory, anti-inflammatory and antiangiogenic properties, although the precise mechanisms of action are not fully understood.

Clinical trials

Anti-VEGF drugs can show therapeutic efficacy in mouse models of cancer, and in an increasing number of human cancers, but the benefits may be transitory and be followed by a restoration of tumour growth and progression. Anti-VEGF monotherapy may produce increased response rates, but tumours may escape targeting using alternative pro-angiogenic pathways. It has become clear that the mechanisms of anti-VEGF therapy are more complex than initially thought, but clinical evidence is available for three effects:

- anti-VEGF agents can prune tumour vessels in patients, and so kill a fraction of the tumour cells
- anti-VEGF agents can normalise tumour vasculature and microenvironment
- anti-VEGF treatment can reduce the number of blood circulating endothelial cells and progenitor cells

The anti-VEGF combinations that have increased survival have either combined targeting the cancer cells using anti-VEGF (bevacizumab) with chemotherapy, or used broad spectrum multi-targeted small molecule tyrosine kinase inhibitors (sunitinib, sorafenib) which affect pathways involved in both endothelial cell and cancer cell growth or survival.

So, the results from clinical trials have not shown the anti-tumour effects expected from preclinical studies. The reasons are unclear, but if multiple angiogenic molecules are produced by tumours, or if different angiogenic molecules are produced at different stages of tumour development, then blocking a

single angiogenic molecule may have little or no effect on tumour growth.

Combination therapy

Combination therapies have produced encouraging results. For example, combinatorial inhibition of the AKT/mTOR and ERK MAPK signalling pathways in a preclinical mouse model of prostate cancer; and combinatorial inhibition of mTORC1 and MAPK in solid tumours in-vitro, in-vivo and in patients.

Malignant progression and metastasis

Recently, it has been reported that, in mice, angiogenesis inhibitors targeting the VEGF pathway not only demonstrated anti-tumour effects, but also elicited tumour adaptation and progression to stages of greater malignancy, with heightened invasiveness and, in some cases, increased lymphatic and distant metastasis. Further, in several metastatic assays in mice, VEGFR inhibition accelerated metastatic tumour growth and decreased overall survival.

Our group

We have recently tested a small molecule inhibitor of neuropilin 1 (NP1) in-vitro, with promising results. We are also collaborating with other groups on testing their compounds against VEGF, and the relevant signalling pathways.

[1594 words]

Benefits

Under ASPA 5(4), the Secretary of State is required to weigh the likely adverse effects on the animals to be used in the programme against the benefit likely to result from the programme to be specified in the licence.

What are the likely benefits of this project? Why are they worthwhile?
The primary potential benefit relates to new knowledge about the initiation and control of tumour angiogenesis. The aim is to publish the findings in academic journals. The information is likely to be of interest to pre-clinical scientists interested in tumour biology. The secondary potential benefit relates to the value of the results to clinicians, in particular oncologists, and to the possibility that new molecular targets may be identified, for which new pharmaceutical products could be developed. [76 words]

TOTAL NUMBER OF WORDS (PART C): 1730

References

List up to 10 key references and/or regulatory guidelines supporting the need for the work and/or benefits set out above and relevant references for any specific models proposed in your programme of work.
The information in this example has been taken from published articles, but no references have been cited. *In your application, the references must be listed below.*

D. PLAN OF WORK Section 18

The total response to this Part must not exceed **2000 words**

For the purposes of this application, a "plan of work" is defined as a series of steps designed to achieve specified scientific purposes.

Purpose

What are you aiming to achieve, find out, establish, or produce by undertaking this project? Express this either as a single programme purpose, or as an overall aim with one or more key elements. The purpose should be specific to this project, unambiguous, realistic and achievable.
The main objective is to prevent tumour vascularisation and tumour growth by: • direct or indirect targeting of VEGF at the mRNA or protein level • direct targeting of VEGF receptors • blocking downstream signalling pathway components The aim is then to determine whether combinations of anti-VEGF therapy and chemotherapy are more effective than monotherapy in preventing vascularisation and tumour growth. [60 words]

Project plan

• Provide an outline of the stages of the plan of work and indicate clearly, by using the protocol numbers, how each protocol will be used to achieve your objectives. Where it would aid clarity, illustrate the steps of the programme using an annotated flow diagram or process map. • Indicate how in vitro and ex vivo work integrates with the in vivo work, the relationship between each component of the project and the sequence of the work. • In broad terms, what data or products are needed to achieve the purpose of the project? • How will those data or products be generated?
<u>Outline of programme</u> We aim to prevent tumour vascularisation and tumour growth by: • direct or indirect targeting of VEGF at the mRNA or protein level • direct targeting of VEGF receptors • blocking downstream signalling pathway components Having identified useful agents, we will then determine whether combinations of anti-VEGF therapy and chemotherapy are more effective than monotherapy in preventing vascularisation and tumour growth. In-vitro work generally precedes in-vivo work. Protocol 1 allows us to determine the efficacy and vascular mechanisms of anti-VEGF treatment, using the mouse window chamber model. Protocol 2 is used to determine well-tolerated doses of agents (eg VEGF inhibitors, chemotherapeutic drugs). In some cases, the presence of a tumour may modify drug effects: where there is reason to suspect this, the range finding may be done in tumour bearing animals. Protocol 3 allows us to maintain tumour cell lines. Some cell lines may be stored in the frozen state, but may require limited tumour passage to regain their characteristics. All cell lines will be checked to ensure their identity, and that they are free of unwanted viral contaminants. <u>In-vitro work</u>

We run clonogenic assays to assess the effect of drugs (or other agents) on the survival and proliferation of tumour cells. In summary, this involves applying an experimental treatment to a sample of cells which are plated in a tissue culture vessel, after which the colonies produced are fixed, stained and counted to produce a cell survival curve. The tumour clonogenic assay has proven predictive value in the chemosensitivity testing of standard and experimental anticancer drugs.

We also run tissue cultures (eg human bladder carcinoma, colon carcinoma, human umbilical vein endothelial cells, human dermal microvascular endothelial cells) to look at the molecular effects of the experimental treatments, eg on HIF-1 and gene expression, to understand the signal transduction pathways involved. This may involve techniques such as immunofluorescence, Western blotting, electrophoretic mobility shift assays, and semi-quantitative real-time RT-PCR.

In-vitro assays cannot adequately model the complete array of stromal effects which are important in tumour biology. Therefore, further in-vivo work is required.

Animal models

The animal model of tumour vascularisation is the window chamber model, using:
- human tumours in SCID mice
- syngeneic mouse tumours in conventional inbred mice

The window chamber consists essentially of two semicircular titanium plates which are surgically implanted on the dorsal surface of the mouse, where one of the plates has a small porthole. A small piece of skin is surgically removed under the porthole, and a small piece of tumour is implanted there. The tumour, and its vascularisation, can then be observed direct.

Experimental treatments

The experimental treatments use cancer or endothelial cells and anti-VEGF agents, with or without chemotherapy. This includes:
- direct or indirect targeting of VEGF
- direct targeting of VEGF receptors
- blocking downstream signalling pathway components

Experimental Measures

The main in-vivo experimental measures will be:
- tumour vascularisation
- tumour growth

Intravital microscopy will be used to determine the effects of the different treatments on vessel numbers, diameters, mean arterial blood pressure, red cell velocity, vascular resistance and haematocrit. Changes in tumour vascular permeability can be assessed using labelled albumin, to produce a tumour uptake index.

[530 words]

THE 3Rs

Under ASPA 5(5)(a), the Secretary of State cannot grant a project licence unless he/she is satisfied that the purpose of the programme to be specified in the licence cannot be achieved satisfactorily by any other reasonably practicable method not entailing the use of protected animals.

Under ASPA 5(5)(b), the Secretary of State cannot grant a project licence unless he/she is satisfied that the regulated procedures to be used are those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm, and are most likely to produce satisfactory results.

Replacement

- Why is it not possible to achieve the objectives of your project without using animals?
- What alternatives have you considered and why are they not suitable? What alternatives will be used in achieving your objectives?

In-vitro assays (see above) cannot adequately model the complete array of stromal effects which are important in tumour biology. Therefore, further in-vivo work is required.

Angiogenesis assays include the chick chorioallantoic membrane, the cornea pocket assay, sponge implant models, matrigel plugs, the matrigel plus assay, the rat (or mouse) aortic ring model assay, the alginate bead assay, the directed in-vivo angiogenesis assay, and the subcutaneous air sac model. Furthermore, the leech (Hirudo medicinalis) has been used for studies of the angiogenic and anti-angiogenic compounds, as has the zebrafish (the zebrafish yolk membrane angiogenesis assay). However, our aim is not simply to assay compounds for their capacity to cause or retard angiogenesis, so we have rejected the use of these models.

The CAM (chorioallantoic membrane) tumour assay, using fertilised hens' eggs, is similar to standard the CAM assay for angiogenesis, except that tumour cells are placed inside the O-rings along with the test article. Tumour cell survival and angiogenesis can be visualized and recorded. While the CAM assay is a step closer to an in-vivo model, the processes that control embryonic angiogenesis may be significantly different to those responsible for cancer-induced angiogenesis, and the CAM assay can be difficult and time-consuming to quantify, provides poor quality images of the results, and is not very reproducible. We therefore rejected the CAM tumour assay.

Though most of our tumour studies are done initially in-vitro, full testing requires a fully formed vascular network. As it is not feasible to produce an adequate model of the vascular network in-vitro, the next step requires a living animal.

[262 words]

Reduction

- What measures have been or will be taken to ensure that the minimum number of animals will be used in this project?
- Explain the principles of experimental design you will use and any sources of advice you will consult e.g. on statistics

Our use of in-vitro methods (see above) limits the numbers of animals required for the in-vivo investigation stage.

The use of the window chamber allows longitudinal assessment of tumour neovascularisation. This strategy also reduces the number of animals required compared to designs requiring destructive testing, ie serial killing of groups of experimental animals.

Range finding studies are done using a dose escalation design. This requires a smaller number of animals compared to assays using single doses at different dose levels.

When tumour cell lines are not in use, they will be stored in a frozen state. This minimises the numbers of animals required for tumour passage, because there is then no requirement for maintaining a tumour bearing colony of living animals on "tick-over".

The proposed experimental designs and methods of analysis of the results have been discussed with the Statistical Services Unit. The design of individual experiments will generally involve factorial designs, which maximise the information obtained from the minimum resource. Some of the measures (eg vascular anatomy) are essentially qualitative. Others are quantitative (eg repeated measures over time of tumour size), for which statistical analysis (eg RM ANOVA) may be appropriate. For most of the quantitative experiments, sample sizes may be set using power analysis. Generally, the significance level will be 5%, and the power 80%. For example, in a four-group experiment, if the least practicable difference between groups is chosen to be 25% and if the coefficient of variation is estimated to be 15%, then about 7 animals/group would be required. Clearly, the exact numbers of animals required will vary with the particular experimental design, the estimate of the coefficient of variation, and so on. For the qualitative experiments, the amount of material required will be the minimum necessary to provide an adequate description (eg of vascular anatomy).

Intravital microscopy data can be fitted to a multivariate model (MANOVA) with repeated measures to determine the effects of the experimental treatments, after fitting responses to effects using least squares methods. The effect of variation between individual vessels can be accounted for by using a nested design, taking variation between animals as random. Differences in responses caused by treatment or time can be tested for significance using an approximate F test, based on comparison of the matrix for the hypothesis sum of squares and cross products with the matrix of the residual. A t-test can be used to test for differences in tumour uptake indices for different treatment groups.

For each and every experiment, as part of good laboratory practice, we write an experimental protocol which includes:

- a statement of the objective(s)
- a description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals/group), and the experimental material
- an outline of the method of analysis of the results (which may include a sketch of the analysis of variance, an indication of the tabular form in which the results will be shown, and some account of the tests of significance to be made and the treatment differences that are to be estimated)

[516 words]

Refinement

- Explain your choice of species, model(s) and method(s). Explain why they are the most refined for the intended purpose.
- How will you minimise animal suffering in order to achieve your objectives?
- Provide specific justification for any substantial severity protocols

The window chamber model uses SCID mice for human tumours and conventional inbred mice for syngeneic mouse tumours. This method allows the use of a tiny fragment of a tumour, too small to have any effect on the mouse's health. The disadvantage of the method is that the window chamber requires surgical implantation, which is difficult to do well, and which requires post-surgical analgesia. On balance, we think the window chamber is preferable to the other animal models outlined above in terms of least severity, minimum numbers of animals required and better scientific results achievable. Severe combined immune deficient (SCID) mice, which lack functional B and T lymphocytes, allow the use of xenografts, and can therefore be used to study the biology of human malignancies. These

immunocompromised mice will be maintained in IVCs (individually ventilated cages) under barrier conditions, to avoid infections. Syngeneic mouse tumours are used in conventional inbred mice.

To find out the maximum tolerated, effective dose levels of a treatment, the mice are used in an escalating dose design. This may cause slightly more by ways of interventions and adverse effects for those mice, but we believe that the increases in adverse effects are trivial, whereas we can significantly reduce the total numbers of animals required by avoiding an approach where an animal receives one and only one dose level.

Some mice are used to reconstitute tumours which have been kept frozen for storage. Generally, 2-3 passages are necessary before the frozen cell line behaves in the same way as it did before being frozen for storage. The tumours are generally placed subcutaneously, and have minimal effect on the animal.

[273 words]

SPECIAL SPECIES

Cats, dogs, primates and equidae

Under ASPA 5(6), a licence cannot authorise the use of cats, dogs, primates or equidae unless no other species is suitable or it is not practicable to obtain animals of another suitable species.

If you intend using cats, dogs, primates or equidae, explain why no other species is either suitable for the purpose or practicably available

Endangered species

Under ASPA 10(3)(c), no vertebrate of an endangered species may be used unless the Secretary of State considers an exception justified.

If you intend using an endangered species, explain why no other species is either suitable for the purpose or available

Animals taken from the wild

Under ASPA 10(3)(d), no protected animal taken from the wild may be used unless the Secretary of State considers an exception justified. *Note that animals undergoing work in the wild are <u>not</u> regarded as having been taken from the wild.*

If you intend using wild-caught animals, explain why no other animals are available or suitable for the purpose

USE OF NEUROMUSCULAR BLOCKING AGENTS

Under ASPA 17, neuromuscular blocking agents may only be used if expressly authorised by the personal and project licences under which the relevant regulated procedure is carried out and may not be used instead of an anaesthetic.

If you intend using neuromuscular blocking agents in any part of this project give details of how they will be used and provide justification for their use.

TOTAL NUMBER OF WORDS (PART D): 1579

TRANSFER OF ANIMALS – the following gives authority to transfer animals from a previous project to this project, and/or to export / import rodents, normal and genetically altered, genetically altered zebra fish and genetically altered Xenopus sp. Provided these conditions can be met there is no need to seek prior authorisation for such transfers from the Home Office.

Authority is hereby given to acquire rodents (including genetically altered animals), genetically altered zebra fish and genetically altered Xenopus sp. from non-designated establishments and transfer animals undergoing regulated procedures under the licence(s) specified at 'Continuation of Work' in part A to this project for continued use in the relevant protocols.

Export of genetically altered rodents, genetically altered zebra fish and genetically altered Xenopus sp
Genetically altered rodents, genetically altered zebra fish and genetically altered Xenopus sp. bred and/or maintained under the authority of this project may be transferred to scientific establishments outside the United Kingdom only if:
1. The transfer will be made to a recognised scientific research establishment with a scientific requirement for genetically altered animals (or their controls) of that type; and where appropriate veterinary care can be provided as necessary; and
2. Sending tissue, gametes or embryos is not practicable or carries a higher potential welfare cost than moving live animals; and
3. Animals will be transported in accordance with all relevant regulations regarding welfare of animals in transit or the import or export of animals; and
4. Animals will be inspected by a competent person before transfer; and
5. A veterinary surgeon will confirm that he/she is not aware of any reason why these animals might suffer by virtue of the fact of being moved to another recognised scientific establishment.
6. Any transport related problems with the welfare of the animals will be notified to the Home Office promptly.

Acquisition of rodents (including genetically altered animals) genetically altered zebra fish and genetically altered Xenopus sp. from non-designated establishments
Rodents (including genetically altered animals), genetically altered zebra fish and genetically altered Xenopus sp. may be obtained from recognised scientific and breeding establishments outside the United Kingdom for use under this project licence only if:
1. The purpose for which animals are imported is consistent with the programme of work specified on the schedule: and
2. Attempts have been made to obtain the animals from Designated Sources in the UK but they are not available or animals from Designated Sources in the UK are not suitable for the purpose; and
3. Receiving tissue, gametes or embryos is not practicable or carries a higher potential welfare cost than moving live animals; and
4. Animals are transported in accordance with all relevant regulations regarding welfare of animals in transit or the import or export of animals; and
5. Animals will be inspected by a competent person after transfer
6. Any transport related problems with the welfare of the animals will be notified to the Home Office promptly.

Details of each transfer shall be recorded and made available to the Home Office on request. These records should contain the information set out in paragraph 4.30 of the HO guidance, and include the reasons for obtaining animals from non-designated sources.

E. PROTOCOLS Section 19

Under ASPA 5(1), a project licence must authorise the application of specified regulated procedures to animals of specified descriptions.

The term "protocol" is used to describe a single or a series of regulated techniques applied for a particular experimental or other scientific purpose to a protected animal. In most cases a protocol will involve all regulated procedures applied to the animal until the animal is killed or released from the controls of ASPA. Depending on the complexity of your work you may need one or several protocols. Different protocols are usually needed where different types of experimental procedures are to be used to achieve your objective(s). For example a project licence may have a protocol for the breeding and maintenance of genetically altered animals. These animals may then be transferred to another protocol in which, for example, treatments are evaluated in disease models.

ASPA 5(5)b states that: The Secretary of State shall not grant a project licence unless he is satisfied that the regulated procedures to be used are those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm, and are most likely to produce satisfactory results.

To add extra lines to the Summary place the cursor at the end of the line and press ENTER

To add extra protocols copy and paste new protocol sheets into the application. Each protocol should start on a new page.

Summary. Section 19a

Protocol no.	Short title	Species of animals	Estimated numbers over the duration of the project	Severity limit
1	Window chamber	Mouse	500	Moderate
2	Range finding	Mouse	100	Mild
3	Tumour passage	Mouse	100	Mild

Version Date		PPL No. /

Applicant to complete version date

PROTOCOL NUMBER. Section 19b : 1

Title:	Window chamber
Species of animals (state if genetically altered):	Mouse - SCID, WT
Severity limit:	Moderate

If the animals have been used, bred or surgically prepared under the authority of this or any other project licence, briefly describe what has been done to them and indicate whether the use now proposed represents 'continued-use' or 're-use' - refer to the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 and Home Office guidance on Use, Continued Use and Re-use of Animals.

Continued use: genetically altered animals for use in this project may be obtained from projects with authority to breed genetically altered animals of the type above.

List each of the steps in this protocol. Note: It is accepted that the order of steps may be varied according to scientific need. Indicate which steps are optional and for each give the anaesthetic code. If appropriate indicate the method of killing, Schedule 1 or non-Schedule 1. Give brief details of non-Schedule 1 methods e.g. perfusion fixation (AC).

Purpose: to determine the efficacy and vascular mechanisms of anti-VEGF treatment

Description of procedure

1. Under general anaesthesia with recovery (AB)
   - implantation of a dorsal skin-flap window chamber, including removal of a small disc of skin
   - implantation of tumour cells (as cell suspension, cell clump or tumour fragment, ~1mm^3) into skeletal muscle or connective tissue in the area of the window
   - subcutaneous implantation of slow release pellets, fibroblasts or osmotic minipump

2. Post-operatively, maintenance at 24-34^{o}C (to promote tumour growth) (AA).

3. Administration of substances or cells (on one or more occasions):
   - topically (AA)
   - orally, by gavage or in feed or drinking water (AA)
   - by injection (subcutaneous, intramuscular, intravenous, intraperitoneal) (AA or AB)
   - via an osmotic minipump, placed subcutaneously under general anaesthesia (AB)

4. Physical restraint in a jig (~30 mins duration) (on one or more occasions) (AA) and:
   - intra-vital microscopy
   - administration of substances or cells by injection (subcutaneous, intramuscular, intravenous, intraperitoneal)
   - withdrawal of blood from superficial blood vessels

5. Under terminal general anaesthesia (AC):
   - physical restraint in a jig
   - intra-vital microscopy

- administration of substances or cells
- withdrawal of body fluids (including exsanguination)
- removal of tissues or organs

Fate of animals not killed at the end of the protocol

Indicate the proposed fate of animals which are not killed at the end of the protocol.

- ☐ **Continued use in another protocol under this or another project licence** - give details below and ensure that you give an appropriate cross reference in the protocol sheet under which the continued use will occur.
- ☐ **Kept alive at the designated establishment.** Note that any subsequent re-use must be authorised in the relevant project licence.
- ☐ **Discharge from the controls of the Act at a PODE site** – e.g. setting free in the wild.
- ☐ **Other** – give details below

Adverse effects

List the likely adverse effects of each of the regulated procedures described above. Indicate how you will manage these effects to minimise severity. There is no need to list uncommon or unlikely adverse effects or effects from procedures that cause no more than transient discomfort and no lasting harm, for example intravenous injection. For each adverse effect indicate:

- the likely incidence
- how the adverse effect will be recognised
- the measures you will take to prevent or control occurrence and severity
- practicable and realistic humane end-points.

The typical animal is surgically fitted with a dorsal window chamber, containing a small tumour fragment. The tumours are small (a few mm) and without systemic effect. Maintenance in a warm room (up to 34 ^{o}C) has had no adverse effect on either SCID mice or normal mice. The experimental treatments generally have little clinical effect on the animal, because the doses chosen are less than a maximum tolerated dose. The periods of restraint are relatively brief (about 30 minutes) and are nearly always well tolerated by the animal. Analgesics are given, to minimise post-operative discomfort. Hence, we do not expect to see anything more than subtle changes in the clinical condition or the behaviour of the animal - and if more obvious changes in clinical condition or behaviour occur then the animal will be killed humanely.

Restraint. Initially, animals may be agitated when placed in the jig restraint. However, most animals quickly become accustomed to restraint in the jig: animals that do not will not be used further.

Window chamber. The procedure involves raising a dorsal skin flap (where the skin is taut but not stretched), removing a small disc of skin (so the underside of the other part of the skin flap can be seen through the window), and fixing the twin titanium plates comprising the window chamber to the skin (using sutures, and penetrating bolts). Because skin swells within 24 hours of incision, the bolts will initially be left slack, and will not be tightened until the skin swelling has subsided. Post-operative pain will be controlled using analgesics. A properly fitted window chamber should remain within ±30^{o} of the vertical: angles greater than this are sometimes associated with skin excoriation (through friction), with impaired mobility,

and with behavioural frustration. Animals with signs such as skin excoriation, impaired mobility or behavioural frustration will be removed from the study and be killed humanely. Animals bearing window chambers will be kept in cages with high sides, so that the window chamber cannot get caught on the cage lid or feed hopper.

Tumour cells. The volume of the tumour cells will be small: tumour volume will not be such that the tumour outgrows the window. Tumours which secrete cachetic factors will not be used. Hence, the tumour is not intended or expected to have any material effect on the well-being of the animal.

Substances. The substances to be used are: anti-angiogenic agents; anti-tumour agents including standard chemotherapeutic drugs; tumour vascular disrupting agents; vasoactive markers; pharmacological activators or inhibitors; gene activators or de-activators; and fluorescent dyes. Doses will be no more than the maximum tolerated dose (MTD - defined as that associated with weight loss of no more than 10% of the animal's initial bodyweight). The commonest side effects of chemotherapeutic agents are leukopaenia or gastro-intestinal disturbance. Other side effects of chemotherapeutics include: bruising or bleeding, anaemia, nausea, loss of appetite, bladder irritation, lethargy; doxorubicin may also cause discoloured urine (pink-red, for 48 hours) skin darkening (excess production of pigment), and paclitaxel may cause peripheral neuropathy. Tumour vascular disrupting agents may cause hypertension. The other agents are not expected or intended to have any material effect on the well-being of the animal. The slow release pellets are small (<5 mm diameter) and cause no appreciable effects. The level of hormone (eg oestrogen) is chosen so that it does not cause a systemic effect on the animal but is sufficient to promote the growth of hormone-dependent tumours. Similarly for the fibroblasts.

Intravital microscopy. The technique itself involves observation through the window chamber of the tumour and its vasculature. It involves restraint of the animal or general anaesthesia.

Cells. The cells to be used are fluorescent labelled cells: immune cells, haematopoietic or mesenchymal stem cells, or RBC. They are not expected to affect the well-being of the animal.

Injection. All the injection routes will cause momentary needle-stick pain. A small osmotic minipump will be used where feasible in preference to multiple needle-stick administration.

Withdrawal of blood. Blood will be taken from superficial blood vessels: to avoid hypovolaemia or anaemia, not more than 10% of the total blood volume (TBV) will be withdrawn on any one occasion and no more than 15% TBV in any 28-day period.

Terminal general anaesthesia. In the terminal phase of the procedure, the animal will be insentient throughout.

SCID mice. Severe combined immune deficient (SCID) mice lack functional B and T lymphocytes. They will be maintained in IVCs under barrier conditions, to avoid infections.

PROTOCOL NUMBER. Section 19b : 2

Title:	Range finding
Species of animals (state if genetically altered):	Mouse - SCID, WT
Severity limit:	Mild

If the animals have been used, bred or surgically prepared under the authority of this or any other project licence, briefly describe what has been done to them and indicate whether the use now proposed represents 'continued-use' or 're-use' - refer to the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 and Home Office guidance on Use, Continued Use and Re-use of Animals.

Continued use: genetically altered animals for use in this project may be obtained from projects with authority to breed genetically altered animals of the type above.

List each of the steps in this protocol. Note: It is accepted that the order of steps may be varied according to scientific need. Indicate which steps are optional and for each give the anaesthetic code. If appropriate indicate the method of killing, Schedule 1 or non-Schedule 1. Give brief details of non-Schedule 1 methods e.g. perfusion fixation (AC).

Purpose: to determine well-tolerated doses of agents (eg VEGF-inhibitors, chemotherapeutic drugs)

Description of the procedure

1. Under general anaesthesia, with recovery (AB):
   - administration of tumour cells (as suspension, cell clump or tumour fragment) subcutaneously
   - subcutaneous implantation of slow release pellets, fibroblasts or osmotic minipump

2. Administration of substances (on one or more occasions):
   - orally, by gavage or in feed or drinking water (AA)
   - by injection (subcutaneous, intramuscular, intravenous, intraperitoneal) (AA or AB)
   - via an osmotic minipump, placed subcutaneously under general anaesthesia (AB)

3. Kill by Schedule 1 method.

Version Date		PPL No. /

Applicant to complete version date

Fate of animals not killed at the end of the protocol

Indicate the proposed fate of animals which are not killed at the end of the protocol.

- ☐ **Continued use in another protocol under this or another project licence** - give details below and ensure that you give an appropriate cross reference in the protocol sheet under which the continued use will occur.
- ☐ **Kept alive at the designated establishment**. Note that any subsequent re-use must be authorised in the relevant project licence.
- ☐ **Discharge from the controls of the Act at a PODE site** – e.g. setting free in the wild.
- ☐ **Other** – give details below

Adverse effects

List the likely adverse effects of each of the regulated procedures described above. Indicate how you will manage these effects to minimise severity. There is no need to list uncommon or unlikely adverse effects or effects from procedures that cause no more than transient discomfort and no lasting harm, for example intravenous injection. For each adverse effect indicate:

- the likely incidence
- how the adverse effect will be recognised
- the measures you will take to prevent or control occurrence and severity
- practicable and realistic humane end-points.

The typical animal receives the compound of interest as an escalating series of dose levels. The series is ended when a maximum tolerated dose is reached (a dose level at which the animal's behaviour changes from normal, or at which there is a change in bodyweight of more than 10% from the initial bodyweight) or a fixed dose level (eg 500 mg/kg) is reached. Occasionally, range finding is done in a tumour bearing animal.

Anti-VEGF therapy is likely to have cytotoxic effects. The dose limiting sign for anti-VEGF agents will be a 10% loss in bodyweight. The experimental doses of some chemotherapeutics are published in the literature (e.g. cyclophosphamide). The doses of others will have to be determined. Typical side effects of chemotherapeutics include bruising or bleeding, anaemia, nausea, loss of appetite, bladder irritation, and lethargy - if any of these occur the animal will be killed humanely. Doxorubicin may also cause discoloured urine (pink-red, for 48 hours) or skin darkening (excess production of pigment): no treatment is necessary. Paclitaxel may cause peripheral neuropathy: if this causes signs of spontaneous pain such as excessive paw licking, guarding of the limb etc, or causes autotomy, then the animal will be killed humanely. The general dose limiting sign will be a 10% loss in bodyweight.

Subcutaneous tumours may grow up to 12.5 mm diameter, at which point the animal will be humanely killed by an appropriate Schedule 1 method.

The slow release pellets are small (<5 mm diameter) and cause no appreciable effects. The level of hormone (eg oestrogen) is chosen so that it does not cause a systemic effect on the animal but is sufficient to promote

the growth of hormone-dependent tumours. Similarly for the fibroblasts. Hence, we do not expect to see anything more than subtle changes in the clinical condition or the behaviour of the animal - and if more obvious changes in clinical condition or behaviour occur then the animal will be killed humanely.

If any tumour ulcerates, or limits the animal's normal behavioural repertoire, or if the animal loses more than 10% of its bodyweight, then the animal will be killed using an appropriate Schedule 1 method.

SCID mice are immunocompromised: to avoid unwanted infections they will be maintained in IVCs in a barrier environment. If the mice develop unwanted infections they will be killed humanely.

Version Date		PPL No. /

Applicant to complete version date

PROTOCOL NUMBER. Section 19b : 3

Title:	Tumour passage
Species of animals (state if genetically altered):	Mouse - SCID, WT
Severity limit:	Mild

If the animals have been used, bred or surgically prepared under the authority of this or any other project licence, briefly describe what has been done to them and indicate whether the use now proposed represents 'continued-use' or 're-use' - refer to the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 and Home Office guidance on Use, Continued Use and Re-use of Animals.

Continued use: genetically altered animals for use in this project may be obtained from projects with authority to breed genetically altered animals of the type above.

List each of the steps in this protocol. *Note: It is accepted that the order of steps may be varied according to scientific need.* Indicate which steps are optional and for each give the anaesthetic code. If appropriate indicate the method of killing, Schedule 1 or non-Schedule 1. Give brief details of non-Schedule 1 methods eg perfusion fixation (AC).

Purpose: to maintain tumour cell lines

Description of procedure

1. Under general anaesthesia, with recovery (AB):
   - administration of tumour cells (as suspension, cell clump or tumour fragment) subcutaneously
   - subcutaneous implantation of slow release pellets, fibroblasts or osmotic minipump

2. Kill by a Schedule 1 method.

Fate of animals not killed at the end of the protocol

Indicate the proposed fate of animals which are not killed at the end of the protocol.

- ☐ **Continued use in another protocol under this or another project licence** - give details below and ensure that you give an appropriate cross reference in the protocol sheet under which the continued use will occur.
- ☐ **Kept alive at the designated establishment.** Note that any subsequent re-use must be authorised in the relevant project licence.
- ☐ **Discharge from the controls of the Act at a PODE site** – e.g. setting free in the wild.
- ☐ **Other** – give details below

Adverse effects

List the likely adverse effects of each of the regulated procedures described above. Indicate how you will manage these effects to minimise severity. There is no need to list uncommon or unlikely adverse effects or effects from procedures that cause no more than transient discomfort and no lasting harm, for example intravenous injection. For each adverse effect indicate:

- the likely incidence
- how the adverse effect will be recognised
- the measures you will take to prevent or control occurrence and severity
- practicable and realistic humane end-points.

The typical animal is given a small subcutaneous tumour, as a suspension of cells or a small tumour fragment. The tumour is then allowed to grow to a reasonable size, at which point the animal is humanely killed.

Subcutaneous tumours may grow up to 12.5 mm diameter, at which point the animal will be humanely killed by an appropriate Schedule 1 method. If any tumour ulcerates, or limits the animal's normal behavioural repertoire, or if the animal loses more than 10% of its bodyweight, then the animal will be killed using an appropriate Schedule 1 method.

The slow release pellets are small (<5 mm diameter) and cause no appreciable effects. The level of hormone (eg oestrogen) is chosen so that it does not cause a systemic effect on the animal but is sufficient to promote the growth of hormone-dependent tumours. Similarly for the fibroblasts. Hence, we do not expect to see anything more than subtle changes in the clinical condition or the behaviour of the animal - and if more obvious changes in clinical condition or behaviour occur then the animal will be killed humanely.

SCID mice are immunocompromised: to avoid unwanted infections they will be maintained in IVCs in a barrier environment.

F. DECLARATIONS

1. Declaration by the applicant

I hereby apply for a project licence in respect of the studies described in this application form. To the best of my knowledge and belief all the information I have provided in this application form is correct and complete.

Signature of applicant:

Date:

2. Declaration by the certificate holder at the primary availability

I confirm that this application has completed my establishment's ethical review process.

If licensed, I accept 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'. I am aware of, and will carry out, my responsibilities as set out in the published 'Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 (HC321)'.

Name of PCD holder:

Signature of PCD holder:

Date:

3. Declaration by the certificate holder at the additional availability

I confirm that the relevant parts of this application have completed my establishment's ethical review process.

If licensed, I accept 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'. I am aware of, and will carry out, my responsibilities as set out in the published 'Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 (HC321)'.

Name of PCD holder:

Signature of PCD holder:

Date:

Make further copies of box 3 if you have more than one additional availability. Each additional availability must have a declaration signed by the relevant Certificate Holder.

Version Date		PPL No. /

Applicant to complete version date

G: PROJECT ABSTRACT

NOTE: This abstract will not form any part of the licensed programme of work. However, the Secretary of State considers the project abstract an essential step towards greater openness and expects them to be provided in every case. Use lay terms and avoid confidential material or anything that would identify you or your place of work. This abstract will be placed on the Home Office website at http://scienceandresearch.homeoffice.gov.uk/animal-research/. Examples of other abstracts can be viewed on this site.

NAME OF APPLICANT

Prof Black

DESIGNATED ESTABLISHMENT

University of X

PROJECT TITLE (Section 1) (<50 characters including spaces)

Tumour Neovascularisation

In no more than 500 words:

- Summarise your project (1-2 sentences)
- Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.
- Outline the general project plan.
- State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.
- Explain how you will ensure that you use the minimum number of animals. Indicate approximately how many animals of each species you propose to use.
- Explain why the protocols and the way they are carried out should involve the least suffering.
- Explain why you chose the particular species of animal.
- Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.
- Outline in a few sentences how science will advance, or people or animals will benefit from this project.

Our main objective is to determine whether combinations of anti-VEGF (vasoendothelial growth factor) treatments and chemotherapy are more effective than any single treatment in preventing tumour vascularisation and tumour growth.

Cancer is a leading cause of death worldwide. Primary tumours are the result of extensive deregulation of gene expression, leading to a loss of control of key cellular regulatory processes. But for tumours to grow, become invasive and metastasise, they must first develop a blood supply, primarily through a process called angiogenesis (generation of new blood vessels). The aim is to interfere with tumour angiogenesis so that the blood supply to the tumour is cut off. Drugs targeting tumour angiogenesis are effective in mouse models of cancer, and in some human cancers. However, the benefits may be brief, followed by a resumption of tumour growth and progression. We need to understand the reasons why this happens, and to find more effective treatments. For example, if multiple angiogenic molecules are produced by tumours, or if different angiogenic molecules are produced at different stages of tumour development, then blocking a single angiogenic molecule may have little or no effect on tumour growth. Early clinical results suggest that a combination of angiogenic inhibitors with conventional therapeutic approaches may be more effective than monotherapy.

The plan is to identify useful agents that prevent tumour vascularisation and tumour growth, and then determine whether combinations of them are more effective than single treatments. This involves a lot of in-vitro laboratory work, using tissue culture techniques. Full testing requires a fully formed vascular

network. As it is not feasible to produce an adequate model of the vascular network in-vitro, the next step requires a living animal. We expect to use about 700 mice for this project.

The mouse window chamber model involves the surgical implantation of a small porthole onto the back of a mouse, under which is placed a tiny fragment of a tumour. The tumour is too small to have any effect on the mouse's health. The tumour, and the response to treatment, can then be seen using a microscope. Other mice are used to find out the right dose of a treatment, or to reconstitute tumours which have been kept frozen for storage. Normal mice can be used for studying mouse tumours, and immunodeficient mice can be used for studying human tumours. The immunodeficient mice are kept in individually ventilated cages, to avoid infections.

The primary potential benefit relates to new knowledge about the initiation and control of tumour angiogenesis. The aim is to publish the findings in academic journals. The information is likely to be of interest to pre-clinical scientists interested in tumour biology. The secondary potential benefit relates to the value of the results to clinicians, in particular oncologists, and to the possibility that new molecular targets may be identified, for which new pharmaceutical products could be developed.

[476 words]