

2) Identification Of Novel Tissue Specific Targets For Drug Delivery By Phage Display In Vivo

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Key Objectives: To identify and target tissue specific determinants for drug delivery. Although the advent of biologics has dramatically improved the treatment of RA, the use of systemic drugs to treat what is principally a joint disease exposes patient to a considerable burden of potential side effects. Tissue specific drug delivery therefore remains an attractive therapeutic option. In recent years, the development of phage display technology has allowed the successful targeting of tissue specific microvascular endothelial (MVE) determinants of the seven different organs probed in mice, as well as for tumour vasculature [8;9]. We have pioneered a similar in vivo approach in the human/SCID mouse transplantation model for human vasculature [10] [11]. Using the "Nissim" single-chain antibody variable fragment (scFv) phage display library in this model Panagiotis Kamperidis (year 3 OB student) has isolated for the first time, a series of 9 scFv homing antibodies capable of specifically localising to synovial MVE grafts, via the mouse circulation, in preference to other human (skin grafts) or mouse tissues. Sequence analysis of the encoding DNA for the CDR2 and CDR3 regions enabled identification of 3 prevalent clones that when re-tested in recirculation studies in double transplanted animals showed over 100 fold synovial homing properties. This work has generated three novel strands of research suitable for PhD projects:

a) The utilisation of the identified sequences to construct targeting devices to concentrate therapeutic/diagnostic materials specifically to the synovium. This is a very exciting, highly translational project where the student will develop innovative therapeutics by conjugating the synovial homing scFv Ab to: (i) liposomes which can be used as drug shuttles and (ii) radionuclides for synovial imaging or tissue specific radiotherapy and (iii) anti-inflammatory cytokines in collaboration with Prof Yuti Chernajovsky (see 4.2.2 below). We have recently validated this approach in the human/SCID mouse transplantation model at the William Harvey Research Institute (WHRI) using liposomes armed with an anti-human E-selectin Ab (1.2B6 – in collaboration with Prof D Haskard and A George) and demonstrated over 10 fold increased delivery to the grafts compared to control. We have also completed experiments with the radiolabelled 1.2B6 Ab labelled with ¹¹¹In detected using the NanoSPECT/CT system, a powerful imaging system available at WHRI that allows precise localisation with tri-dimensional reconstruction and quantification of the uptake of the labelled compound in the transplanted synovial tissue. In this project, conjugated scFv Ab will be tested for their capacity to act as tissue specific drug delivery systems in double transplanted (synovium and skin, test and control tissue respectively) SCID animals, as previously described [10].

b) The biological/biochemical characterisation and isolation of synovial MVE receptor(s). This project will involve the use of the newly identified scFv Ab as probes to determine: (i) The biochemical properties of the synovial MVE receptor following treatment of tissue samples with various proteases and sialidase, N and O glycosidase as described [12]. (ii) The modulation in vivo of the synovial MVE receptor(s) following intra-graft injection of TNF α and other pro-inflammatory cytokines (e.g. IL-1), chemokines (e.g. SDF-1), bacterial products (e.g. LPS) or thrombin. Potential down-modulatory factors such as TGF β will also be tested. The final part of this project will be aimed to (iii) isolate the synovial MVE receptor(s) using either a classical biochemical approach (immunoaffinity purification) using His-tagged scFv to capture the receptor, followed by matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry analysis, or expression cloning using a modification of the "panning" strategy of Arrufo and Seed [13]. The identification of synovial MVE receptor(s) will be followed by cloning and chromosomal localisation using standard techniques.

c) Novel tissue specific target identification by in vivo phage display selection in the SCID mouse transplantation model. In this project we propose to extend the strategy successfully used for synovial tissue by Panagiotis Kamperidis and [10] to other tissues that we have

demonstrated to be successfully transplanted into SCID mice such as skin[10] and lymph nodes [14] using synovial tissue and synovial homing phage as controls.

Potential benefits: these studies have pioneered a new approach to human tissue targeting using phage display technology in vivo in the h/SCID mouse chimera model. They might lead the development of innovative therapeutic and imaging delivery systems. The OB student will be directly involved in the molecular, cellular and functional development of the area and train in a wide range of cutting edge technologies.