The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	Characterisation and preclinical evaluation of a fibroblast-activating protein (FAP) targeted TRUCK CAR T-cell therapy for malignant pleural mesothelioma (MPM)
Short Project Title:	TRUCK CAR T-cell therapy for malignant mesothelioma

SUPERVISORY TEAM	
Primary Supervisor(s):	Astero Klampatsa
Associate Supervisor(s):	Alan Melcher
Backup Supervisor: (must have IRS status)	Clare Isacke
Lead contact person for the project:	Astero Klampatsa
DIVISIONAL AFFILIATION	
Primary Division:	Cancer Therapeutics
Primary Team:	Thoracic Oncology Immunotherapy Group

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Malignant mesothelioma (MM) is a highly lethal malignancy involving the body's mesothelial surfaces, mainly the pleura. Conventional tri-modality treatments including surgery, radiotherapy and chemotherapy fail to provide long term survival benefit to MM patients, whose prognosis remains dismal at 12-18 months from the time of diagnosis.

Chimeric Antigen Receptors (CARs) are membrane-spanning fusion receptors composed of a targeting moiety, which binds directly to tumour-associated antigens (TAAs), as well as co-signalling intracellular domains that allow activation and effector function when these CARs are engineered into T cells. Although CAR T cell therapy for blood malignancies has shown remarkable results, resulting in FDA approval of the first ever cellular therapy in 2017, the use of CAR T cell therapies in solid malignancies is proving to be a harder task due to the multiple immunosuppression factors within the tumour microenvironment (TME) (1). CAR T cells targeting an array of cancer specific antigens have shown promise, but clinical response rates have been low. Developing better CAR T cell therapies with enhanced efficacy against solid tumours, like MM, is thus a major unmet need (2).

Multiple barriers unique to solid tumours contribute to the lack of efficiency of CAR T cells. The TME is a hostile territory full of immunosuppressive features including the stroma, cytokines and chemokines, suppressive regulatory cells and hypoxic conditions, which can all pose formidable barriers for the effective anti-tumour function of CAR T-cells (1). Understanding these hurdles, the aim of this project is to develop a CAR T cell therapy that can overcome one or more immunosuppressive TME factors, resulting in direct tumour cell killing or in facilitating tumour eradication by subsequent treatments.

PROJECT AIMS

- Characterize novel murine and human TRUCK CARs that target both stromal fibroblasts and TGF-beta in the tumour microenvironment
- Develop appropriate models (cell lines, 3D spheroids) to test efficacy of these CARs in vitro
- Undertake toxicity and mechanistic studies in immune-compromised and syngeneic mouse models respectively
- Use the developed CAR T cell therapy in combination with checkpoint inhibitors

RESEARCH PROPOSAL

In this PhD project, the student will characterize and test an augmented CAR T cell therapy approach by using what has been termed a "TRUCK (**T**-Cell-**R**edirected for **U**niversal **C**ytokine **K**illing)" CAR. Currently the most innovative IN CAR T cell designs, TRUCK CARs aim to both target an antigen and deliver a cargo to the tumour at the same time (3). In this project, the student will (i) characterize our lab's FAP-targeting CARs, which are 'armed' to produce peptides that will block the ability of TGF-beta to bind to its receptors on the CARTs, the endogenous lymphocytes, CAFs, myeloid cells and tumours, (ii) evaluate efficacy of the developed TRUCK CAR in MM cell lines stably transduced with human FAP, (iii) test efficacy and toxicity of the TRUCK CAR in animal models and (iv) use the TRUCK CAR in combination with systemic immunotherapeutic agents

(i) A number of TGF-beta inhibitory peptides have been identified that have good in vitro TGF-beta blocking activity. Since TGF-beta is very highly conserved, these peptides should work for both mouse and human TGF-beta (4). A number have been used in animal models of cancer and fibrosis after systemic injection and have shown some activity (5). The student will work with plasmids, which contain DNA sequences that encode for one or more of these peptides, and which have be placed upstream of the FAP CAR sequence separated by a T2A sequence. He/she will package the plasmids into lentiviruses or retroviruses and transduce them into human or mouse T cells respectively.

(ii) Functional studies evaluating the efficiency of the developed TRUCK CARs will be done using murine MM cell lines stably transduced with human FAP. For in vitro toxicity studies, human cell lines with variable expression of FAP will be used. 3D in vitro studies using "tumour+fibroblast" spheroid models will also be performed. In all in vitro studies, TRUCK CAR efficiency will be measured with killing and cytokine production assays.

(iii) The efficacy and toxicity of the TRUCKs will be tested in animal models. To test the human TRUCK CARs, the student will utilize both orthotopic and subcutaneous MM models in immunodeficient (NSG) mice. Established human MM tumours will be treated with FAP-targeting CAR T cells which either contain or not the TRUCK. Assessment of tumour growth/regression will be done by BLI imaging and/or calliper measurements, depending on the model. Mechanistic in vivo studies will be performed in a subcutaneous MM syngeneic mouse model. Similarly to the human studies, established murine tumours will be treated with FAP-CARTs or FAP-TRUCKS. Response to therapy will be assessed as per tumour volume measurements using callipers. If tumours are not completely regressed, they will be harvested for analysis, including: a) IHC to assess stromal destruction, necrosis and T cell infiltration, b) flow cytometry to phenotype the TME, c) IHC of other organs to determine off-tumour toxicity.

(iv) Preclinical studies have demonstrated that CAR-T cell therapy and PD-1 blockade was highly synergistic, leading to long-term survival without causing any signs of pathology in vivo (6). The student will test this in our system, by combining the FAP-targeting TRUCK-CAR with checkpoint blockade antibodies anti-CTLA4 and anti-PD1. These studies will be conducted in flank and orthotopic i.p. MM tumours in NSG mice, where each therapy will be tested alone and in combination, to assess treatment efficiency and toxicity. Mechanistic studies involving the TRUCK-CAR will be conducted in syngeneic MM models, in Balb/c and/or C57BL/6 strains. Of particular interest and novelty in this system will be the study of the effects this combination treatment will have on the persistence and function of T cells, given the simultaneous targeting of tumour stroma, TGF-beta and inhibitory receptors. Further studies will be performed upon completion of the animal experiments, where intratumoural T cells will be isolated and re-stimulated ex vivo to access function.

Summary and Expected Outcome

CAR T cell therapies has been used in blood malignancies with remarkable success; however this cannot currently be translated into solid tumours due to a number of obstacles including poor trafficking to the tumour site and loss of function due to the hostile TME. In MM, CAR T cell therapies had limited clinical response. In this project, a strategy is proposed by which CAR T cells are directly attacking the MM tumour stroma, thus disrupting major tumour growth processes, and additionally block intratumoural TGF-beta by the means of locally-secreted inhibitory peptides, inhibiting TGF-beta driven immunosuppression. The expected outcome will be that this double-targeting strategy will render CAR T cells more efficacious and possibly more persistent within the TME, ultimately augmenting the therapeutic effect of this therapy in MM.

LITERATURE REFERENCES

1. Beatty, G.L and O'Hara M. Chimeric antigen receptor-modified T cells for the treatment of solid tumours: Defining the challenges and next steps. *Pharmacol Ther* (2016); 166: 30-39.
2. Klampatsa, A, *et al*. Chimeric antigen receptor T cell Therapy for malignant pleural mesothelioma. *Cancers (Basel)* 2017; 9(9): 115.
3. Chmielewski, M. and Abken, H. TRUCKs: the fourth generation of CARs. *Expert Opin Biol Ther* 2015; 15(8): 1145-54
4. Dotor, J., et al. Identification of peptide inhibitors of transforming growth factor beta 1 using a phage-displayed peptide library. *Cytokine* 2007; 39: 106-15.
5. Llopiz, D., et al. Peptide inhibitors of transforming growth factor-beta enhance the efficacy of antitumor immunotherapy. *Int J Cancer* 2009; 125: 2614-23.
6. Liu, X., et al. A chimeric switch-receptor targeting PD1 augments the efficacy of second generation CAR T-Cells in advanced solid tumors. *Cancer Res* 2016; 76(6): 1578-90.

CANDIDATE PROFILE

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)

Pre-requisite qualifications of applicants: e.g. BSc or equivalent in specific subject area(s)	BSc Hons (First or 2:1) in Cancer, Cell or Molecular Biology or Immunology
Intended learning outcomes:	• CAR cloning methods • CAR T cell engineering • Experience in tissue culture in vitro assays • Experience in flow cytometric analysis • Skills and knowledge in in vivo cancer immunotherapy models, in vivo experiments and imaging analysis • Skills and knowledge in developing 3D in vitro models and imaging analysis

ADVERTISING DETAILS

Project suitable for a student with a background in:	X Biological Sciences ☐ Physics or Engineering

	☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science x Other (Immunology)
Keywords:	**1. Immunotherapy**
	2. CAR T cell
	3. mesothelioma
	4. tumour microenvironment
	5.
	6.