MONASH University

MONASH INNOVATION

IMPROVED ADOPTIVE CELL THERAPY: Enhanced CD8+T cell formation and function

A method of generating functionally improved cytotoxic T lymphocytes (CTLs) *ex vivo* independent of CD4+ T helper cells for use in adoptive cell therapy. Inhibiting the tyrosine phosphatase PTPN2 achieves enhanced CTL formation and tumour killing.

- **Enhance existing cell therapy platforms**
- **Compatible with the *ex vivo* reinvigoration of 'tolerised' T cells or tumour antigen specific CAR-T cells**
- **'Proof of Mechanism' *in vivo* efficacy**
- **Potential use in single agent and combination therapy applications**

THE CHALLENGE

Adoptive transfer of naturally occurring or gene-engineered T cells can mediate tumour regression in patients with cancer.

In adoptive cell therapy (ACT), tumour infiltrating CTLs, which are naturally active to tumour antigens, can be isolated from a patient. Alternatively, T cells can be genetically modified to express chimeric antigen receptors (CARs) on their cell surface, combining the exquisite specificity of a monoclonal antibody fragment for a tumour-associated target, with T-cell receptor activation.

Essential for ACT is that T cells are expanded and stimulated *ex vivo* before being transferred back into the patient. This generates functional CTLs that specifically attack the tumour.

While ACT has tremendous promise, complete tumour regression is rare, in part due to inhibitory signals limiting T cell activation.

THE TECHNOLOGY

The Monash University research team led by Prof. Tony Tiganis, have identified PTPN2 as a negative regulator of T-cell receptor signaling. By inhibiting PTPN2, CD8+ T cells acquire CTL activity, independent of CD4+ help.

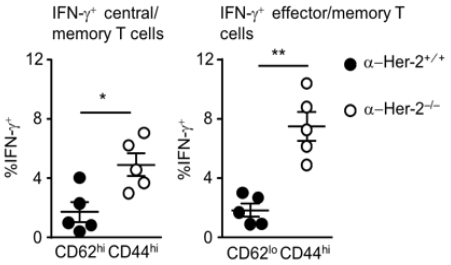

Figure 1. PTPN2-deficiency enhances CAR T cell activation of central and effector memory T cells. Her-2-specific control versus Her-2-specific PTPN2-deficient CD8+ CAR T cells were incubated with Her-2 expressing 24JK sarcoma cells. Antigen-specific T cell activation was assessed by monitoring for intracellular IFN-γ in CD8+ central/memory ($CD62L^{hi}CD44^{hi}$) and effector/memory ($CD62L^{lo}CD44^{hi}$).

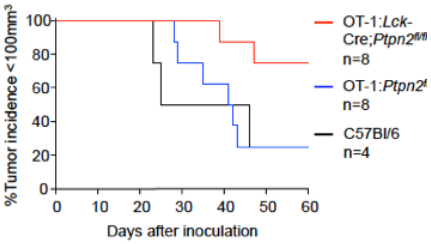

Figure 2: PTPN2-deficiency enhances the activity of OVA-specific OT-1 T cell. OVA-specific OT-1;Ptpn2fl/fl versus OT1;Lck-Cre;Ptpn2fl/fl T cells were adoptively transferred into C57BL/6 mice bearing OVA expressing B16.F10 melanoma cells on the skin and tumor incidence was monitored.

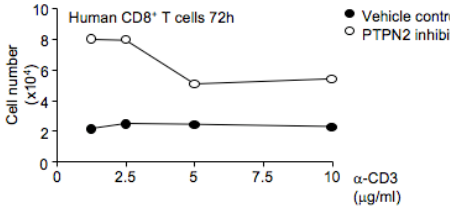

Figure 3: Inhibition of PTPN2 in human CD8+ lymphocytes enhances TCR-mediated proliferation. Freshly isolated human PBMCs were stimulated with plate-bound anti-CD3 in the presence or absence of PTPN2 inhibitor.

Summary of research to date
PTPN2-deficient CD8+ T cells have enhanced cytotoxic function ex vivo
PTPN2-deficient CAR-T cells extend survival in a xenograft model
PTPN2-deficiency enhanced the generation of long lasting central memory T cells (T_{CM}) and the tumor-killing effector T cells (T_{EM})
Pharmacological inhibition of PTPN2 enhanced the cytotoxic function of human CD8+ T cells ex vivo
PTPN2 deficient CAR-T cells do not persist longer than wild-type counterparts *in vivo* - **Safety**

The team has generated data supporting PTPN2 inhibition as an approach to enhancing CTL formation and function. Genetic deficiency of PTPN2 enhances the generation and activation of effector/memory T cells ex vivo (Fig 1.) and enhances the activity of antigen specific CD8+ T cells in the context of adoptive transfer (Fig 2.). Furthermore, pharmacological inhibition of PTPN2 in human CD8+ T cells enhances TCR-mediated proliferation (Fig. 3).

Current experiments are aimed at exploring methods of inhibiting PTPN2, using CAR-T cells specific for HER2, both *in vitro* and *in vivo* and in combination studies with anti-PD1 or anti-CTLA4 inhibitors.

Intellectual property: National phase applications filed (US, EP, JA, AU) for PCT/AU2015/050318.

THE OPPORTUNITY

Monash University seeks a commercial partner to develop its technology within an ACT platform. The method has the potential to greatly improve CTL production, and consequently, ACT therapy success.

CONTACT US

Monash Innovation
T: +61 3 9905 9910
E: innovation@monash.edu

GROUP OF EIGHT AUSTRALIA

CRICOS provider: Monash University 00008C
Produced by Monash Innovation: June 2017