

Development of early-phenotype CAR-T cells for treatment of canine osteosarcoma

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Chimeric Antigen Receptor (CAR)-T cell therapy has changed the treatment paradigm for treatment-resistant haematological cancers in people. There are currently six approved CAR-T treatments for lymphoma, myeloma and leukemia in Australia, which can cure between 30-90% of people that would otherwise not have a curative treatment option. However, CAR-T treatment for solid tumours has been of limited benefit in clinical studies in people to date. Reasons for this include heterogeneity of tumour antigen expression, an immunosuppressive tumour microenvironment, limited trafficking of CAR-T cells to the tumour, and importantly, lack of CAR-T cell persistence following infusion into patients.

A new type of CAR-T therapy derived from stem cell memory, or naïve T cells, termed T_{STEM}-like CAR T cells has been developed by our group (Figure 1). These T_{STEM}-like CAR-T cells showed superior proliferative capacity and cytokine secretion compared with conventional CAR-T cells.¹ These cells also demonstrated long-term persistence *in vivo* and resulted in regression of established solid tumours when administered alone, or when combined with an anti-PD-1 monoclonal antibody.¹ Recently published by our group in *Nature*, overexpression of the transcription factor *FOXO1* also resulted in CAR-T cells with improved stemness.² These CAR-T cells showed improved metabolic fitness and superior control of solid tumours *in vivo* compared with unedited CAR-T cells.²

Pet dogs that develop spontaneous solid tumours, including osteosarcoma (OSA), could significantly benefit from cancer immunotherapy. In addition, canine appendicular OSA shows striking similarity to paediatric OSA in terms of clinical behaviour, histologic/morphologic features and expression of tumour-associated antigens such as HER-2 and B7H3. Work by Professor Nicola Mason and the immunotherapy research group at the University of Pennsylvania have demonstrated that anti-CD20 CAR-T therapy for the treatment of diffuse B-cell lymphoma in dogs is feasible and safe.^{3,4} Current treatments for both canine and paediatric OSA has not advanced for

decades, and outcomes have failed to improve, with metastatic disease claiming the lives of most pet dogs and about 40-50% of paediatric patients.⁵ The tumour-associated antigen B7H3 has been shown to be highly expressed in OSA and represents an attractive target for CAR-T therapy. One group has reported *in vitro* efficacy of conventional anti-B7H3 CAR-T against canine OSA cell lines, however *in vivo* persistence was poor, resulting in limited antitumour efficacy.⁶ This limited persistence was also observed by Professor Mason and her team, with tumour-bearing dogs treated with anti-CD20 CAR-T showing loss of detectable circulating CAR-T cells by day 28 post-infusion.³ A significant limitation in existing veterinary CAR-T studies is a lack of T cell phenotyping. My work has demonstrated that conventional canine T cells very rapidly shift to an exhausted phenotype in culture, and this likely represents another reason why canine CAR-T cells fail (Figure 2). Thus, it is likely that shifting canine CAR-T cells towards a memory/stem-like phenotype will result in improved persistence and anti-tumour efficacy, as has been demonstrated for human and murine CAR-T cells.

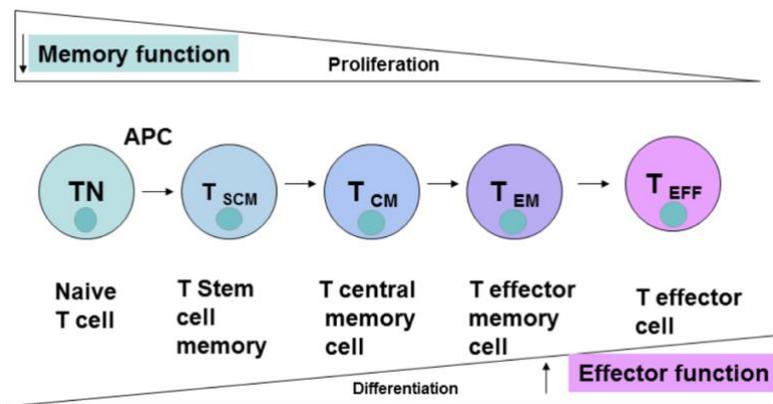


Figure 1. Illustrating the differentiation of naive T cells towards an effector phenotype. As T cells become activated and exposed to antigen, they expand more rapidly, but also become prone to exhaustion.⁷

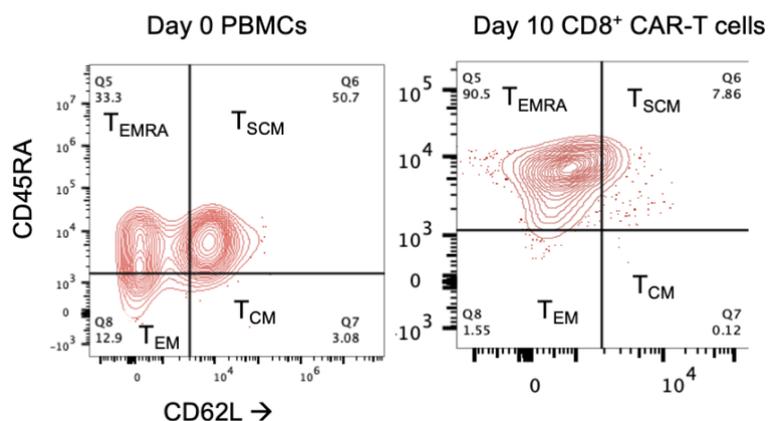


Figure 2. Phenotype of untransduced canine PBMCs prior to activation (left) and stimulated anti-LeY CD8⁺ CAR-T cells day 10 post-activation (right). Almost all canine CAR-T cells have shifted to an exhausted phenotype by day 10 in culture. T_{SCM} stem cell memory; T_{CM} central memory; T_{EM} effector memory; T_{EMRA} effector memory re-expressing CD45RA

I will present the progress of my PhD to date, including validation of the commonly used human T cell phenotyping markers (CD45RA and CD62L) which identify naïve versus non-naïve canine T cells, as well as the successful generation of conventional canine CAR-T cells. I will also discuss the current barriers limiting the success of CAR-T for solid cancer therapy and strategies our lab are exploring to improve efficacy.

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