CRISPR/Cas9 mechanism as a molecular tool to enhance the immune system for cancer therapy

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Introduction
Programmed death-1 (PD-1) pathway is one of the most critical checkpoint pathways responsible for mediating tumour-induced immune suppression, normally involved in promoting tolerance and preventing tissue damage in settings of chronic inflammation. Many human solid tumours express PD-1 ligand 1 (PD-L1), and this is often associated with a worse prognosis. Tumour-infiltrating lymphocytes from patients with cancer typically express PD-1 and have impaired anti-tumour functionality.

To date, several studies have revealed that the blockade of PD-1/PD-L1 pathway shows remarkable anti-tumour responses in patients with advanced melanoma and lung cancer with durable clinical responses. However, the long-term systemic administration of the blocking antibody carries the risk of breaking immune tolerance and, thus, causing immune attack.

To overcome the above shortcomings, the aim of this project is to propose a proof-of-concept in a lung squamous cell carcinoma mouse model to enhance the immune system disrupting PDCD1 gene in autologous mouse CD8+ T-cells by CRISPR/Cas9 system, thereby reaching a more specific and long-term therapy.

Conclusions
PDCD1 gene disruption on tumour-specific CD8+ T cells presents important advantageous features over the current PD-1 treatment, PD-1 antibodies:
- **Increase in specificity**: since only anti-tumour - effector T cells will undergo such disruption, thus preventing unspecific reactions towards healthy tissues.
- **Long-term therapy**: once modified T-cells are reinfused back to the patient, in secondary organs they may differentiate into memory T cells, long-lived cells that give an enhanced response to antigens, thereby yielding protection from subsequent challenges by the same type of tumour.

References

Figure: Proof-of-concept of a CRISPR-Cas9-based therapeutic strategy against cancer. In some tumours, constitutive oncopgenic signalling can upregulate PD-L1 expression on all tumour cells (innate immune responses), whereas in other tumours, PD-L1 is not constitutively expressed, but it is induced in response to inflammatory signals that are produced by an active anti-tumour immune response (adaptive immune responses). In this study, peripheral blood lymphocytes are collected from brachial vessels at the axillary region of mouse and isolated through a Ficol-hygase gradient. Afterwards, they are analysed for reactivity against predicted tumour epitopes (neoantigens obtained from whole-exome sequencing of tumour cells from the mouse) presented by mouse APCs. T cells expressing the activation marker are then purified using flow cytometry, cultured and knock-out by CRISPR/Cas9 system in the laboratory (PD-1 knockout). Finally, modified T-cells are expanded ex vivo in presence of IL-2 and re-infused back into the mouse, followed by IL-2 administration. Blocking the PD-L1-PD-1 pathway by FDCD1 disruption would suppress cancer cell survival and enhance the anti-tumour responses of T cells, leading to tumour regression and rejection. BMBC: Peripheral Blood Mononuclear Cells; APC: Antigen-presenting cells.