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

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Introduction

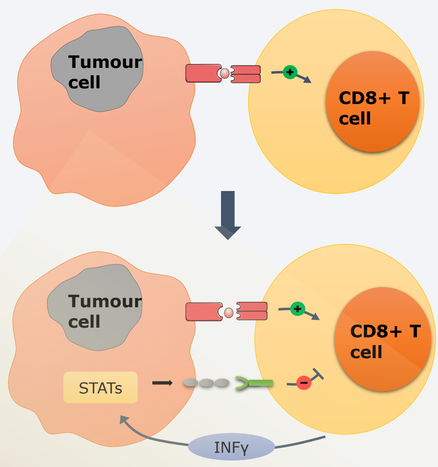
Programmed death-1 (PD-1) pathway is one of the most critical checkpoint pathways responsible for mediating tumour-induced immune suppression, normally involved in promoting tolerance and preventing tissue damage in settings of chronic inflammation. Many human solid tumours express PD ligand 1 (PDL1), 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 shortcoming, 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.

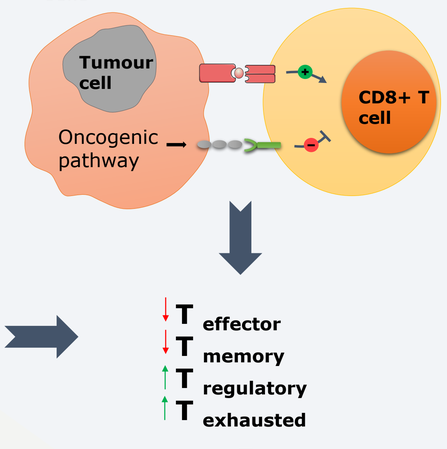
Adaptive immune resistance

T cell-induced PDL1 upregulation in tumour cells



Innate immune resistance

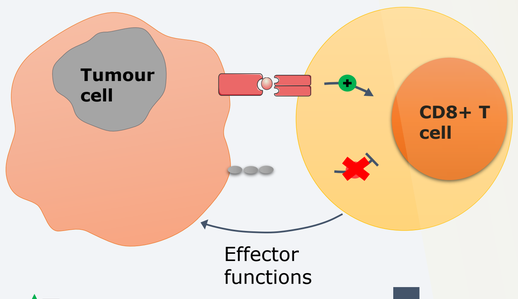
Constitutive oncogenic signalling induces PDL1 expression in tumour cells



T effector
T memory
T regulatory
T exhausted

Cancer cell survival
T cell inhibition

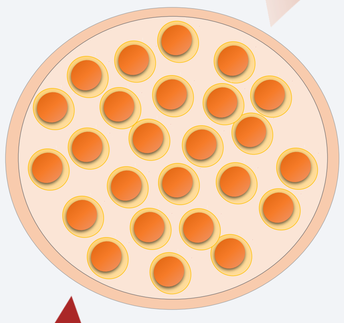
Tumour tolerance

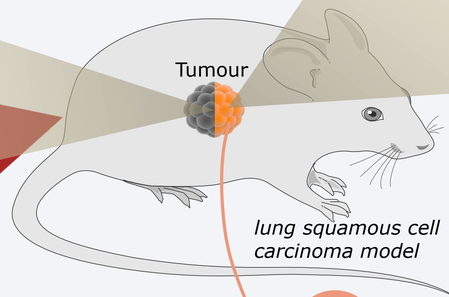


T effector
T memory
T regulatory
T exhausted

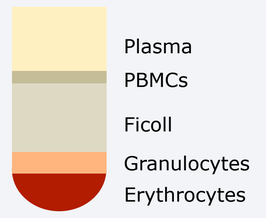
Cancer cell apoptosis
T cell activation

Tumour rejection





1 **Isolation of peripheral blood lymphocytes** through a Ficoll-hypaque gradient



2.a Tumour sample extraction

2.b Extract DNA from normal and malignant tissue

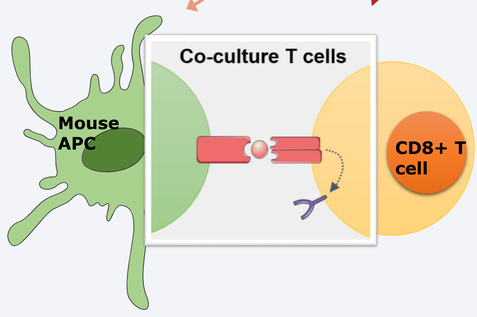
2.c Exome sequencing to identify cancer mutations

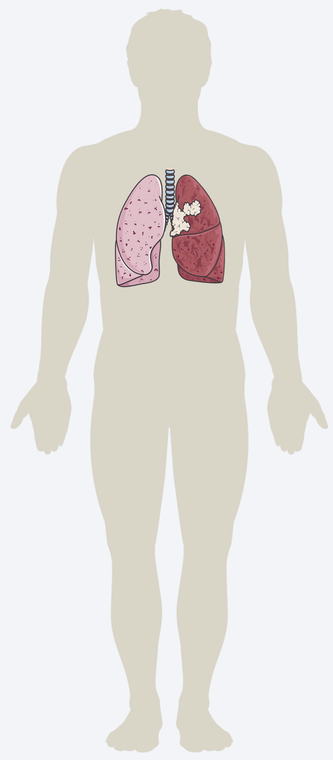
2 **Selection of T cells recognizing tumour-specific mutations**

Flow cytometry selection of T cells that express the activation marker 41BB

2.d Tandem minigenes or synthetic peptides of all mutations introduced to mouse APCs

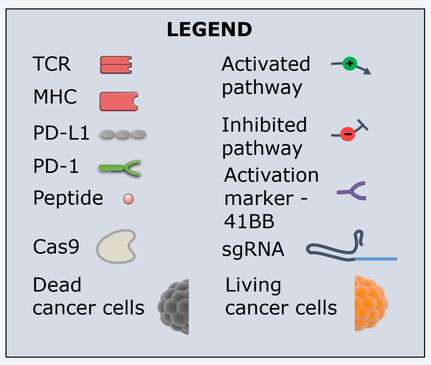
2.e Some mutations are presented by mouse APCs





3 **PD-1 disruption**

Electroporation of plasmids carrying nuclease and sgRNA expression



4 **Validate genetic modification**

- Cell culture positive selection with puromycin
- PCR amplification and Sanger sequencing

5 **Modified T-cells expansion** in presence of IL-2

6 **T cells Re-infusion,** followed by IL-2 administration

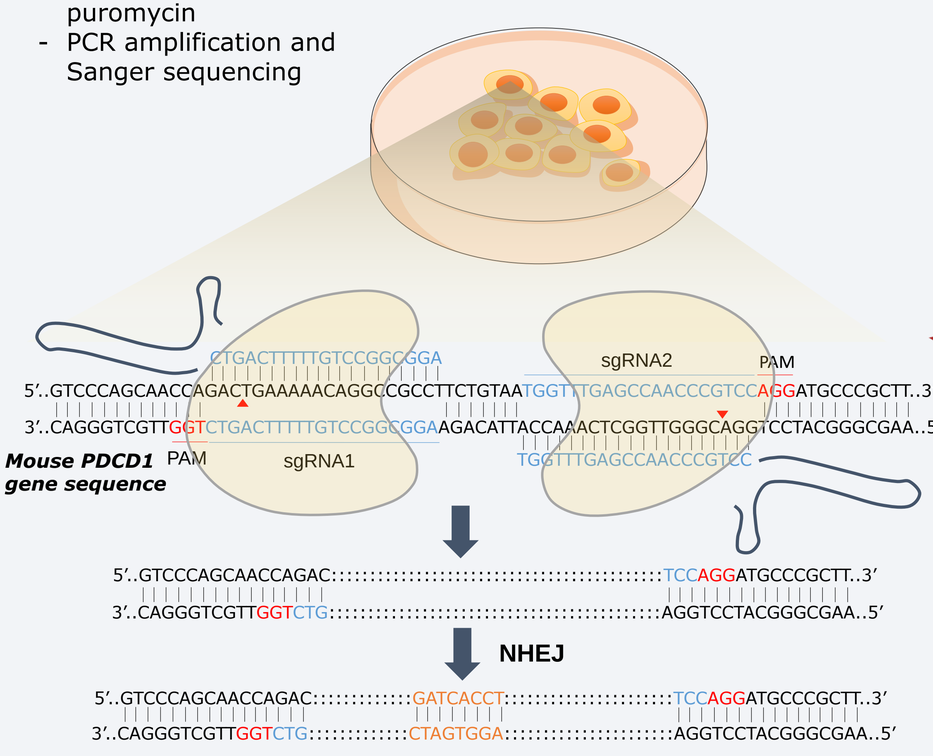


Figure: Proof-of-concept of a CRISPR-Cas9-based therapeutic strategy against cancer. In some tumours, constitutive oncogenic signalling can upregulate PDL1 expression on all tumour cells (innate immune resistance); whereas in other tumours, PD-L1 is not constitutively expressed, but rather it is induced in response to inflammatory signals that are produced by an active anti-tumour immune response (adaptive immune resistance). In this strategy, peripheral blood lymphocytes are collected from brachial vessels at the axillary region of mouse and isolated through a Ficoll-hypaque 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 knocked-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-1/PD-L1 pathway by PDCD1 disruption would suppress cancer cell survival and enhance the anti-tumour responses of T cells, leading to tumour regression and rejection. *PBMCs: Peripheral Blood Monoclonal Cells*; APCs: Antigen-presenting cells.

Determinants of PD-1 disruption

Parameters needed for therapy response:

- **Tumour foreignness**
  Increased intratumoural genetic heterogeneity
- **Good general immune status**
  High PBLs levels
- **Immune infiltration**
  Marked infiltration of T cells
- **Presence of checkpoints**
  High PD-L1 levels
- **Absence of inhibitory tumour metabolism**
  Low intratumoural hypoxia and glucose depletion
- **Tumour sensitivity to immune effectors**
  MHC-I expression

Conclusions

PDCD1 gene disruption on tumour-specific CD8+ T cells presents important advantageous features over the current 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

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