Engineered immune-mobilising T cell receptors against viruses

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<th>Relations that could be relevant for the meeting</th>
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Challenges for HIV immunotherapy

- Low or absent viral antigen expression on reservoir cells:
  - transcriptional / translational silence
  - Nef-mediated HLA class I downregulation
- ‘Kick and kill’ unsuccessful to date – both components need to be improved
- Archived mutants: reactivated virus may not be recognised by CD8+ T cells
- Sanctuary sites in lymphoid tissue: CD8+ T cell effectors excluded from B cell follicles
- Cells harbouring replication-competent viruses resistant to CD8+ T cell killing?
ImmTAV®: new class of bispecific biologic

- Platform redirects all T cells to kill infected cells
- Developed for HLA-A2 – potential to extend to other HLA class I alleles
- Scalable, easy to manufacture (‘drug in a vial’)

Specific
T cell receptor: targets infected cells that normally escape immune system detection

Soluble
Disulphide bond: stabilises molecule

Potent
Effector end: CD3-specific scFv recruits and activates cytolytic T cells to form a synapse leading to killing of the target cell
The T cell receptor (TCR) recognises intracellular proteins offering a broader range of targets specific to infectious diseases.
The process of ImmTAV® generation

**T cell cloning***

* TCRs also isolated from naive libraries by phage display

**TCR gene cloning**

PCR products of TCR genes

**TCR validation**

WT TCR

KD in µM range

**Affinity maturation**

Phage-TCR Inhibition ELISA

**Further affinity enhancement**

CDR mutations combination (soluble TCR)

Anti-CD3 scFv fusion

Biocore analysis: affinity enhancement

**Biological evaluation**

In vitro testing

Harper et al. PLOS One 2018
Lowe et al. Cancer Treatment Reviews 2019
First HIV TCR (‘m121’): high affinity and recognition of viral variants

Gag p17-specific TCR isolated from HLA-A*02:01+ patient

TCR exhibited unusually high affinity and broad recognition of viral variants

Several TCRs with pM affinity generated by phage display

Yang et al. Molecular Therapy 2016
Key results with first Gag-specific ImmTAV

- Potency in primary cell *in vitro* infection model
- Potency against *ex vivo* CD4+ T cells from ART-treated patients at ImmTAV concentration as low as 10 pM and E:T 1:10
- Detectable killing in presence of NRTIs
- Resting CD4+ cells within bulk cultures also eliminated
Live cell imaging of Gag ImmTAV killing of HIV-infected targets

Targets: HIV infected Jurkat cells  Effectors: CD8+ T cells (healthy donor)  HIV ImmTAV: 10nM
Gag ImmTAV detects HIV-infected cells expressing <50 target epitopes

Yang H et al. Molecular Therapy 2016
Isolate resting CD4+ T cells from ART-treated patients → Reactivate HIV with latency reversing agents (LRA) → Co-culture with CD8+ T cells +/- ImmTAV or control TCR → Measure released HIV

Gag ImmTAV redirects CD8+ T cells to eliminate HIV reservoir cells \textit{ex vivo}

Yang H \textit{et al.} Molecular Therapy 2016

- No drugs
- LRAs only
- LRAs + ImmTAV
- LRAs + control TCR

HIV RNA copies/ml (culture supernatant)
Resting cell infection model to investigate kill without a kick

HLA-A*02:01+ primary resting CD4+ T cells from healthy donor (CD25-/CD69-/HLA-DR-)

Spinoculate with HIV

Over 4 days cells express Gag but do not produce infectious virus

Killing assay with healthy donor CD8+ T cells + ImmTAV (m121)

Pace M et al. PLOS Pathogy 2012
ImmTAV-redirected healthy donor CD8+ T cells can eliminate resting Gag+ CD4+ T cells

Targets: resting Gag+ CD4+ T cells
Effectors: healthy donor polyclonal CD8+ T cells
Control TCRs: m231 – non-binding anti-CD3; m232 – irrelevant retargeting TCR
48 hour co-culture
Elimination of Gag+ cells quantified by flow cytometry
Key messages

- ImmTAV® are potent molecules for redirected T cell killing of virus-infected cells
- First HIV Gag-specific ImmTAV showed efficacy in ex vivo kick and kill
- Gag ImmTAV redirects CD8+ T cells to kill resting infected primary CD4+ T cells
- Further work to probe epitope density on reservoir cells from patients
- New ImmTAV molecules targeting other HIV epitopes are in development
Development of ImmTAV® for HBV cure

* First-in-class biologic for chronic HBV infection: goal is interferon-free cure
* Novel mode of action: re-directed T cell elimination of hepatocytes containing HBV DNA through recognition of viral peptide presented by HLA class I

Non-exhausted T cells are engaged and activated, leading to killing of HBV Ag+ cells

Release of lytic granules

Exhausted HBV-specific T cells are unable to kill or produce antiviral cytokines

healthy hepatocyte

Infected hepatocyte

Hepatocyte with integrated HBV-DNA

healthy hepatocyte

Source of HBV

Major source of HBsAg

cccDNA

HBV-DNA

HBV

HBsAg
HBV ImmTAV mediates potent and specific eradication of HBV Ag$^+$ cells
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**HBV programme**: potent and specific molecule selected as clinical candidate; first in human trial planned for 2020

- Proprietary *in vitro* toxicology package to address specific challenges of entirely human bispecific molecules
Acknowledgements

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People living with HIV