Natural Killer (NK) cells and Programmed cell death protein-1 (PD-1)

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OUTLINE

Background -

- Natural Killer (NK) Cells
- Programmed Death (PD-1) Receptor
- PD-1 and NK Cells
- Anti-PD1 scFv

Specific Aims

Results

Conclusion

Future Direction
NK Cells

- Large, granular lymphocytes of the innate immune system

- Diverse function:
  - ‘First response’ cytolytic effector function against virus-infected and tumor transformed cells
  - Does not require prior exposure or priming
  - T-cell, macrophages, Dendritic cell activation/inhibition

- Regulated by various activatory and inhibitory receptors
Programmed Cell Death (PD-1) Receptor

- Immune check-point regulator e.g. PD-1, CTLA-4
- Inhibitory receptor
- Inducibly expressed by T-cells and B-cells
- Frequently exploited by tumor cells to evade immune response
- Ligands PD-L1/PD-L2

- Current clinical use: Anti-PD-1Ab in Melanoma, NSC lung cancer
  - Pembrolizumab (Keytruda)
  - Nivolumab (Opdivo)
- Well characterized in T-cells
- PD-1 expression and regulation of NK cells unclear
- Questions?
  - Expression/Inducibility
  - Cytotoxicity
  - Cytokine secretion
  - Proliferation
  - Downstream signaling pathway

Anti-PD-1 scFv

Anti-PD1 Antibody

Fab

Fc

Anti-PD1 scFv

V<sub>H</sub> V<sub>L</sub>

V<sub>H</sub> V<sub>L</sub>

T-cell

NK cell

Tumor cell

TCR

MHC-1

PD-L1/PD-L2

PD-1

SHP-2

Signaling Kinases
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**Aim 1:** Determine if, and to what extent the checkpoint receptor, programmed death-1 (PD-1) is expressed in peripheral blood NK cells from healthy individuals.

**Aim 2:** Investigate the inducibility of PD-1 expression under various activating conditions including cytokine stimulation and the inflammatory tumor microenvironment.

**Aim 3:** Determine the functional relevance of PD-1 on peripheral blood NK cells in response to tumor targets expressing its ligand, PD-L1 using conventional and novel PD-1 antagonistic antibodies.
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PD-1_PD-L1/L2 Expression

- Isotype control
- aPD-1 scFv
- aPD-1

PD-1
- THP-1_iso
- THP-1_PD-L1
- THP-1g_PD-L1

PD-L1
- THP-1_iso
- THP-1_PD-L1

PD-L2
- THP-1_iso
- THP-1_PD-L2
- THP-1g_PD-L2
Full-length vs scFv

- %CD107a+ NK

- %IFNγ+ NK
NK ADCC response

[Graph showing CD107a+ NK and IFNγ+ NK percentages across different conditions and treatments.]
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1. The anti-PD-1 scFv construct demonstrates superior target affinity and specificity as compared to commercial full-length anti-PD1 antibody.

2. PD-1 is expressed on all peripheral blood NK cells.

3. PD-1 blockade using scFv construct enhances the cytolytic, cytokine production, and ADCC function of NK cells.

4. The NK cell PD-1 pathway involves downstream signaling component (pAKT) parallel to that of the well characterized T-cell PD-1 signaling pathway.

5. The anti-PD-1 scFv has potential implications in the development of novel BiKE/TriKE constructs that could be therapeutically used to enhance NK cell function.
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**BiKE construct**

- CD33
- CD16

**TriKE construct**

- CD33
- PD-1
- CD16

**BiKE construct**

- CD33
- CD16

**TriKE construct**

- CD33
- PD-1
- CD16

**NK Cell**

- CD33
- PD-1
- CD16

**Tumor Cell**

- Redirected Lysis

**ADCC**

- PD-1 blockade
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