Natural Killer Cell Engagers
Disclosure

• Innate Pharma
• Co-founder + CSO
Today’s presentation

• The cancer innate immunity cycle
• What’s Next? Natural Killer Cell Engagers
  • First generation: NKCE$^3$
  • NKCE$^3$ to NKCE$^4$, new data from next generation technology
• The ANKET™ platform
  Antibody-based NK cell engager therapeutics
A pivotal role of T cells in tumor immunity

References:
- Pages et al., NEJM, 2005
- Chen & Mellman, Immunity 2013
- Schumacher & Schreiber, Science 2015
- Sharma & Allison, Science 2015
- Chen & Mellman, Nature 2017
T cells are not autonomous in their anti-tumor functions
The cancer innate immunity cycle

Demaria et al., Nature 2019
# Innate Lymphoid cells

**Stimuli**
- Tumors, intracellular microbes (Virus, bacteria, parasites)
- Large extracellular parasites and allergens
- Mesenchymal organizer cells (Retinoic acid, CXCL13, RANK-L)
- Extracellular microbes (Bacteria, fungi)

**Mediators**
- IFN-γ
- Granzymes
- Perforin
- IL-4
- IL-5
- IL-13
- IL-9
- AREG
- RANK
- Lymphotixin
- TNF
- IL-17
- IL-22
- IL-22
- IL-17
- GM-CSF
- Lymphotixin

**Immune function**
- **Type 1 immunity** (Macrophage activation, cytotoxicity)
- **Type 2 immunity** (Alternative macrophage activation)
- **Formation of secondary lymphoid structures**
- **Type 3 immunity** (Phagocytosis, antimicrobial peptides)

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*Vivier et al., Nature Immunol. 2008*
*Vivier et al., Science 2011*
*Vivier et al., Cell 2018*
The cancer innate immunity cycle

Demaria, Gajewski, Vivier, SITC in press
Antibodies are great medicines

A. Mullard, Nature Reviews Drug Discovery 2021
Antibodies in cancer immunotherapy

**Blocking antibodies**
- e.g. anti-PD-(L)1 mAbs
- anti-NKG2A mAbs
- anti-TIGIT mAbs

**Cytotoxic antibodies**
- e.g. anti-CD20 mAbs
- anti-EGFR mAbs
- anti-HER2 mAbs

**Antibody-dependent cell phagocytosis (ADCP)**

**Antibody-dependent cell cytotoxicity (ADCC)**
Boosting cytotoxic antibodies against cancer
Boosting cytotoxic antibodies against cancer

Blocking mAbs: Anti-NKG2A mAbs (Monalizumab)

Gauthier & Vivier, Cell 2020

André et al. Cell 2018
Boosting cytotoxic antibodies against cancer

Agonist mAbs: NKp46 NK cell engagers
Gauthier et al. Cell 2019

Blocking mAbs: Anti-NKG2A mAbs
(Monalizumab)
André et al. Cell 2018
Trifunctional Natural Killer Cell Engagers (NKCE³)

Gauthier & Vivier, Cell 2019
Rationale for engaging NK cells through NKp46 1/3

NKp46 is expressed by all NK cells
Rationale for engaging NK cells through NKp46 2/3

NKp46 triggers potent signaling pathways

- NK cell activation +
  - cytotoxicity

- NK cell activation +++
  - cytotoxicity
  - cytokines and chemokines

Lanier et al., Nature Immunol 2008
Vivier et al., Science, 2004
Rationale for engaging NK cells through NKp46 3/3

NKp46 expression is conserved on tumor-infiltrating NK cells

Solid tumors
(HN, HCC, NSCLC, Urothelial RCC)
**NKCE\(^3\): the first generation**

### Efficacy

- Activity in preclinical in vivo models
- Efficacy NKCE\(^3\) > approved benchmark antibodies in a cancer model in vivo

![Graph showing efficacy comparison between NKCE, Obinutuzumab, and PBS](image1)

### Mode of Action

- Optimized killing activation by co-engagement of NKp46 and CD16
- Increased NK cell number in the tumor

![Images showing control and NKCE conditions](image2)

_Gauthier et al., Cell 2019_
From NKCE$^3$ to NKCE$^4$

NKCE$^3$

NKCE$^4$

Gauthier et al., Cell 2019
Rationale for including IL-2v in NKCE

Complementarity between ITAM and IL-2Rγ (γc) signaling pathways
IL-2v in NKCE⁴ format does not bind to IL-2Ra but still binds to IL-2Rb

CD25 (IL-2Rα)

CD122 (IL-2Rβ)
IL-2v is functional in NKCE\textsuperscript{4} format and limits Treg activation.

- **Cells:** PBMC from HD
- **Stimulation:** 20 min
- **Read-out:** STAT5 phosphorylation by flow cytometry
NKCE promotes specifically IL-2R activation on NK cells

- **Cells**: PBMC from HD
- **Stimulation**: 20 min
- **Read-out**: STAT5 phosphorylation by flow cytometry
Binding to CD16 and NKp46 is required for optimal IL-2R signaling by NKCE⁴

- Cells: PBMC from HD
- Stimulation: 20 min
- Read-out: STAT5 phosphorylation by flow cytometry

**Cells:**

- NKp46
- CD16
- IL-2R

**Graph:**

- x-axis: Concentration (nM)
- y-axis: pSTAT5 positive cells (%)
- y-axis: EC50 pSTAT5 (nM)

**Legend:**

- Green: NKp46
- Blue: CD16
- Orange: IL-2R

**Statistical Tests:**

- *p < 0.05
- **p < 0.01

**Notes:**

- EC50 values indicate the concentration at which half of the maximum effect is observed.
NKCE\textsuperscript{4} induces NK cell proliferation

- **Cells:** purified NK cells
- **Stimulation:** 5 days
- **Read out:** proliferation, CTV dilution

CTV dilution
NKCE induces cytokine production upon target engagement

- **Effector**: Freshly purified NK cells from HD
- **Target**: Raji B cell line
- **Incubation**: 4h with NKCE + Golgi Stop, E:T=0.5:1
- **Read out**: Intracellular FACS

**Graphs:****

- **Left Graph**: IFN-$\gamma$+ NK cells
- **Right Graph**: MIP1$\beta$ MedFI (on NK cells)

**X-axis**: Concentration (nM)
**Y-axis**: % IFN-$\gamma$+ NK cells for the left graph and MIP1$\beta$ MedFI (on NK cells) for the right graph.
NKCE^4 induces cytokine production upon target engagement

- **Effector**: purified NK cells from HD
- **Target**: Raji B cell line
- **Read out**: Calcein release assay, 4h, E:T=10:1
NKCE4 induces potent cytotoxicity by mouse NK cells

- **Effector**: purified NK cells from HD
- **Target**: Raji B cell line
- **Readout**: Calcein release assay, 4h, E:T=10:1

![Graph showing % Specific lysis against Concentration (nM)]

- **Concentration (nM)**: 10^-3, 10^-2, 10^-1, 10^0, 10^1, 10^2
- **% Specific lysis**:
  - 0, 20, 40, 60

**Legend**:
- Tag
- NKp46
- Fc
- IL-2v
- Obinutuzumab

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**Details**:
- **Tag**: Tagged constructs
- **NKp46**: Natural Killer cell activation
- **Fc**: Immunoglobulin Fc domain
- **IL-2v**: Interleukin-2v
- **Obinutuzumab**: Monoclonal antibody

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NKCE\textsuperscript{4} anti-tumor efficacy: solid tumor model

**Model**: Raji, $5 \times 10^6$ in matrigel, sc

**Mice**: CB17 SCID
CD20-NKCE⁴ is more potent than obinutuzumab

**Model:** Raji, 5x10⁶ in matrigel, sc  
**Mice:** CB17 SCID
Mechanisms of NKCE⁴ anti-tumor efficacy. 1

NKCE⁴ induces accumulation of activated NK cells at the tumor bed

**Model**: Raji, 5x10⁶ in matrigel, sc

**Mice**: CB17 SCID or RAGko huNKp46Tg (immunodeficient)

**Treatment**: single injection 3 days before analysis
Mechanisms of NKCE^4 anti-tumor efficacy. 2

- **Model:** Raji, 5x10^6 in matrigel, sc
- **Mice:** CB17 SCID
- **NK depletion:** anti-asialo GM1, q1w
Mechanisms of NKCE⁴ anti-tumor efficacy. 3

Optimal efficacy requires all arms in a single NKCE⁴ molecule
NKCE\textsuperscript{4} anti-tumor efficacy: disseminated tumor model

- Model: huCD20-B16F10, 5x10\textsuperscript{5}, i.v.
- Mice: C57BL/6
- Treatment: D1
- Lung analysis: D13

Vehicle

Obinutzumab
CD20-NKCE\textsuperscript{4} induces circulating B cell depletion in NHP

UMAP whole blood NHP treated with CD20 NKCE\textsuperscript{4} 0.5 mg/kg, n=4

![Graph showing B cell depletion over Days with different treatment groups: Vehicle, 0.05 mg/kg, 0.5 mg/kg.]

- **Pre-dose**
  - B cells
  - NK cells
  - Pre-dose Day7
  - 3. CD8 T cells
  - 4. CD4 T cells

- **Day7**
  - B cells (% of baseline) vs. Days
  - Vehicle
  - 0.05 mg/kg
  - 0.5 mg/kg
Absence of CD20-NKCE⁴ toxicity in NHP

Clinical data

- Behavioral scoring
- Body weight (kg)
- Rectal temperature (°C)
- Heart rate (bpm)
- Oxygen saturation (%SpO₂)

- Vehicle
- 0.05 mg/kg
- 0.5 mg/kg
Absence of CD20-NKCE\textsuperscript{4} toxicity in NHP

**Biological data**

- IFN-\(\gamma\) (ng/mL)
- IL-6 (ng/mL)
- IL-8 (ng/mL)
- TNF (ng/mL)
- MCP-1 (ng/mL)
- IL-10 (ng/mL)
- IL-6 (ng/mL)

**Time Points:**
- Day -7 pre-dose
- 2h
- 6h
- 24h
Absence of CD20-NKCE^4 toxicity in NHP

**Biological data**

- **IFN-γ (ng/mL)**
- **IL-6 (ng/mL)**
- **IL-8 (ng/mL)**
- **IL-10 (ng/mL)**
- **TNF (ng/mL)**
- **MCP-1 (ng/mL)**

**CD20 TCE > 1 ng/mL**

**CD20 TCE > 5 ng/mL**

**CD20 TCE > 8 ng/mL**

**CD20 TCE > 10 ng/mL**

**CD20 TCE > 1.7 ng/mL**

**CD20 TCE > 500 ng/mL**

**MCP-1 = CCL2**

*Engelberts et al., EBioMedicine, 2020*
Natural Killer cell engagers

• NKCE technology provides efficient engagement of NK cells against tumors

• NKCE engage NK cells through **NKp46**, the most NK cell-specific activating receptor

• Trifunctional NKCE\(^3\) **co-engage NKp46 and CD16 on NK cells** and a tumor antigen on cancer cells; this leads to potent NK cell activation, cytotoxicity and efficient control of tumor growth in various preclinical mouse models (Gauthier et al., *Cell*, 2019).

• NKCE\(^4\) also induce NK cell proliferation and in vitro cytolytic activity against malignant cells expressing the targeted antigen.

• NKCE\(^4\) have long PK and show in vivo anti-tumor efficacy in several preclinical tumor models
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Anti-tumor immunity induced by tetrafunctional Natural Killer cell engagers armed with not-alpha IL-2 variant

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