Dendritic - NK cell mechanisms

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Scientific Advisory Boards 2021

Alector
Atreca
Dragonfly
DrenBio
Nkarta
Obsidian
SBI
There’s a natural killer inside everyone with the potential to take on multiple myeloma.
NK cell functions are controlled by a balance of inhibitory and activating receptors.

Cytotoxicity
Cytokines
Proliferation

Inhibition

Activating receptor

Activating ligand

MHC I
β2m

Inhibitory Receptor

Fail-safe prevents NK cells from attacking healthy self tissues.
ITAM-based activating NK receptors

- **CD16**
  - ITAM
  - FcεRIγ or CD3ζ

- **NKp30**
  - ITAM
  - FcεRIγ or CD3ζ

- **NKp44**
  - DAP12

- **NKp46**
  - FcεRIγ or CD3ζ
Co-activating NK receptors

NKG2D

DNAM-1

2B4

ITSM

SAP or EAT2

PKC

Fyn

TRAFs

Co-activating NK receptors

DAP10

DAP10

YINM

p85 PI3-kinase

Grb2 - Vav - SLP76

DNAM-1

SAP or EAT2

PKC

Fyn

TRAFs

Miller & Lanier Ann Rev Cancer Biology 2019
NK cells like to kill cells lacking MHC class I – “missing-self”

NK Cells Reject Tumors Lacking MHC Class I

Class I+ tumors grow in vivo
Class I- tumors are rejected
Class I- tumors in NK-depleted mice grow in vivo

Karre et al. 1986 Nature 319:675
MHC class I Inhibitory Receptors on Human NK cells

- KIR
- CD94
- NKG2A
- ITIMs
- LILRB1
Tumors can escape CD8+ T cell surveillance by loss of MHC class I

*Membrane MHC class I expression on primary human melanoma cells ranges from 100 to 0% (median, 70%)

Lack of MHC class I expression on most of malignant cells (>50%) was observed in 34 of 92 cases (37%)

Due to transcriptional down-regulation of HLA-A,-B,-C and β2-microglobulin –not mutation

How do MHC class I-negative tumors escape NK cell recognition and elimination?

How can we re-engage NK cells against these tumors?
NK cell functions are controlled by a balance of inhibitory and activating receptors.

- Cytotoxicity, Cytokines, proliferation
- Inhibition

Fail-safe prevents NK cells from attacking healthy self tissues.
Why don’t NK cells kill HLA class I-negative tumors arising in cancer patients?

* Tumors lack ligands for activating receptors

* Redundant inhibitory receptors other than for class I dampen NK cell responses

* NK cells kill some tumors, but without cytokines don’t expand – then become “de-sensitized”

* Tumor microenvironment suppresses NK cell (e.g. NK cells hate Transforming Growth Factor β)
ITAM-based activating NK receptors

Miller & Lanier Ann Rev Cancer Biology 2019
Co-activating NK receptors

Miller & Lanier Ann Rev Cancer Biology 2019
Why don’t NK cells kill HLA class I-negative tumors arising in cancer patients?

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Non-MHC Inhibitory Receptors on Human NK cells

- LAIR-1
- KLRG1
- CEACAM1
- TIM3
- LAG3
- PD1
- CTLA-4

- YVKM

I T S M
Why don’t NK cells kill HLA class I-negative tumors arising in cancer patients?

*Tumors lack ligands for activating receptors

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*Tumor microenvironment suppresses NK cell (e.g. NK cells hate Transforming Growth Factor β)

*NK cells kill some tumors, but without cytokines don’t expand – then become “de-sensitized”
Why don’t NK cells kill HLA class I-negative tumors arising in cancer patients?

*Tumors lack ligands for activating receptors

*Redundant inhibitory receptors other than for class I dampen NK cell responses

*Tumor microenvironment suppresses NK cell (e.g. NK cells hate Transforming Growth Factor β, hypoxia)

*NK cells kill some tumors, but without cytokines don’t expand – then become “de-sensitized”
In vivo model of anergy NK cells induced in tumor MHC class I – negative tumor environment

Tumor injection

Day 0

Anergy NK cells are induced

Day 7
Day 10
Day 14

Sacrifice

B2m-ko RMA
$1 \times 10^6$

RMA
$1 \times 10^6$
NK cells infiltrating B2m-ko RMA tumor are hypo-responsive ex vivo

In progress – RNA-Seq on NK cells infiltrating RMA versus B2m-ko RMA
Engaging NK cells to kill MHC class I-negative tumors

*Chronic exposure to MHC class I-negative tumors can render NK cells tolerant

*Blocking KIR or NKG2A MHC class I inhibitory receptors alone in cancer patients may simply result in NK cell tolerance

*Activation of NK cells with cytokines (IL-12 and others) can brake the tolerance and allow kill of MHC class I-negative tumors
STRATEGIES FOR THERAPEUTICALLY MODULATING NK CELL FUNCTION
Factors boosting NK cell lytic activity

- Type I IFN
- IL-2
- IL-12
- IL-15
- IL-18
- IL-21
- FLT3
- Immunosuppressive drugs

- Increased expression of activating receptors
- Increased expression of perforin, granzyme B, CD95L and TRAIL
- Less dependence on co-activation from multiple NK-cell activating receptors
Why don’t NK cells kill HLA class I-negative tumors arising in cancer patients?

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Inhibitory Receptors on Human NK cells

- KIR
- LILRB1
- NKG2A
- CD94
- KLRG1
- CEACAM1
- TIM3
- LAG3
- PD1
- LAIR-1
- CTLA-4

ITIMs

YVKM
Checkpoint blockade therapies

- anti-KIR
- anti-NKG2A
- anti-PD1
- anti-Tim3
- anti-LAG3

Inhibitory receptor
Inhibitory ligand
Activating receptor
Stimulatory ligand

NK cell

Tumor cell

Lysis
Antibody-dependent cellular cytotoxicity

NK cell + Lysis + CD16 + Tumor antigen

MHC class I

Inhibitory receptor

Tumor cell

Antibodies

rituximab, trastuzumab, daratumumab
Bispecific antibodies

- anti-tumor x anti-NK activating receptor

NK cell + Lysis + Activating receptors

MHC class I

Inhibitory receptor

Bispecific antibodies – anti-tumor x anti-NK activating receptor

Tumor cell

Activating receptors

Tumor antigen
Therapies that up-regulate stress-induced ligands on tumors or agents that activate NK cells

**NK cell**

- **Activating** receptors
- **Stimulatory** ligands

**Inhibitory receptor MHC class I**

**Stressed or damaged cell**

- **Lysis**

**Interferon-α/β**

- **IL-15**
- **IL-12**

**Agonist co-stimulatory antibodies (e.g. CD137, NKG2D)**

**Irradiation**

**Chemotherapy**
CAR NK cells

Chimeric antigen receptors

Inhibitory receptor

MHC class

Tumor

anti-CD19-cytoplasmic CD137-CD3ζ

Lysis
A natural killer–dendritic cell axis defines checkpoint therapy–responsive tumor microenvironments

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Melanoma patients with more “stimulatory” dendritic cells have better survival
“stimulatory” dendritic cell gene expression tracked with FLT3LG cytokine expression
FLT3LG expression correlates with NK cells in melanoma and head & neck cancer patients.
Response to PD1 blockade in melanoma correlates with NK cells and DC in TIL
NK cell-mediated cytotoxicity contributes to tumor control by a cytostatic drug combination

Marcus Ruscetti1*, Josef Leibold1*, Matthew J. Bott1*, Myles Fennell1, Amanda Kulick2, Nelson R. Salgado3, Chi-Chao Chen1, Yu-jui Ho1, Francisco J. Sanchez-Rivera, Judith Feucht3, Timour Baslan1, Sha Tian1, Hsuan-An Chen1, Paul B. Romesser1, John T. Poirier2,4, Charles M. Rudin2,4, Elisa de Stanchina2, Eusebio Manchado1, Charles J. Sherr5,6, Scott W. Lowe1,6†

Molecularly targeted therapies aim to obstruct cell autonomous programs required for tumor growth. We show that mitogen-activated protein kinase (MAPK) and cyclin-dependent kinase 4/6 inhibitors act in combination to suppress the proliferation of KRAS-mutant lung cancer cells while simultaneously provoking a natural killer (NK) cell surveillance program leading to tumor cell death. The drug combination, but neither agent alone, promotes retinoblastoma (RB) protein-mediated cellular senescence and activation of the immunomodulatory senescence-associated secretory phenotype (SASP). SASP components tumor necrosis factor-α and intercellular adhesion molecule-1 are required for NK cell surveillance of drug-treated tumor cells, which contributes to tumor regressions and prolonged survival in a KRAS-mutant lung cancer mouse model. Therefore, molecularly targeted agents capable of inducing senescence can produce tumor control through non-cell autonomous mechanisms involving NK cell surveillance.
NK cells are required for optimal chemotherapy (MEK and CDK4/6 inhibitors) in transplantable mouse KP lung tumor model