Neo-antigen recognition as a major ingredient in clinically effective cancer immunotherapies

Evidence & Implications
I have the following financial relationships to disclose:

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Stockholder in: AIMM Therapeutics, Kite Pharma
Part time employee of: Kite Pharma

I will not discuss off label use and/or investigational use in my presentation.
Value of mobilizing endogenous tumor-specific T cell responses

1. TIL therapy

- TIL are grown from melanoma tumors
- Rapid Expansion
- Infusion of TIL + IL-2
- Patient pretreated with lymphodepleting chemotherapy
- 50% response rate in trials in multiple centers (US, Israel)
- Clinical effect at least partially mediated by CD8 T cells

Value of mobilizing endogenous tumor-specific T cell responses
1. TIL therapy
Value of mobilizing endogenous tumor-specific T cell responses

1. TIL therapy

1. Checkpoint blockade
Value of mobilizing endogenous tumor-specific T cell responses

1. TIL therapy

J. Haanen, NKI-AVL

1. Checkpoint blockade

C. Robert, NEJM 2015
Which human cancer antigens could be key to these clinical effects?

1. Self antigens; Non-mutant proteins to which tolerance is incomplete

2. ‘Neo-antigens’; Epitopes that arise as a consequence of tumor-specific mutations
Predictions:

1). If recognition of neo-antigens is an important ingredient to immunotherapy, one would expect that, in tumor types that are responsive to IT, the immune system is often able to recognize mutant antigens.

2). If recognition of neo-antigens is an important ingredient to immunotherapy, one would expect that the extent of DNA damage correlates with the clinical effects of cancer immunotherapy.
Tools for high-throughput analysis of neo-antigen specific CD8 T cell responses:


Analyzing the neo-antigen-specific T cell response in human melanoma
Generate map of tumor-specific mutations (ExomeSeq)

↓

Determine which mutated genes are expressed (RNASeq)

↓

Predict epitopes for each mutation/each HLA-allele in silico

↓

Screen for T cell recognition of mutated epitopes
Pt 002: Partial response upon anti-CTLA4 treatment

pre-treatment

A
August 2010

post-treatment

December 2010

80.39 mm

40.11 mm

64.30 mm

27.75 mm

S100 (mg/L)

Days after start of therapy
Pt 002: Partial response upon anti-CTLA4 treatment

- Resected tumor material
- Isolate tumor cells
- Isolate tumor-infiltrating T cells
- Screen with MHC multimer technology
- Identify tumor-specific mutations
- Predict potential epitopes
Strong T cell response against an ATR_{S>L} neo-epitope within the tumor

- Resected tumor material
- Isolate tumor cells
- Isolate tumor-infiltrating T cells
- Identify tumor-specific mutations
- Predict potential epitopes

Screen with MHC multimer technology

Graph showing percentage of cells reacting with the ATR_{S>L} peptide.
Increased magnitude of neo-antigen-specific T cell response upon anti-CTLA4

van Rooij et al. JCO 2013
Pt 008: CR upon TIL therapy

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- Isolate tumor cells
- Isolate tumor-infiltrating T cells
- Screen with MHC multimer technology
- Identify tumor-specific mutations
- Predict potential epitopes
Pt 008: CR upon TIL therapy

Infusion TIL product

0.172%

\[ \text{RASSF1}_{R>C} \]

23%

\[ \text{DHX33}_{R>W} \]
Pt 008: CR upon TIL therapy

>5000 fold increase in neo-antigen specific T cell reactivity upon TIL therapy
Evidence for neo-antigen reactive CD4 T cells?

Oncogene-immortalized autologous APC platform

Linnemann et al. Nat Med 2014
Neo-antigen reactive CD4 T cells in clinically effective ACT products? (6m PR upon TIL therapy)

Tumor-infiltrating lymphocytes (TIL) are grown from melanoma tumors

Patient pretreated with lymphodepleting chemotherapy

Rapid Expansion

Infusion of TIL + IL-2
Neo-antigen reactive CD4 T cells in clinically effective ACT products? (6m PR upon TIL therapy)
Neo-antigen reactive CD4 T cells in a clinically effective ACT product (>7yr CR upon T cell therapy)

Tumor-reactive T lymphocytes are grown by culture of PB T cells with autologous melanoma

Expansion

Infusion of T cells + IFNα


TNIK_{S>F} 0.77% 2.21% 7.89% 2.02%

RPS12_{G>A} ZC3H18_{G>R}

CD4 IFN-γ
How often does the immune system ‘see’ neo-antigens in melanoma?

- The T cell based immune system frequently interacts with the consequences of DNA damage in human melanoma.

**CD8 T cells:** 11 pts analyzed, neo-antigen specific reactivity in 9. Not all alleles covered, exome coverage incomplete, epitope predictions imperfect…

**CD4 T cells:** 5 pts analyzed, neo-antigen specific reactivity in 4
Does the extent of DNA damage correlate with the clinical effects of cancer immunotherapy?
Does the extent of DNA damage correlate with the clinical effects of cancer immunotherapy?

Alexandrov et al, Nature 2013
Regression of NSCLC upon PD-1 blockade
Regression of NSCLC upon PD-1 blockade is accompanied by induction of neo-antigen specific T cell reactivity.
Mutational load correlates with clinical outcome to anti-PD-1 in NSCLC

Discovery cohort

\[ p = 0.02 \]

# nonsynonymous mutations/tumor

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<th>Durable clinical benefit</th>
<th>Non-durable benefit</th>
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<td>Mutations/tumor</td>
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\[ \text{p} = 0.02 \]
Mutational load correlates with clinical outcome to anti-PD-1 in NSCLC

Discovery cohort

p = 0.02

Validation cohort

p = 0.04

Rizvi et al. Science 2015
Conclusions:

1). Neo-antigen recognition is frequent in melanoma

2). Mutational load correlates with response to checkpoint blockade in a way that is consistent with a simple neo-antigen lottery model
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Can we provide direct evidence that neo-antigen specific T cells (or TCRs) are superior?

*Goal: Develop humanized mouse models to directly compare the effect of different autologous T cells/TCRs on growth of the same tumor.*
Do neo-antigen specific TCRs outperform C/G antigen specific TCRs?

1). From a single patient, isolate:
   2 TCRs against CDK4 and GCN1L1 neo-epitopes
   4 TCRs against 1 MAGE-A10 epitope, 3 MAGE-C2 C/G epitopes

2). Put TCRs into fresh T cells

3). Measure ability to control TCR-autologous tumor in PDX model
Do neo-antigen specific TCRs outperform C/G antigen specific TCRs?

1) Create human melanoma PDX model (NSG-mice)

2a) treat with T cells transduced with autologous C/G Ag specific TCRs

2b) treat with T cells transduced with autologous Neo Ag specific TCRs
Do neo-antigen specific TCRs outperform C/G antigen specific TCRs?
Conclusions:

1). Neo-antigen recognition is frequent in melanoma

2). Mutational load correlates with response to checkpoint blockade in a way that is consistent with a simple neo-antigen lottery model

3). Neo-antigen specific TCRs outperform C/G antigen specific TCRs in a patient-derived xenograft model of cancer immunotherapy

- Based on these data, it is plausible that – at least in tumors with substantial DNA damage – neo-antigen recognition is the major ingredient of effective cancer immunotherapy
What is the relevance of neo-antigens across human malignancies?
What is the relevance of neo-antigens across human malignancies?

A neo-antigen repertoire may only be frequent in some human cancers

Formation of neo-antigens

Generally

Regularly

Occasionally

Caveat: It is possible that a ‘neglected’ repertoire of neo-antigens exists
We may be underestimating…

Schumacher and Schreiber Science 2015
PRECISION CANCER IMMUNOTHERAPY

Combine strategies that *enhance* T cell activity with strategies that *direct* this T cell activity towards cancer neo-antigens.

Schumacher and Schreiber *Science* 2015

**A**
Induce tumor cell destruction
Provide checkpoint blockade

**B**
Identify potential neoantigens
Create synthetic vaccine (RNA, DNA, peptide)
Provide in combination with adjuvant and checkpoint blockade

**C**
Identify potential neoantigens
Induce or expand neoantigen specific T cells
Provide in combination checkpoint blockade

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Tumor destruction + Checkpoint blockade

Neo-ag vaccine + Checkpoint blockade

Neo-ag specific T cell product + Checkpoint blockade

Schumacher and Schreiber *Science* 2015
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**Tumor control**
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**Binding strength**

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   2 TCRs against CDK4 and GCN1L1 neo-epitopes
   4 TCRs against 1 MAGE-A10 epitope, 3 MAGE-C2 C/G epitopes

2). Put TCRs into fresh T cells

3). Measure binding strength of these TCRs to cognate pMHC
Do neo-antigen specific TCRs outperform C/G antigen specific TCRs?

**Binding strength**
Do neo-antigen specific TCRs outperform C/G antigen specific TCRs?

**Ranking:** (1) Neo      (2) Neo      (3) C/G     (4) C/G     (5/6) C/G

**Binding strength**

- CDK4\textsubscript{R24L}
  - 4°C: >500s
  - RT: 229s

- GCN1\textsubscript{L12330P}
  - 4°C: 236s
  - RT: 67s

- MAGE C2\textsubscript{V1W}
  - 4°C: 195s
  - RT: 34s

- MAGE C2\textsubscript{LLF}
  - 4°C: 154s
  - RT: 34s

*Kelderman et al. Unpublished*