Immunotherapy beyond anti-PD-1 inhibitors

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Disclosures

iTeos Therapeutics
eTheRNA
BMS
AMGEN
Ludwig Institute
Sopartec-UCL

Scientific Advisory Board
Scientific Advisory Board
Educational tasks
Educational sessions
Revenues from licensing
Revenues from licensing
Cancer immunotherapy

- Main players
  - T lymphocytes
  - tumor antigens
  - activatory and inhibitory co-receptors

- Current modalities
  - immunostimulatory antibodies
    - manipulating co-receptors (‘Checkpoint Blockade’)
      - BiTE antibodies
  - adoptive T cell therapy
  - vaccines

- Tumor resistance
  - too low antigenicity
  - tumor-driven immunosuppression
  - numbers game
Principal immune effectors in cancer immunotherapy: cytolytic T lymphocytes

CTL need other cells for full effectiveness: antigen-presenting cells, CD4 T cells.
(These other cells may be critical limiting factors)

Other modalities of tumor cell killing:
- NK cells without antitumor antibodies
- NK cells with antitumor antibodies (ADCC)
- Antitumor antibodies (complement)
- Macrophages, neutrophils

Exquisite specificity
Memory
Spontaneous anti-tumor T cell responses in cancer patients

How do we know that spontaneous antitumor T cell responses exist?

- Antitumor CTL are present in patients prior to any treatment.
  - in blood
  - in tumors (among TILs: Tumor-Infiltrating Lymphocytes)
- Higher incidence of tumors in immunosuppressed patients.
- Frequent HLA losses in some types of tumors.
  (likely resulting from immunoselection)
- Prognostic value of TILs, likely due to the presence of tumor-specific T cells.

Obviously these responses are insufficient.
Darwinian selection of resistant tumor cells.
Spontaneous anti-tumor T cell responses in cancer patients

How do we know that spontaneous antitumor T cell responses exist?

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- Prognostic value of TILs, likely due to the presence of tumor-specific T cells.

Currently used immunostimulatory antibodies boost these responses.
Mounting an anti-tumor T cell response

Release of tumor antigens

adapted from Chen & Mellman - 2013 - Immunity
Mounting an anti-tumor T cell response

- Release of tumor antigens
- Tumor antigen presentation
- Dendritic cells
- Tumor
Mounting an anti-tumor T cell response

T cell priming

Tumor antigen presentation

Release of tumor antigens

lymph node

tumor
Mounting an anti-tumor T cell response

- **T cell priming**
- **Tumor antigen presentation**
- **Release of tumor antigens**
- **Trafficking to tumors**
- **Infiltration into tumors**

**Steps:**
1. T cell priming
2. Tumor antigen presentation
3. Release of tumor antigens
4. Trafficking to tumors
5. Infiltration into tumors
Mounting an anti-tumor T cell response

- T cell priming
- Tumor antigen presentation
- Release of tumor antigens
- Tumor cell recognition
- Infiltration into tumors
- Trafficking to tumors
- T cell activation
Mounting an anti-tumor T cell response

- T cell priming
- Tumor antigen presentation
- Release of tumor antigens
- Tumor cell recognition
- Tumor cell killing
- Infiltration into tumors
- Trafficking to tumors
Mounting an anti-tumor T cell response

Each step is necessary but not sufficient. Several concurrent blockades are possible, → combine immunotherapies.
Cancer immunotherapy

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Cytolytic T lymphocytes (CTL) recognize on the surface of target cells **peptides** presented by **HLA class I** molecules (HLA-A, B, C).

Which peptides are derived from a given protein? **Difficult** to predict.

Which peptides can bind to given HLA molecules (A2 or A24 or B7 …)? **Easy** to predict.
Classes of tumor antigens recognized by T cells

(T. Boon, Brussels)

‘mutated’ antigens (neoepitopes)

- DNA mutations (mostly non synonymous single nucleotide variations) > amino acid change in a protein.

- Mostly at random: passenger mutations.

- A peptide containing a mutated amino acid can be presented by HLA molecules and recognized by T cells.
Antigens resulting from mutations (single nucleotide variations)

Only ±5% of mutated sequences are antigenic.
(a ‘mutated’ peptide displayed on surface HLA molecules)

adapted from Vogelstein et al. - 2013 - Science
Classes of tumor antigens recognized by T cells

‘mutated’ antigens (neoepitopes)  MAGE-type antigens

‘Cancer-germline’ genes
- Expressed in tumors.
- Silent in normal adult tissues, except for male germline cells (HLA-negative).
- Reason for this pattern of expression: DNA demethylation.
- Examples:
  - MAGEA1, -A2, -A3, ... -A12
  - MAGEC1, -C2
  - LAGE1 = NYESO1
  - BAGE, GAGE
  - SSX1, -2
  - ...

Genetic process:
- mutation
- tumor-specific gene expression

Genotype:  
- all normal cells  
- other normal cells  
- spermatocytes spermatogonia trophoblast

Phenotype:  
- no protein  
- no HLA
Expression levels of gene **MAGEA3** (*RNA-seq*)

Gene expression level (normalized according to RPKM)

**Normal tissues (GTEx)**

**Cancerous tissues (TCGA)**
Expression levels of gene **CTAG1B (NY-ESO-1)** (RNA-seq)

Gene expression level (normalized according to RPKM)

Normal tissues (GTEx)

Cancerous tissues (TCGA)
Classes of tumor antigens recognized by T cells

- ‘mutated’ antigens (neoepitopes)
- MAGE-type antigens
- Viral antigens

Genetic process:
- Mutation
- Tumor-specific gene expression
- Oncogenic virus

Tumor cells:
- Mutated antigens
- MAGE-type antigens
- Viral antigens

Normal cells:
- All normal cells
- Other normal cells
- Spermatocytes spermatogonia trophoblast

No HLA
Classes of tumor antigens recognized by T cells

- ‘mutated’ antigens (neoepitopes)
- MAGE-type antigens
- viral antigens
- differentiation antigens (melanoma, B cells)

Genetic process:
- mutation
- tumor-specific gene expression
- oncogenic virus
- tissue-specific gene expression

Tumour cells:
- no protein
- demethylation

Normal cells:
- no HLA
- spermatocytes spermatagonia trophoblast
- all normal cells
- other normal cells
- melanocytes
Classes of tumor antigens recognized by T cells

- 'mutated' antigens (neoepitopes)
- MAGE-type antigens
- viral antigens
- differentiation antigens (melanoma, B cells)
- overexpressed antigens

Genetic process:
- mutation
- tumor-specific gene expression
- oncogenic virus
- tissue-specific gene expression
- gene overexpression

Tumor cells:
- mutation
- tumorderived
- demethylation
- no protein

Normal cells:
- no protein
- no HLA

Overexpressed antigens:
- melanocytes
- other normal cells
Expression levels of gene CEACAM5 (RNA-seq)

Gene expression level (normalized according to RPKM)

Normal tissues (GTEx)
Cancerous tissues (TCGA)

Adipose
Adrenal
Artery
Bladder
Brain
Breast
Cervix
Cholangiocarcinoma
Colon
Esophagus
Fallopian tube
Heart
Kidney
Liver
Lung ad
Lung sq
DLBCL
Mesothelioma
Ovary
Pancreas
Pheochromocytoma
Prostate
Rectum
Sarcoma
Melanoma skin
Stomach
Testis
Thymus
Thyroid
Uterus
Melanoma uveal
Expression levels of gene **CEACAM6** (RNA-seq)

Gene expression level (normalized according to RPKM)

- **Normal tissues (GTEx)**
- **Cancerous tissues (TCGA)**

Gene expression levels for various tissues, including:
- Adrenals
- Bladder
- Glioma (low gr)
- Breast
- Cervix
- Cholangiocarcinoma
- Colon
- Esophagus
- Glioma (high gr)
- H & N
- Kidney
- Liver
- Lung ad
- Lung sq
- DLBCL
- Mesothelioma
- Ovary
- Pancreas
- Pheochromocytoma
- Prostate
- Rectum
- Sarcoma
- Melanoma skin
- Stomach
- Testis
- Thymus
- Thyroid
- Uterus
- Melanoma uveal
Gene expression level (normalized according to RPKM)

Expression levels of gene **ERBB2** (RNA-seq)
Expression levels of gene **MAGEA3** *(RNA-seq)*

Gene expression level (normalized according to RPKM)

Normal tissues (GTEx)

Cancerous tissues (TCGA)
Expression levels of gene *ERBB2* (*RNA-seq*)

**Normal tissues (GTEx)**

**Cancerous tissues (TCGA)**

Gene expression level (normalized according to RPKM)
Expression levels of gene $TP53$ (RNA-seq)

Gene expression level (normalized according to RPKM)

Normal tissues (GTEx) vs. Cancerous tissues (TCGA)
Classes of tumor antigens recognized by T cells

**High tumor specificity**
- 'mutated' antigens (neoepitopes)
- MAGE-type antigens
- Viral antigens

**Low tumor specificity**
- Differentiation antigens (melanoma, B cells)
- Overexpressed antigens

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Genetic processes:
- Mutation
- Tumor-specific gene expression
- Oncogenic virus
- Tissue-specific gene expression
- Gene overexpression

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Tumor cells:
- All normal cells
- Other normal cells
- Spermatocytes
- Spermatogonia
- Trophoblast
- All normal cells
- Melanocytes
- Other normal cells

Normal cells:
- No protein
- Demethylation
- No HLA

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Diagram shows the relationship between genetic processes and tumor antigens with emphasis on the high and low tumor specificity classes.
Cancer immunotherapy

- Main players
  - T lymphocytes
  - tumor antigens
  - activatory and inhibitory co-receptors

- Current modalities
  - immunostimulatory antibodies
    manipulating co-receptors (‘Checkpoint Blockade’) 
    BiTE antibodies
  - adoptive T cell therapy
  - vaccines

- Tumor resistance
  - too low antigenicity
  - tumor-driven immunosuppression
  - numbers game
T cell differentiation following activation by antigen

- **Priming** (first activation)
  - proliferation
  - differentiation

- **Effector function** (subsequent activations)

**T lymphocyte** (naive)

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**professional antigen-presenting cell** (dendritic cell)

---

**target cell** (tumor cell)

---

'naive'

---

'TCR'

---

'antigen experienced'
T lymphocyte (naive) → Priming (first activation) → Effector function (subsequent activations)

Consequence: T cell priming is controlled by DC.
CTLA-4: - surface expression 4-48h following T cell activation
- physiological brake during T cell activation

T lymphocyte (naive) → **Priming** (first activation) → **Effector function** (subsequent activations)

- proliferation
- differentiation

T lymphocyte (naive)

professional antigen-presenting cell (dendritic cell)

TCR

CD28

antigen

B7

4-48h after antigen recognition

4-48h following T cell activation

physiological brake during T cell activation

target cell (tumor cell)

TCR

CD28

antigen

CTLA-4

'naive'

'antigen experienced'
PD-1: - expressed on activated effector T-cells
- dampens T cell activation

T lymphocyte (naive) → Priming (first activation) → Effector function (subsequent activations)

professional antigen-presenting cell (dendritic cell)

T lymphocyte

'naive'

4-48h after antigen recognition

++

+++
PD-L1 expression:
- constitutive (on tumor cells)
- induced (on many cell types) by activated T cells (and NK cells)

T lymphocyte (naive) → Priming (first activation) → Effector function (subsequent activations)

professional antigen-presenting cell (dendritic cell)

IFN-γ

target cell (tumor cell)

4-48h after antigen recognition

TCR

CD28

B7

CTLA-4

PD-L1

PD-1
Antigen-presenting cell (tumor cell)

Stimulatory or inhibitory co-receptors

Antigen

TCR

Proliferation
Survival
Effector function
Cytokine production
Memory

T-cell
Stimulatory or inhibitory co-receptors

Antigen-presenting cell (tumor cell)

Stimulatory co-receptors

4-1BB, GITR, OX40, CD40, CD70, CD80, CD86

4-1BB, GITR, OX40, CD40L, CD27, CD28

TCR

Proliferation
Survival
Effector function
Cytokine production
Memory
Antigen-presenting cell (tumor cell)

Proliferation
Survival
Effector function
Cytokine production
Memory

Stimulatory or inhibitory co-receptors

4-1BB, GITRL, OX40L, CD40, CD70, CD80, CD86

Stimulatory co-receptors

4-1BB, GITR, OX40, CD40L, CD27, CD28

TCR

Proliferation
Survival
Effector function
Cytokine production
Memory

Inhibitory co-receptors

CTLA-4, PD-1, TIM-3, LAG-3, BTLA-4

Role: fine tuning of T-cell activation in time and space.
Stimulatory or inhibitory co-receptors

Antigen-presenting cell (tumor cell)

agonistic antibodies

blocking antibodies

T-cell

Proliferation
Survival
Effector function
Cytokine production
Memory

adapted from Vogelstein et al. - 2013 - Science
anti-CTLA-4

T cell priming

Tumor antigen presentation

Release of tumor antigens

Tumor cell killing

Tumor cell recognition

Infiltration into tumors

Blood vessel

Trafficking to tumors

lymph node

Tumor
anti-PD-1
anti-PD-L1
Cancer immunotherapy

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  - immunostimulatory antibodies
    - manipulating co-receptors (‘Checkpoint Blockade’)
    - BiTE antibodies
  - adoptive T cell therapy
  - vaccines

- Tumor resistance
  - too low antigenicity
  - tumor-driven immunosuppression
  - numbers game
Pembrolizumab in treatment-refractory progressive metastatic CRC (anti-PD-1 IgG4)

Change in CEA level (%)

- Mismatch repair–proficient colorectal cancer
- Mismatch repair–deficient colorectal cancer
- Mismatch repair–deficient noncolorectal cancer

Days
Cancer immunotherapy

- Main players
  - T lymphocytes
  - tumor antigens
  - activatory and inhibitory co-receptors

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    - manipulating co-receptors (‘Checkpoint Blockade’)
  - BiTE antibodies
  - adoptive T cell therapy
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- Tumor resistance
  - too low antigenicity
  - tumor-driven immunosuppression
  - numbers game
BiTE antibodies: Bispecific T-cell Engagers

**Anti-CD3 + anti-CD19**
- ALL (blinatumomab)

**CD20**
- B cell malignancies

**CD123**
- AML

**EpCAM**
- Malignant ascites, solid tumors

**CEA**
- GI adenocarcinomas
Cancer immunotherapy

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  - T lymphocytes
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- Current modalities
  - immunostimulatory antibodies
    manipulating co-receptors (‘Checkpoint Blockade’)
  - BiTE antibodies
  - adoptive T cell therapy (conventional T cells; transduced (TCR, CAR) T cells)
  - vaccines

- Tumor resistance
  - too low antigenicity
  - tumor-driven immunosuppression
  - numbers game
Release of tumor antigens → Tumor antigen presentation → T cell priming → Trafficking to tumors → Infiltration into tumors → Tumor cell recognition → Tumor cell killing → Tumor cell killing
Adoptive cell therapy: current approaches

1. Harvest PBMCs by apheresis
2. Excise tumor mass
3. T cell activation
4. Transduction
5. TIL cell isolation
6. TIL cell expansion
7. Infusion
8. CART cells
9. TCRT cells
10. Host condition chemotherapy
11. Lymphodepleted cancer patient

June et al. - 2015 - Science Transl Med
- Regression of CRC lung metastases after transfer of anti-KRAS G12D HLA-C8-restricted T cells.
  Tran et al. 2016 NEJM

- T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis.
  Parkhurst et al. 2011 Mol Ther
Chimeric Antigen Receptors

**first generation**

**second generation**

CD28

CD3

TCR

scFv

CD3ξ

CD28

CD3ξ
Cancer immunotherapy

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  - adoptive T cell therapy
  - vaccines

- Tumor resistance
  - too low antigenicity
  - tumor-driven immunosuppression
  - numbers game
Vaccines

Release of tumor antigens

Tumor antigen presentation

Tumor cell killing

Tumor cell recognition

Infiltration into tumors

Trafficking to tumors

Blood vessel

Lymph node

T cell priming

Release of tumor antigens
‘Exogenous’ vaccination
- using one or several tumor-specific antigens (soon mutated antigens)
- to prime or re-activate tumor-specific T cells
- difficulty: How to stimulate cytolytic T cells, rather than antibody production?

‘Endogenous’ vaccination
- some chemotherapy regimens, leading to ‘Immunogenic Cell Death’
- radiotherapy (the abscopal effect)
- oncolytic viruses (T-VEC for advanced melanoma)
- ... ... ...

! intratumoral
T cell priming

exogenous vaccines

Tumor antigen presentation

Release of tumor antigens

endogenous vaccines

Tumor cell recognition

Infiltration into tumors

Tumor cell killing

Tumor antigen presentation

Blood vessel

lymph node

Tumor

Trafficking to tumors

Release of tumor antigens
Cancer immunotherapy

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Resistance of tumors to T cell attack

- Immunogenic tumors (bearing antigens that have stimulated T cell responses) have necessarily been selected for resistance mechanisms.

- Darwinian process: random variation + selection.

- Has long been known: ‘immunoselection’.
  Is now often referred to as ‘immunoediting’.
Cancer immunotherapy

- Main players
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  - tumor antigens
  - activatory and inhibitory co-receptors

- Current modalities
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    manipulating co-receptors (‘Checkpoint Blockade’)
    BiTE antibodies
  - adoptive T cell therapy
  - vaccines

- Tumor resistance
  - too low antigenicity  (frequent; reversible or irreversible)
  - tumor-driven immunosuppression
  - numbers game
Too low antigenicity

Downregulation / loss of expression of

- HLA class I heavy chains
- β2-microglobulin

! complete HLA class I loss —> higher sensitivity to NK cells

components of the antigen presentation machinery (TAP1/2, …)

Irreversible: mutations, deletions
**Reversible: IFN-γ** (increases expression of all these genes)
Cancer immunotherapy

- Main players
  - T lymphocytes
  - tumor antigens
  - activatory and inhibitory costimuli

- Current modalities
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    BiTE antibodies
  - adoptive T cell therapy
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- Tumor resistance
  - too low antigenicity
  - tumor-driven immunosuppression
  - numbers game

  - shortage in soluble factors:
    tryptophan
    arginine ...
  - immunosuppressive soluble factors:
    TGFβ
    IL-10
    galectins
    extracellular adenosine
    ...
  - immunosuppressive cells:
    Myeloid-derived Suppressor Cells (MDSC)
    Tregs
    ...

  ! local