Proto-oncogenes: normally basic cellular functions, related to cell division

Changed by somatic mutations (spontaneous/environmental)

Driver mutation $\rightarrow$ Oncogene

$\rightarrow$ Uncontrolled cell growth $\rightarrow$ tumor

$\rightarrow$ Spread of tumor cells $\rightarrow$ metastasis
The cancer mutanome

Large variability in tumor mutation burden (TMB) within tumor types, ranging from 10s to 1000s of mutations

Within the same tumor type different clones accumulate different mutations

Majority of cancer mutations are unique to the individual patient
Cancer mutanome and immune therapy

Cancer mutanome = source of neoantigens

→ can be recognized by the immune system as foreign-like neoantigens presented on MHC molecules
Major Histocompatibility Complex (MHC) proteins in humans

MHC are found on the membrane of most cells
MHC present antigens

- Immune recognition
- Histocompatibility (transplantations)
- Immunological individuality
Class I MHC molecules: β2 subunits \(\rightarrow\) can only be recognized by CD8 co-receptors \(\rightarrow\) Cytotoxic T-cells \(\rightarrow\) cellular immunity

Class II MHC molecules: β1 and β2 subunits \(\rightarrow\) recognized by CD4 co-receptors. \(\rightarrow\) Helper T-Cells \(\rightarrow\) adaptive immunity
Immune response in tumors
Immune response in tumors
Cancer Immunotherapy

- Non-specific Immune stimulation
- Immune-checkpoint blockade
- Adoptive cell transfer
- Vaccination strategies
Cancer Immunotherapy

Activate antigen presenting cells (APCs) with cytokines

→ Alert T cells

→ IFN alpha and IL-2 are approved drugs
Cancer Immunotherapy
Adoptive cell transfer

Direct isolation of immune cells from the tumor

Autologous tumor-infiltrating lymphocytes (TILs)
T cells, B cells, NK cells, macrophages, neutrophils, DCs, mast cells, eosinophils, basophils

T-cells activated by cytokines

Cells multiplied

Transfer into patient
Cancer Immunotherapy

Adoptive cell transfer

isolation of immune cells from the blood

T-cells activated by cytokines

Multiplied

genetic engineering needed to arm the cells with specific receptors
Cancer Immunotherapy

Inject weakened Bacillus Calmette-Guérin (BCG) bacteria against tuberculosis
→ Inflammation
→ General activation of the immune system:
  more immune cells around the cancer

→ helps against bladder cancer
Cancer Immunotherapy

Inject weakened Bacillus Calmette-Guérin (BCG) bacteria against tuberculosis
→ Inflammation
→ General activation of the immune system:
  more immune cells around the cancer
→ helps against bladder cancer

Direct immune cells specifically to the cancer tissue.

Weakened version of Herpes simplex virus modified to produce an immune stimulating factor is used against melanoma and head and neck cancer.

Tumor cell vaccination: extract tumor cells, irradiated, engineered to secrete activating growth factors. When cells are brought back to the patient, the growth factors alert immune cells to target the cancer.

Vaccination with APCs: extract immature APCs from patient, matured outside the body and loaded with tumor antigen. 2010 approved against prostate cancers (Sipuleucel-T).

Vaccination with synthesized long peptides or RNA, DNA plasmid, or viral vector vaccines that encode relevant neoepitope sequences
Cancer Immunotherapy

Remove immune checkpoint blockage

normally dampens down the immune response to avoid immune overreaction and prevent collateral damage to healthy tissue.

Makes cancer cells resistant to T cells

humanized monoclonal antibody targets CTLA4, Enhances T-cell function, proliferation, infiltration of lymphocytes into the tumor and killing of tumor cells. approved 2011 against melanoma

Cytotoxic T-Lymphocyte Antigen 4 (CTLA4) B7 = ligand → combined for immunotherapy
Programmed cell death-1 PD1 receptor PD1 = ligand
Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer

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Science, April 2015

Used removal of checkpoint blockage as cancer therapy
**non–small cell lung cancer (NSCLC)**

Large variability in mutation burden: 10s to 1000s of mutations

More mutations with chronic exposure to mutagens such as UV light, carcinogens in cigarette smoke

Never-smokers generally have fewer somatic mutations than smokers

Hypothesis:
Cancer mutanome of NSCLCs may influence response to anti–PD-1 therapy
Treatment of non-small cell lung cancer patients with anti–PD-1 therapy with **Pembrolizumab** infusion in lung

Immune checkpoint inhibitors, which unleashes a patient’s own T cells to kill tumors

Whole-exome sequencing of non–small cell lung cancers treated with pembrolizumab ($n = 18$)

The median number nonsynonymous mutations
244 in tumors from patients with DCB (durable clinical benefit, partial or stable response lasting >6 months)
125 in those with NDB (no durable benefit)
73% of patients with high nonsynonymous burden experienced DCB

$\rightarrow$ Higher somatic nonsynonymous mutation burden
$\rightarrow$ higher clinical efficacy of pembrolizumab
$\rightarrow$ Higher percentage progression free survival

% of progression free survival is better in patients with more mutations
Examine the landscape of neoantigens

Neoantigen burden in patients with DCB \((n = 14)\) compared to NDB \((n = 17)\)

High neoantigen burden is beneficial
Paper I Summary

The mutational landscape influences the response to anti–PD-1 therapy

More somatic nonsynonymous mutations and neoantigens → better durable clinical benefit (DCB) and higher percentage of progression free disease survival

However, only a fraction of mutations (<0.1%) actually result in productive epitopes triggering a T cell response.

→ Tumors with fewer mutations, have been suggested to be ‘off-limits’ for mutanome-directed therapy (i.e. breast cancer)
A 49-year-old woman with metastatic breast cancer resistant to chemotherapy
Combination of removal of checkpoint blockage, adoptive transfer of antitumor lymphocytes and interleukin (IL)-2
Immune recognition of somatic mutations

1. Sample acquisition: right breast subcutaneous lesion
2. Whole-exome sequencing (WES) and RNA sequencing (RNA-seq) revealed 62 nonsynonymous somatic mutations
3. Tumor-infiltrating lymphocytes (TILs) were cultured and expanded. T cells were screened for reactivity against a series of neoepitopes.
4. They isolated TILs reactive against mutations in four genes (SLC3A2, ECPAS (KIAA0368), CADPS2, and CTSB).
5. They confirmed the reactivity of the different neoepitope-reactive TIL clones.
Clinical study procedure

Neoepitope-reactive TIL clone

Chemotherapy with cytostatic drugs

Humanized monoclonal antibody targets PD-1 receptor of lymphocytes removes immune checkpoint blockage
Complete-durable regression of cancer
ongoing for >22 months

Response curves of target lesions (tumor size measurements).
All lesions resolved 1 year after TIL transfer.
The patient continued to demonstrate complete response 22 months after cell infusion and 20 months after the last dose of pembrolizumab.
Cross-sectional imaging of target lesions

retrosternal mediastinum

Left axilla

Liver

Liver

I week before treatment
Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer

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Nature, July 2017

Vaccination approach to target tumors in a personalized manner by targeting the cancer mutanome
Generate personalized mutanome vaccines

thirteen patients with stage III and IV melanoma
routine tumor biopsies and healthy blood cells
Generate personalized mutanome vaccines

Non-synonymous mutations were identified by comparative exome and RNA sequencing.
Generate personalized mutanome vaccines

**Computational prediction of neo-epitopes**
1. predicted high-affinity binding to autologous HLA class II
2. predicted HLA class I binding
Generate personalized mutanome vaccines

Computational prediction of neo-epitopes
1. predicted high-affinity binding to autologous HLA class II and high expression of the mutation-encoding RNA
2. predicted HLA class I binding.

Engineer vaccine against neo-epitopes
- Unique for each patient
- 10 mutations per patient
- Engineered into two synthetic RNAs
- Translation of RNA-encoded antigens → immune response
Generate personalized mutanome vaccines

RNA-based poly-neo-epitope approach to mobilize immunity against a spectrum of cancer mutations.

- Vaccine against neo-epitope
- Two synthetic pharmacologically optimized RNAs
- each RNA encodes five linker-connected 27mer peptides with the mutation in position 14
- each RNA harboured five mutations (pentatope RNA)
- Translation of RNA-encoded antigens
Clinical study procedure

At least 8 doses with the neo-epitope vaccine were injected percutaneously into inguinal lymph nodes under ultrasound control.

Patients tolerated well without related serious adverse events.
All patients developed T cell responses against multiple vaccine neo-epitopes

The immunogenicity of each of the total 125 mutations was analyzed by IFNγ ELISpot in CD4+ and CD8+ T cells in pre- and post-vaccination blood samples.

T cell response statistics for all 125 mutations on cohort level or per individual patient.
All patients developed T cell responses against multiple vaccine neo-epitopes

The immunogenicity of each of the total 125 mutations was analyzed by IFNγ ELISpot in CD4+ and CD8+ T cells in pre- and post-vaccination blood samples.

- Immunogenic responses were detected in 60% of the predicted neo-epitopes.
- Each patient developed T cells against at least three mutations.
- Pre-existing weak responses against one-third of the immunogenic neo-epitopes were augmented upon vaccination.
- The other two-thirds were *de novo* responses.
- The majority of neo-epitopes resulted exclusively CD4+ responses.
- A smaller fraction was recognized by CD8+ cytotoxic lymphocytes (CTLs) only.
- One-quarter showed concomitant CD4+ and CD8+ responses, recognizing different regions of synthetic RNA
Neo-epitope-specific CD8+ T cells expanded within 2–4 w

ex vivo MHC multimer blood analysis in 4 patients

Mutation-specific CD8+ T cells were stained using
- dextramers carrying 9 or 10 aa-long epitopes from immunogenic mutations
- cell surface markers
- and live-dead staining (DAPI BD)
Disease control by vaccination in melanoma patients with high risk of relapse

Cumulative sum of metastatic events per month before or after neo-epitope RNA vaccination showed a highly significant reduction of longitudinal cumulative recurrent metastatic events

→ sustained progression-free survival of patients.
Conclusion

Efficient RNA and DNA sequencing allows personalized immunotherapy in cancer.

The mutational landscape influences the response to anti–PD-1 therapy.

Combination of removal of checkpoint blockage, adoptive transfer of antitumor lymphocytes and interleukin (IL)-2 led to complete durable regression of cancer.

Neo-epitope vaccines alone may prevent recurrent disease in high risk patients.

It is difficult to predict who might respond to which treatment.

Some immunotherapies show skin reactions, flue like symptoms, swelling, weight gain from extra fluids, heart palpitation as side effects.
THANK YOU