TARGETING THE CANCER MUTANOME

TECHNICAL JOURNAL CLUB

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Mutations and cancer

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

Changed by somatic mutations (spontaneous/ environmental)

Driver mutation → Oncogene

→ Uncontrolled cell growth → tumor

→ Spread of tumor cells → 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
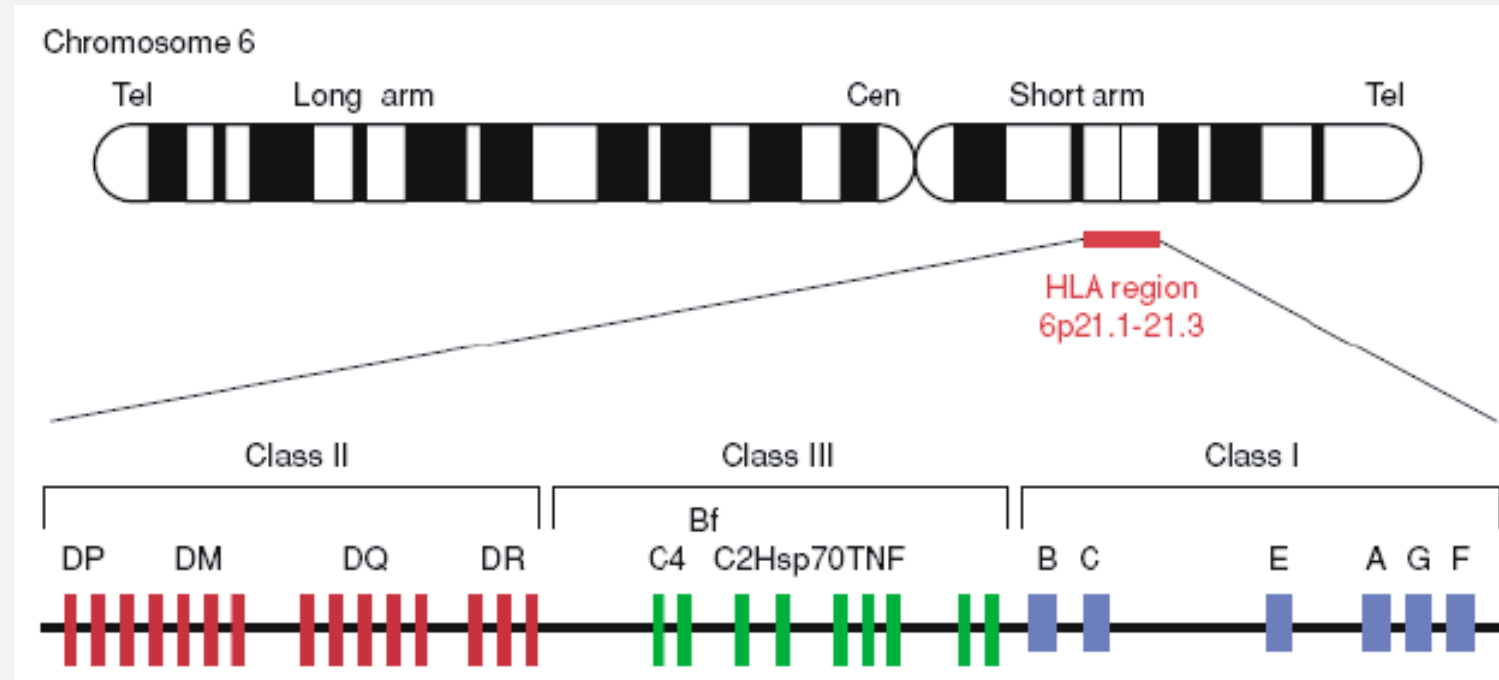
Major Histocompatibility Complex (MHC) proteins in humans

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

➢ Immune recognition

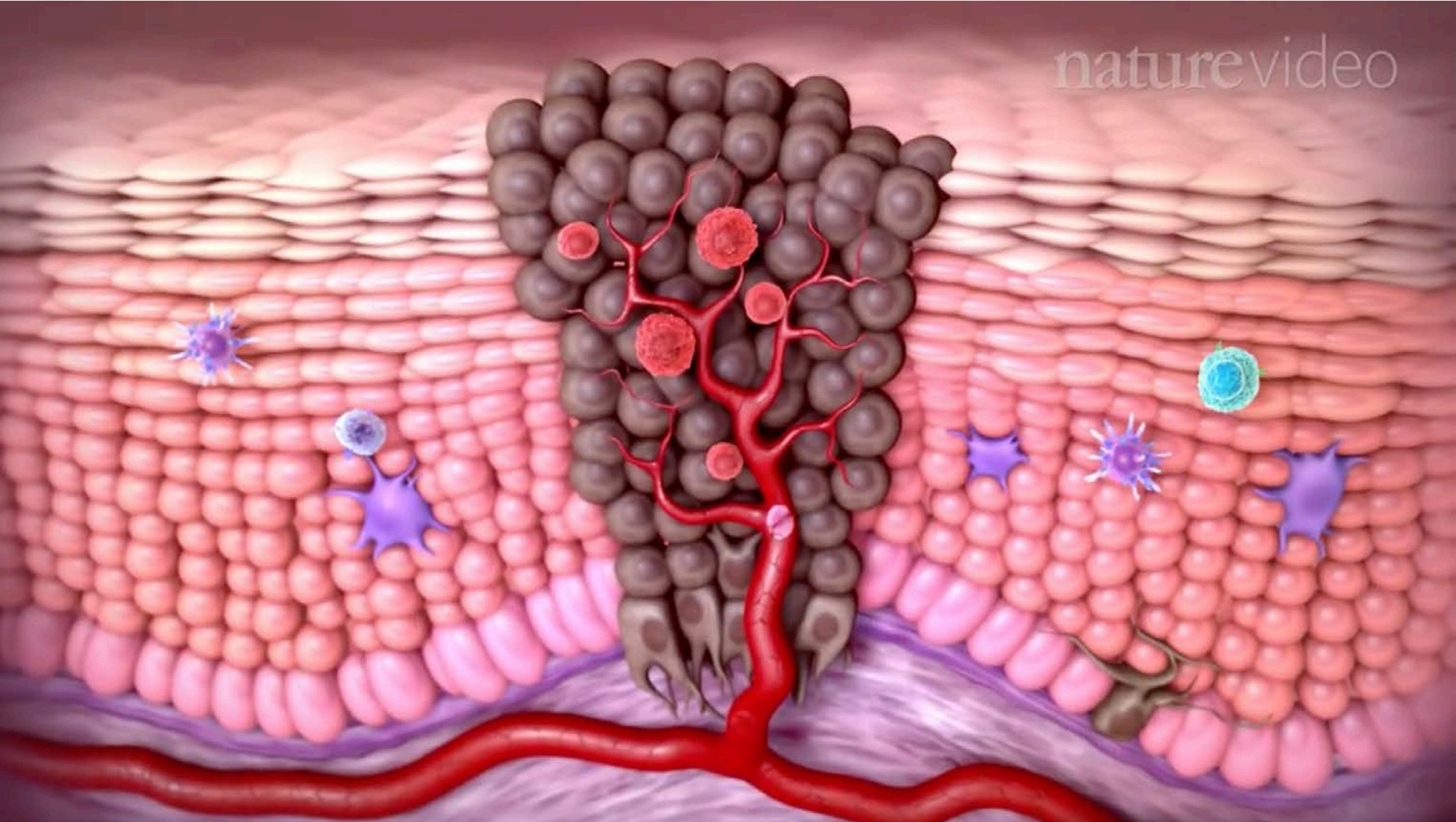➢ Histocompatibility (transplantations)

➢ Immunological individuality

ebi.ac.uk/interpro/potm/2005_3/Page1.htm
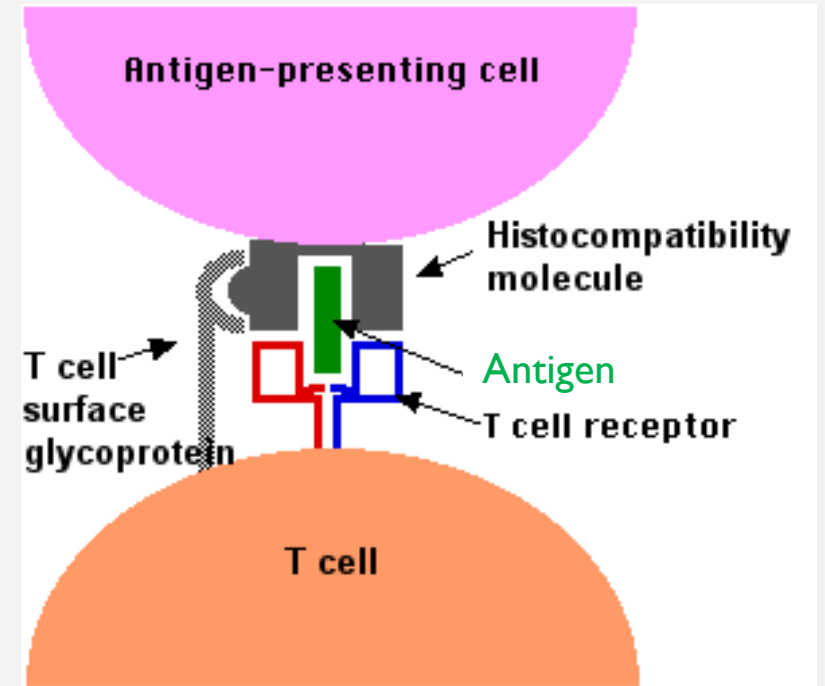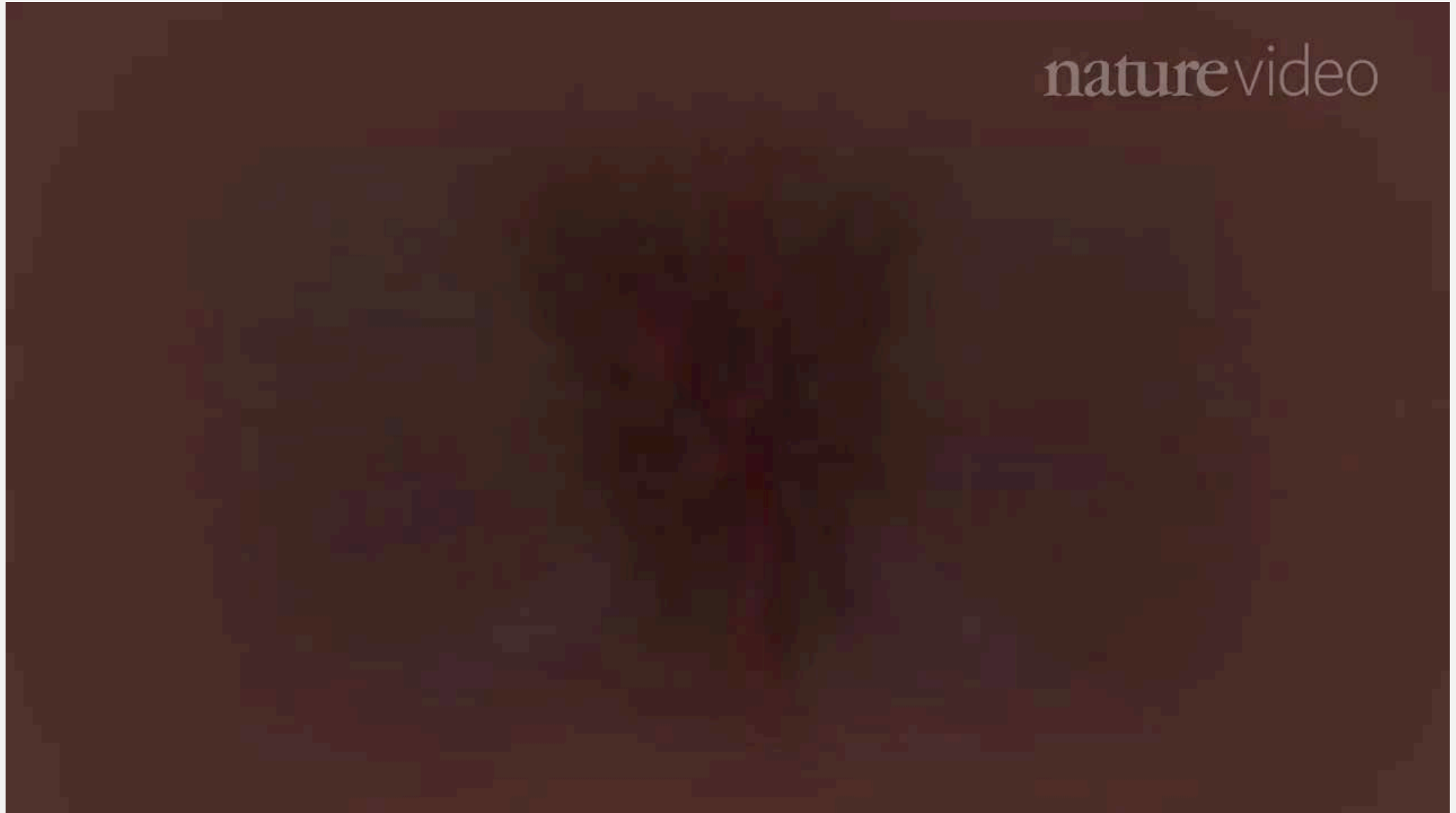
Class I MHC molecules: β2 subunits → can only be recognized by CD8 co-receptors → Cytotoxic T-cells → cellular immunity

Class II MHC molecules: β1 and β2 subunits → recognized by CD4 co-receptors. → Helper T-Cells → adaptive immunity

Immune response in tumors

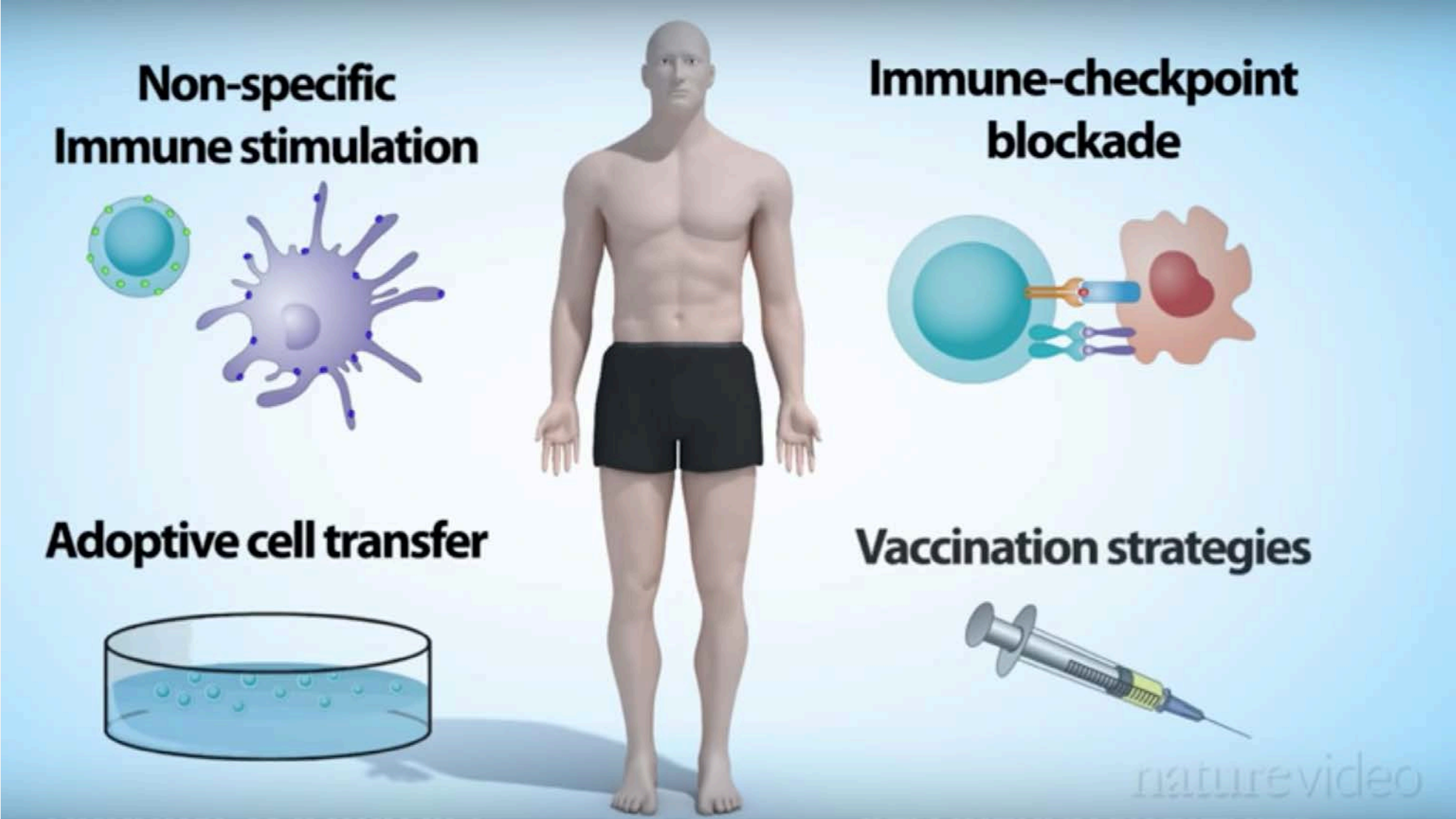# Immune response in tumors

Cancer Immunotherapy

Cancer Immunotherapy

Non-specific Immune stimulation
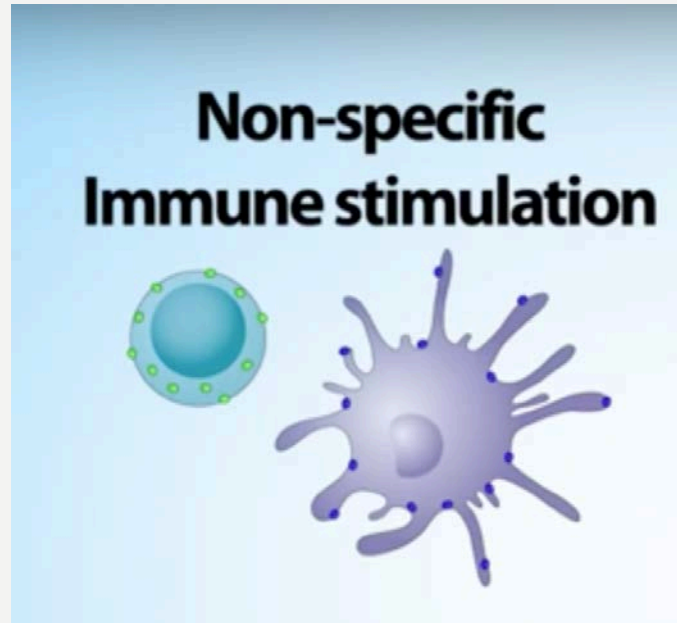
Activate antigen presenting cells (APCs) with cytokines

→ 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
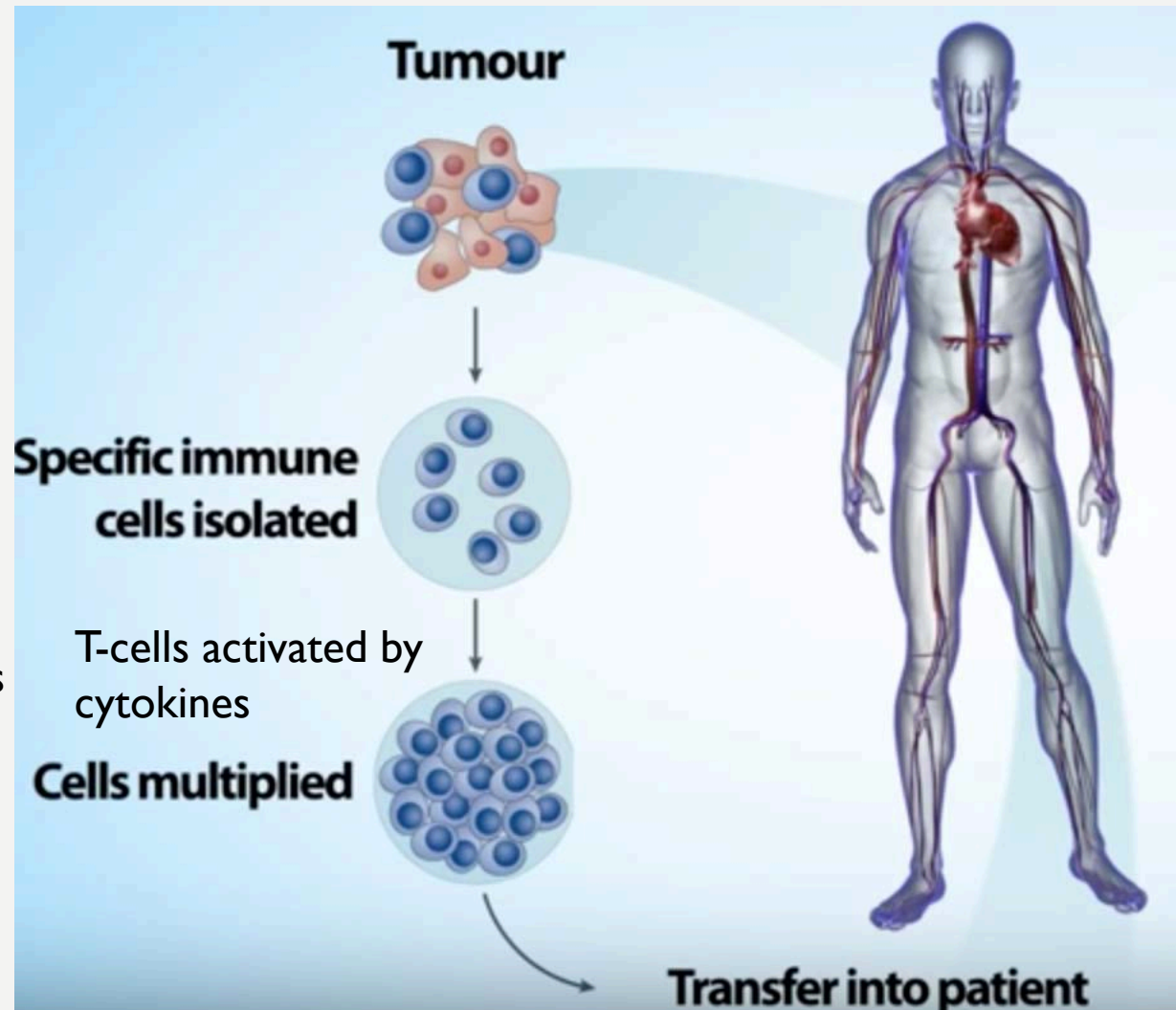
Cancer Immunotherapy

Adoptive cell transfer

isolation of immune cells from the blood

Blood vessel

Non-specific immune cells isolated

genetic engineering needed to arm the cells with specific receptors

T-cells activated by cytokines Multiplied

Transfer into patient
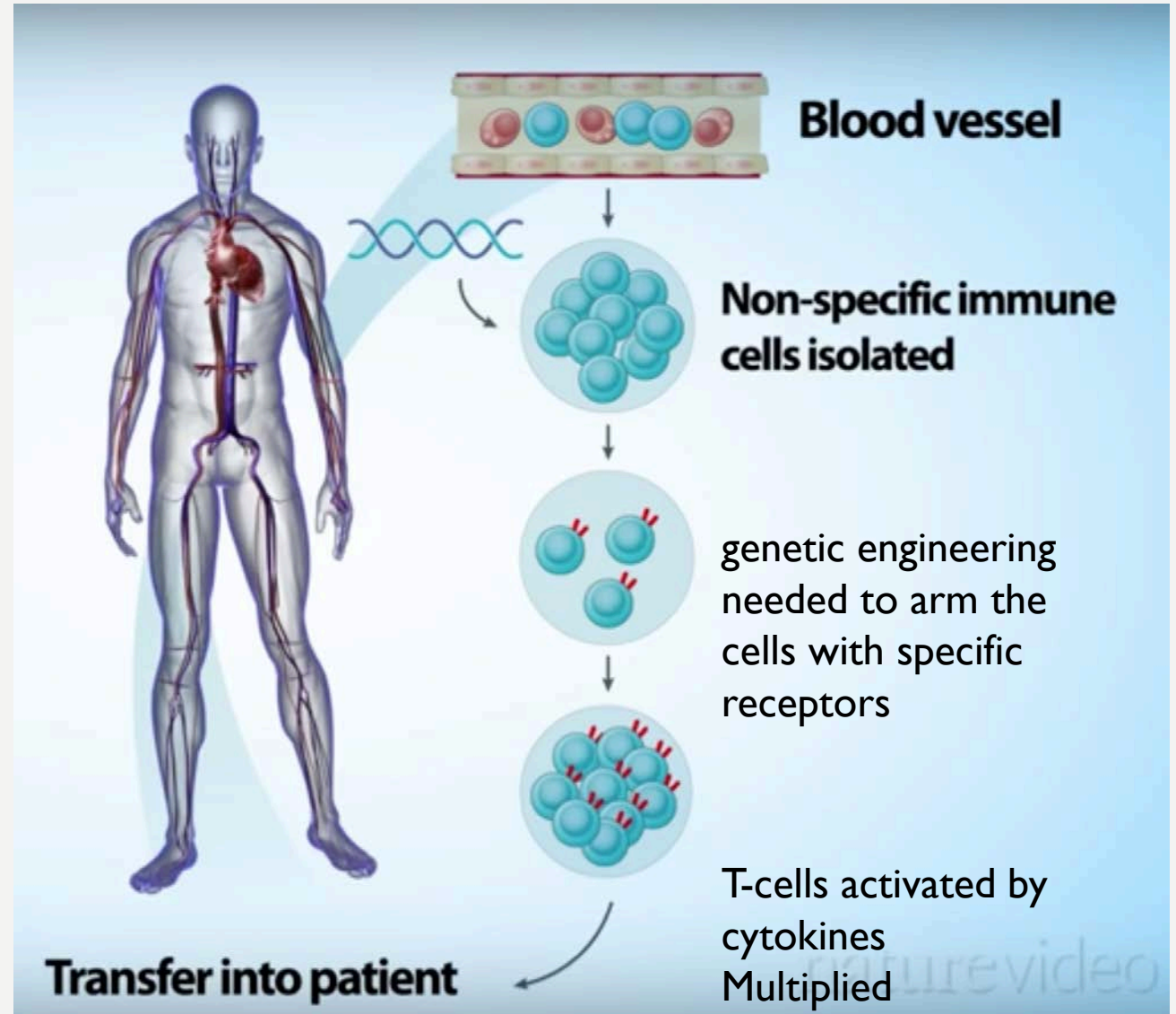
Cancer Immunotherapy

Vaccination strategies

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

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Inject weakened Bacillus Calmette-Guérin (BCG) bacteria against tuberculosis
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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).
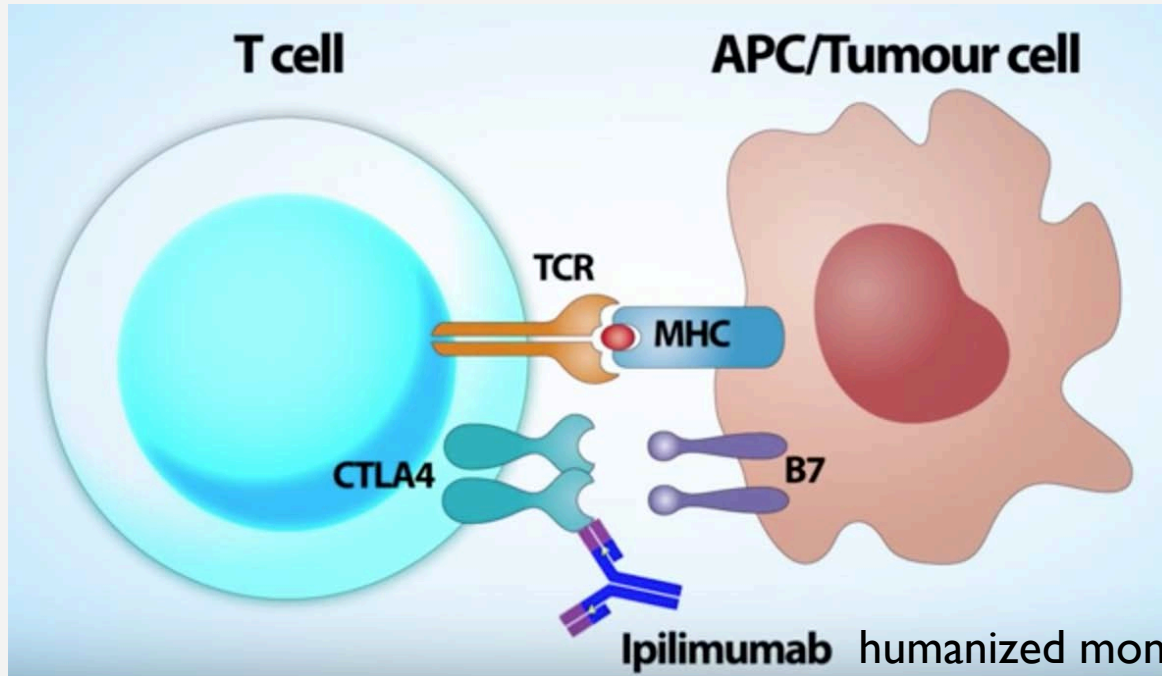
Vaccination with synthesized long peptides or RNA, DNA plasmid, or viral vector vaccines that encode relevant neoepitope sequences

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

Ipilimumab 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   PDL1 = ligand

Paper 1

Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer

Naiyer A. Rizvi[1,2,*,†], Matthew D. Hellmann[1,2,*], Alexandra Snyder[1,2,3,*], Pia Kvistborg[4], Vladimir Makarov[3], Jonathan J. Havel[3], William Lee[5], Jianda Yuan[6], Phillip Wong[6], Teresa S. Ho[6], Martin L. Miller[7], Natasha Rekhtman[8], Andre L. Moreira[8], Fawzia Ibrahim[1], Cameron Bruggeman[9], Billel Gasmi[10], Roberta Zappasodi[10], Yuka Maeda[10], Chris Sander[7], Edward B. Garon[11], Taha Merghoub[1,10], Jedd D. Wolchok[1,2,10], Ton N. Schumacher[4], and Timothy A. Chan[2,3,5,‡]

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
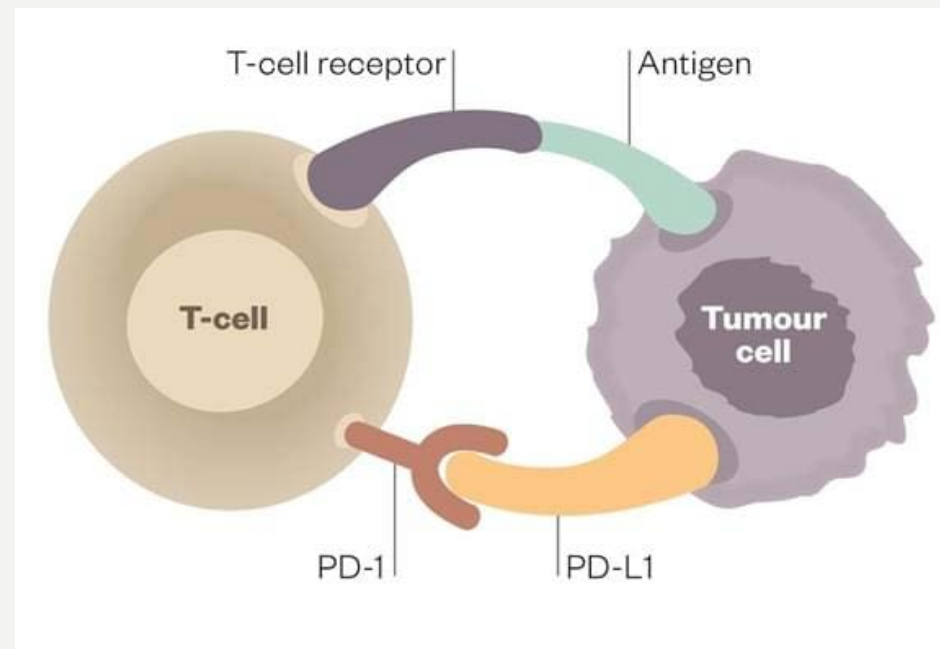
Never-smokers generally have fewer somatic mutations than smokers

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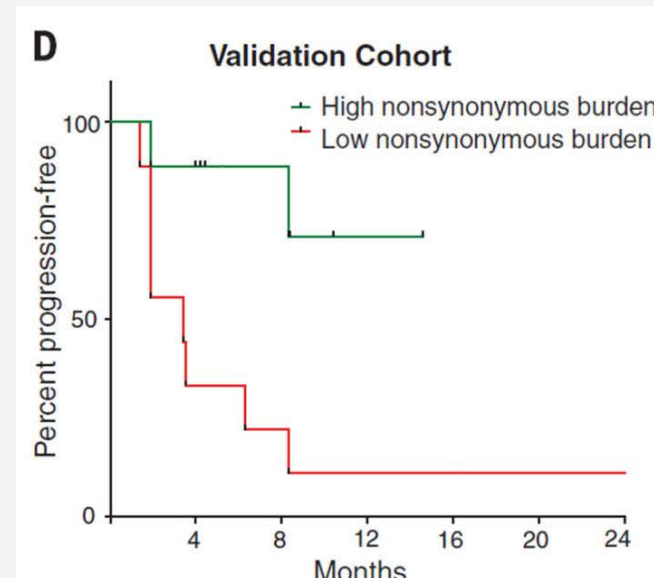
Whole-exome sequencing of non–small cell lung cancers treated with pembrolizumab (*n* = 18)

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
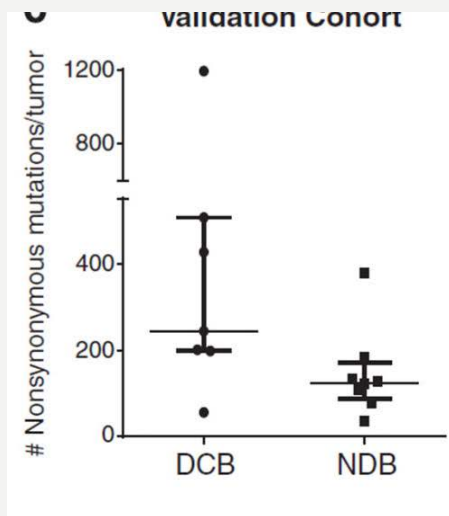
% of progression free survival is better in patients with more mutations

→ Higher somatic nonsynonymous mutation burden
→ higher clinical efficacy of pembrolizumab
→ Higher percentage progression free survival

Examine the landscape of neoantigens

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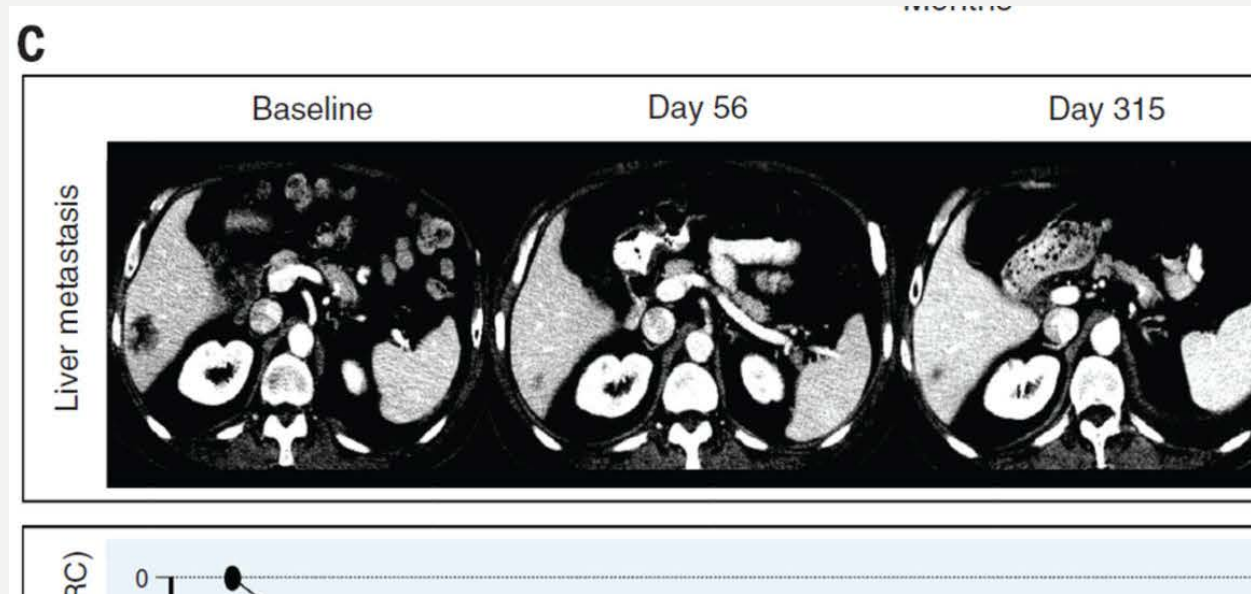

High neoantigen burden is beneficial

Paper 1 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)

Paper II

a-McGowan[2], Robert P. Somerville[1], Paul F. Robbins[1], Steven A. Rosenberg[1]*, Stephanie L. Goff[1] and Steven A. Feldman[1]

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.

I week before treatment

Cross-sectional imaging of target lesions

retrosternal mediastinum

Left axilla

Liver

Liver

Paper III

Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer

Ugur Sahin[1,2,3], Evelyna Derhovanessian[1], Matthias Miller[1], Björn-Philipp Kloke[1], Petra Simon[1], Martin Löwer[2], Valesca Bukur[1,2], Arbel D. Tadmor[2], Ulrich Luxemburger[1], Barbara Schrörs[2], Tana Omokoko[1], Mathias Vormehr[1,3], Christian Albrecht[2], Anna Paruzynski[1], Andreas N. Kuhn[1], Janina Buck[1], Sandra Heesch[1], Katharina H. Schreeb[1], Felicitas Müller[1], Inga Ortseifer[1], Isabel Vogler[1], Eva Godehardt[1], Sebastian Attig[2,3], Richard Rae[2], Andrea Breitkreuz[1], Claudia Tolliver[1], Martin Suchan[2], Goran Martic[2], Alexander Hohberger[3], Patrick Sorn[2], Jan Diekmann[1], Janko Ciesla[4], Olga Waksmann[4], Alexandra-Kemmer Brück[1], Meike Witt[1], Martina Zillgen[1], Andree Rothermel[2], Barbara Kasemann[2], David Langer[1], Stefanie Bolte[1], Mustafa Diken[1,2], Sebastian Kreiter[1,2], Romina Nemecek[5], Christoffer Gebhardt[6,7], Stephan Grabbe[3], Christoph Höller[5], Jochen Utikal[6,7], Christoph Huber[1,2,3], Carmen Loquai[3]* & Özlem Türeci[8]*
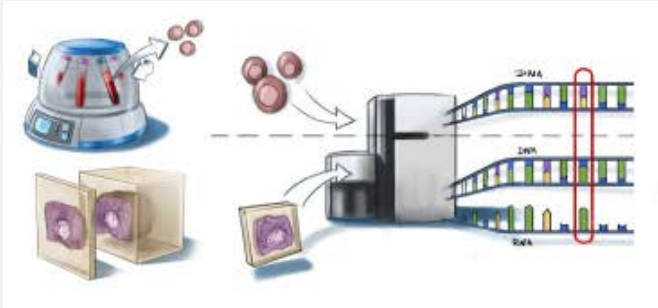
Nature, July 2017

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

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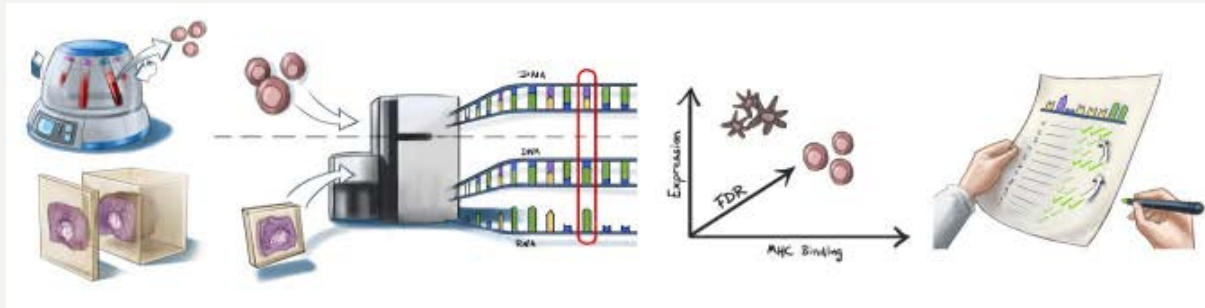
thirteen patients with stage III and IV melanoma
routine tumor biopsies and healthy blood cells

Generate personalized mutanome vaccines

Sample   Mutation

Non-synonymous mutations were identified by comparative exome and RNA sequencing

Generate personalized mutanome vaccines

Sample   Mutation   Neo-epitope   Target

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
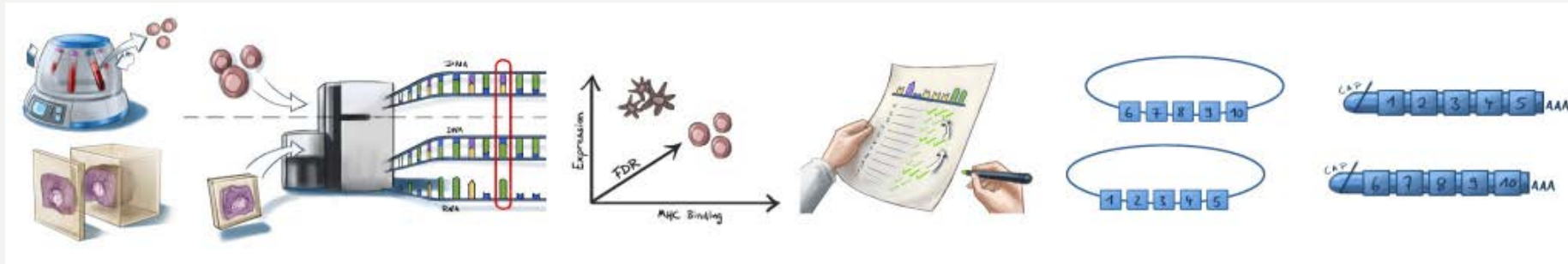
Sample   Mutation   Neo-epitope   Target   Template DNA   GMP RNA

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.

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T cell response statistics for all 125 mutations on cohort level or per individual patient.
- 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