Cancer Immunity

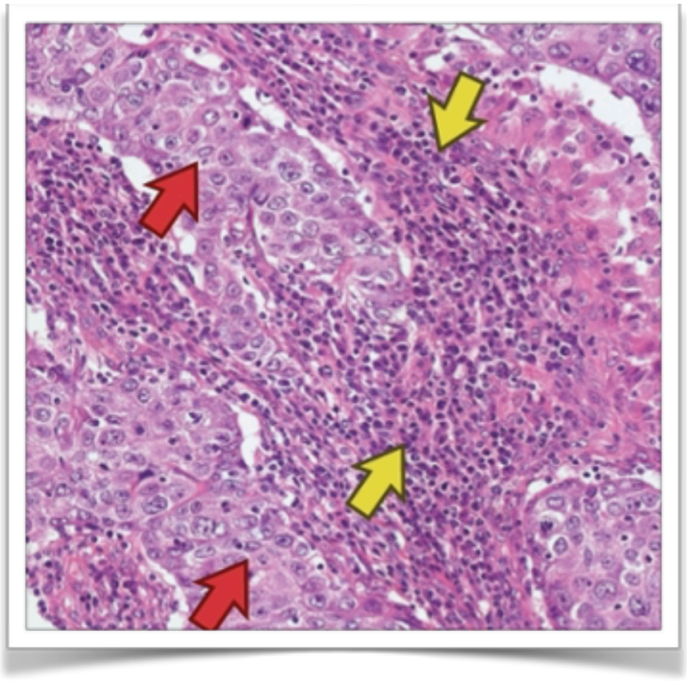
Andrea Ablasser, EPFL

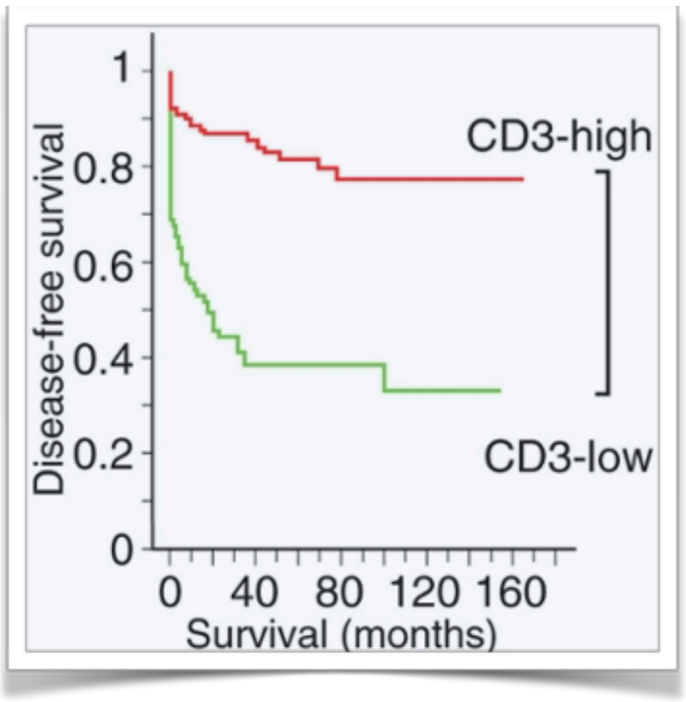
Overview of tumor immunity

- Tumors elicit specific immune responses, which exhibit characteristics of specificity, memory, and a key role of lymphocytes

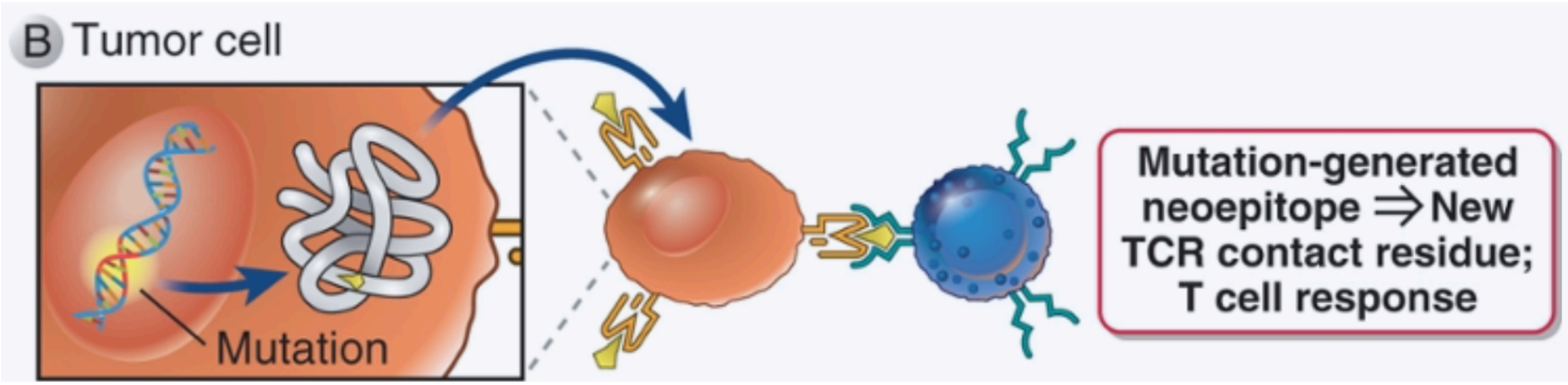
- Immune responses frequently fail to prevent tumorigenesis
  - Immune-regulatory mechanisms by tumors
  - Rapid growth and metastasis

- Reinvigorating antitumor immunity can induce long-lasting effects





Tumor antigens - Neoantigens



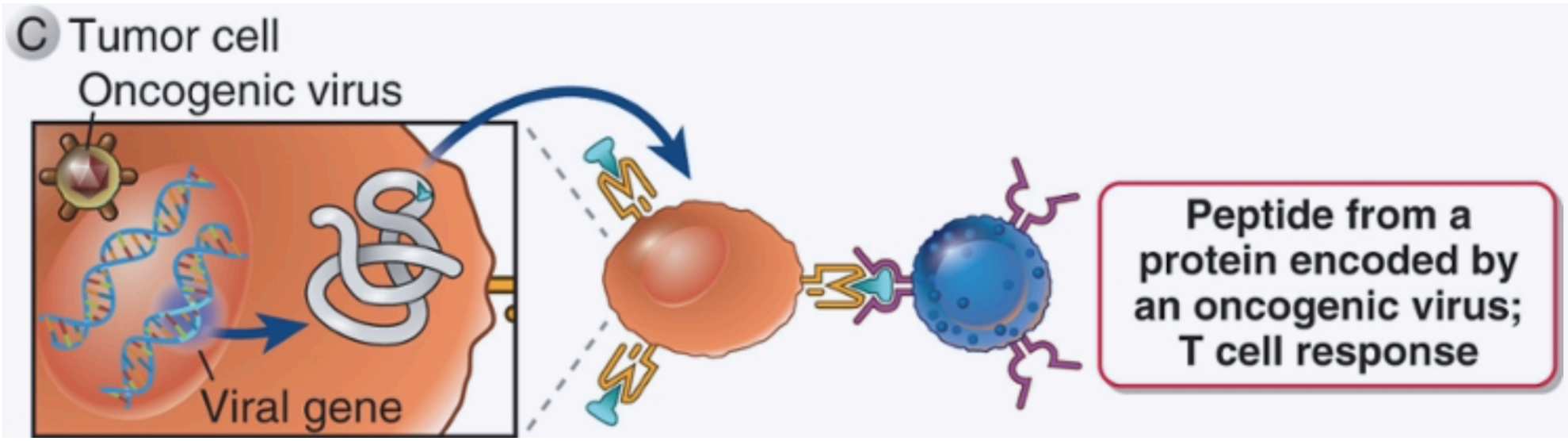
- Neoantigens are products of mutated genes - either randomly mutated genes (passenger mutations) or mutated oncogenes or tumor suppressor genes (driver mutations).

- Neoantigens are mostly presented by MHC I molecules, but if tumor cells are ingested by APCs these neoantigens can also be presented by MHC II molecules

- The same type of tumor in different patients, and even within one patients different tumor cell clones express different neoantigens

- Critical for devising personalized cancer vaccine approaches

Tumor antigens - oncogenic viruses



- Viruses are implicated in many different tumors

- Oncogenic viruses, Epstein-Barr Virus or HPV, can trigger the expression of viral antigens in infected cells, which can serve as antigens for tumor immunity

- The efficacy of virus-specific immunity is largely due to eliminating infected cells before they can turn in malignant cells

- Some viruses, e.g., hepatitis B/C virus, are associated with tumorigenesis by causing a chronic state of inflammation

Tumor antigens-overexpressed genes

~ are not foreign, but can still elicit immune responses or can serve as antigens for antitumor therapies

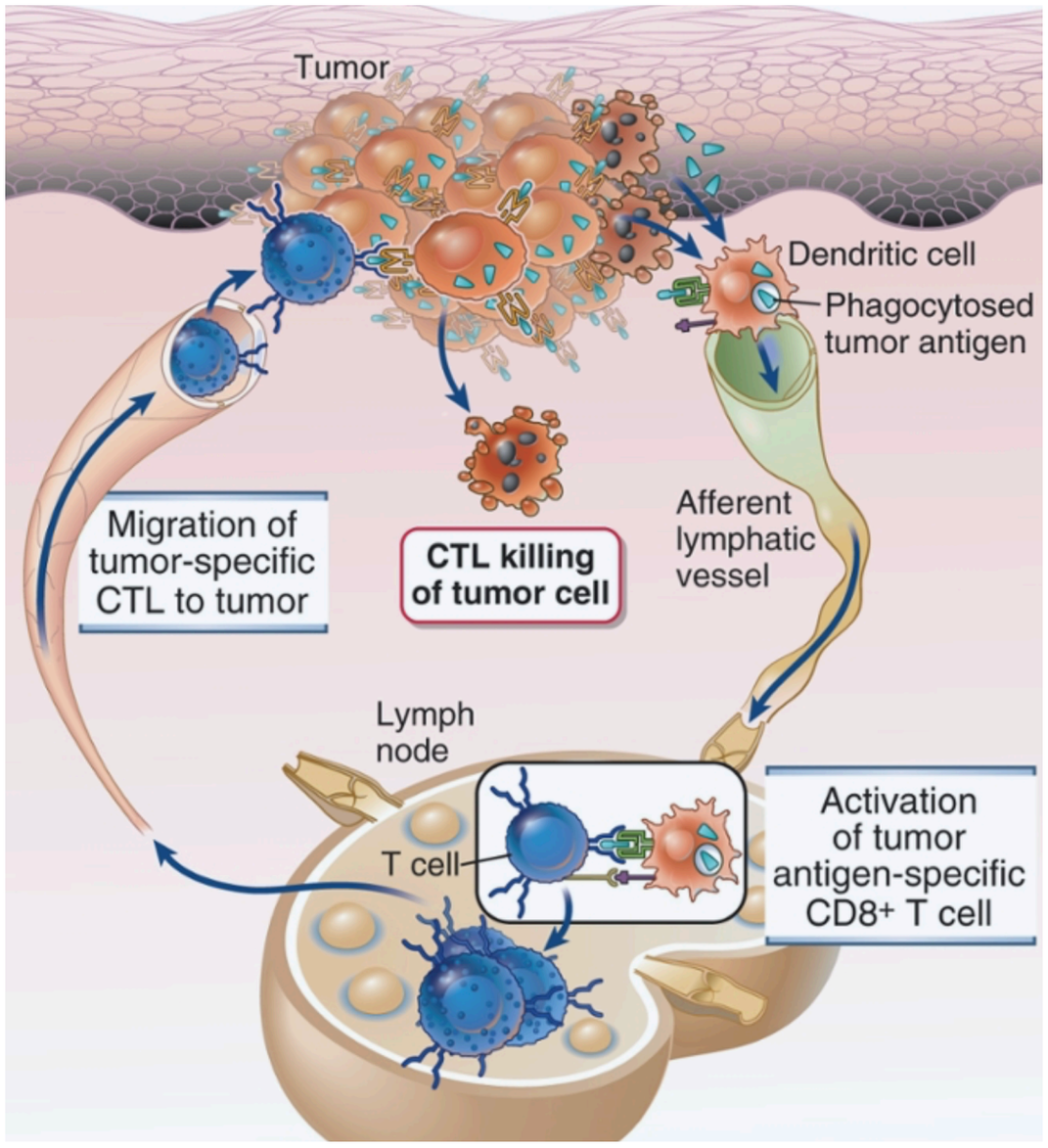
- *Cancer-testis antigens* are expressed in gametes or trophoblasts, but are silenced in most in normal somatic tissue (e.g., MAGE = melanoma-associated antigen)

- Tumor cells may express abnormally high levels of normal proteins (e.g., Her2/Neu, which is an epidermal growth factor) - although the antigen may not be sufficient to induce protective immunity per se, it can be used as a target for passive immunotherapy.

- Differentiation antigens are found on tumor cells and on cells of the same lineage/differentiation stage, but absent in most other cell types (e.g., CD19, tyrosinase, MART-1). Can serve as targets for passive immunotherapy or CAR T cell therapies.

Immune response to tumors

- CTLs are the principal components to host defense against tumors
- CTLs recognize TAAs presented by tumor cells via MHC I molecules
- CTLs are generated by DCs involving cross-presentation of TAAs
- CD4 T cells support antitumor activities of CTLs through cytokine production (IFNg, TNFa) and through enhancing CD8 T cell expansion



Immune responses to tumors

- Innate cells -

- NK cells are important contributors to tumor immunity
- Defects in NK cell biology leads to increased susceptibility of humans and mice to tumorigenesis
- Tumor cells escaping CTL-mediated killing may downregulate MHC I expression, which renders them favorable targets for NK cells
- Tumor cells often express activating NK cell ligands, MIC-A, MIC-B, and ULB
- NK cell inhibitory receptors are favorable targets for enhancing cancer immunotherapy approaches

Regulatory immunity in cancer

Chronic inflammation is a major driver of cancer

1863 R. Virchow noted leukocytes in neoplastic tissue and made the connection between „lymphoreticular infiltrate“ and the emergence of cancer

Today we know that inflammation contributes via several mechanisms towards the development of distinct types of cancer via distinct mechanisms causing inflammatory conditions:
20% of cancers are linked to chronic inflammation

Inflammation and cancer

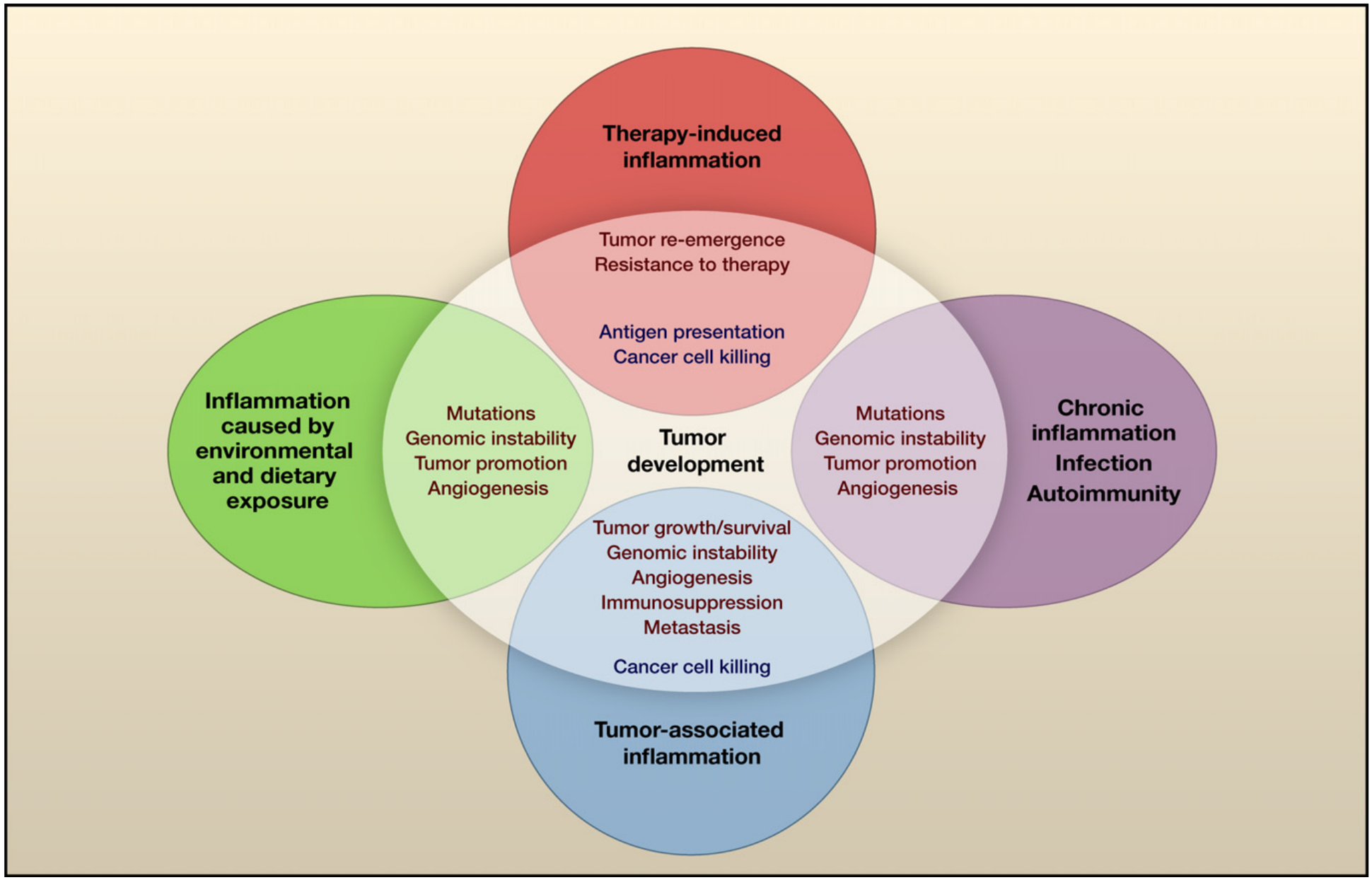


Figure 1. Types of Inflammation in Tumorigenesis and Cancer

Chronic inflammation and cancer

- Infection: Inflammation in this setting is: part of the „normal“ immune response to the infectious agents, yet tumorigenic pathogens persist leading to a chronic inflammatory state

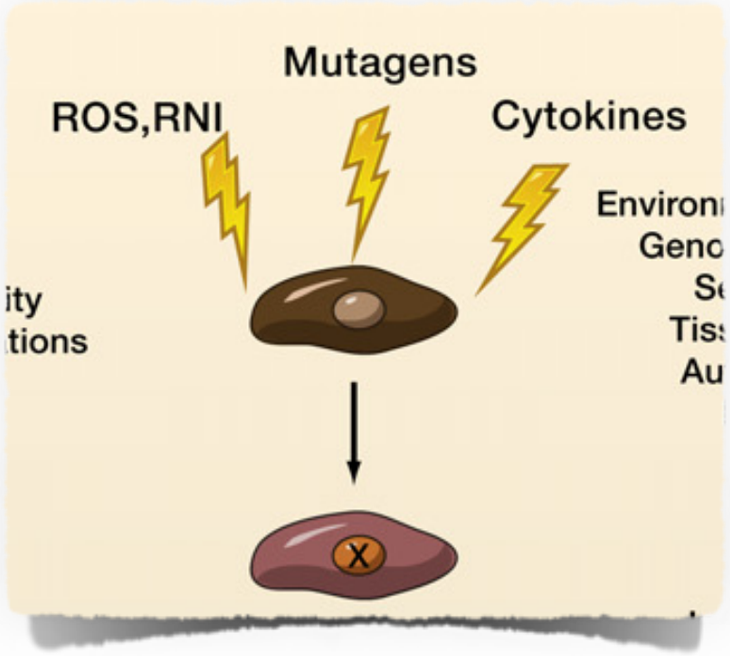
- Autoimmunity (IBD)

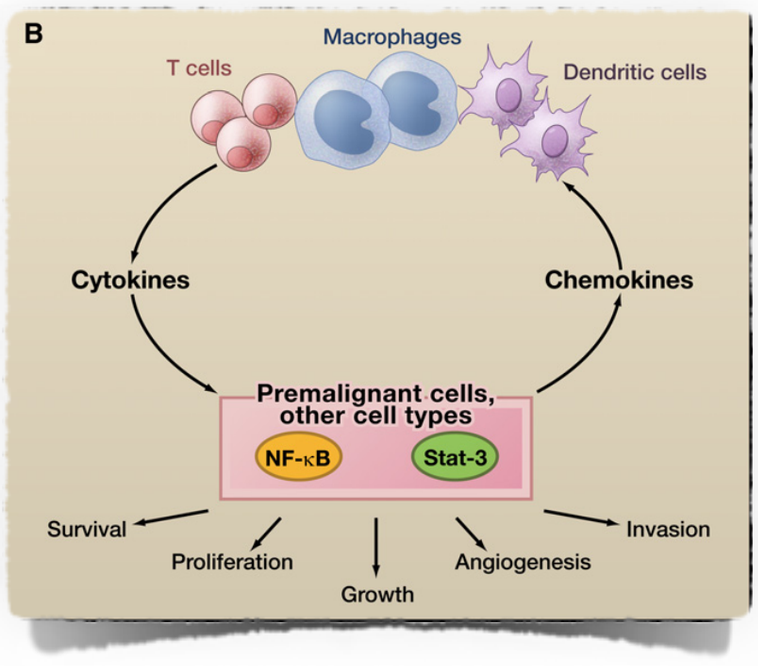
- Environmental pollutants, toxins (e.g., asbest, tobacco smoke)

Panel 1: **Some associations between inflammation and cancer risk**

Malignancy	**Inflammatory stimulus/condition**
Bladder	Schistosomiasis
Cervical	Papillomavirus
Ovarian	Pelvic inflammatory disease/talc/tissue remodelling
Gastric	*H pylori* induced gastritis
MALT lymphoma	*H pylori*
Oesophageal	Barrett’s metaplasia
Colorectal	Inflammatory bowel disease
Hepatocellular	Hepatitis virus (B and C)
Bronchial	Silica, asbestos, cigarette smoke
Mesothelioma	Asbestos
Kaposi’s sarcoma	Human herpesvirus type 8

Causes for tumorigenesis





Chronic inflammation can cause:

- mutagenesis, either directly via activated cells producing ROS and RNIs or indirectly via cytokine-mediated production of ROS in bystander cells
- promote **epigenetic** re-modeling of tumor suppressor genes or oncogenes
- trigger cytokines that stimulate **stem-cell expansion** and induce **stem-cell like phenotypes**
- **pro-tumorigenic signalling mechanisms**

Inflammation associated with cancer

Most, if not all, solid malignancies trigger an intrinsic inflammatory response that creates a protumorigenic environment

Causes:

1. Oncogens (RAS, MYC) can induce a transcriptional program, which has pro-inflammatory features
2. Oxygen and nutrient deprivation due to massive tumor growth induces necrosis and the release of pro-inflammatory factors by tumor cells (HMGB1, IL-1). This in turn provokes angiogenesis and tissue remodeling.
3. Active secretion of molecules by tumor cells

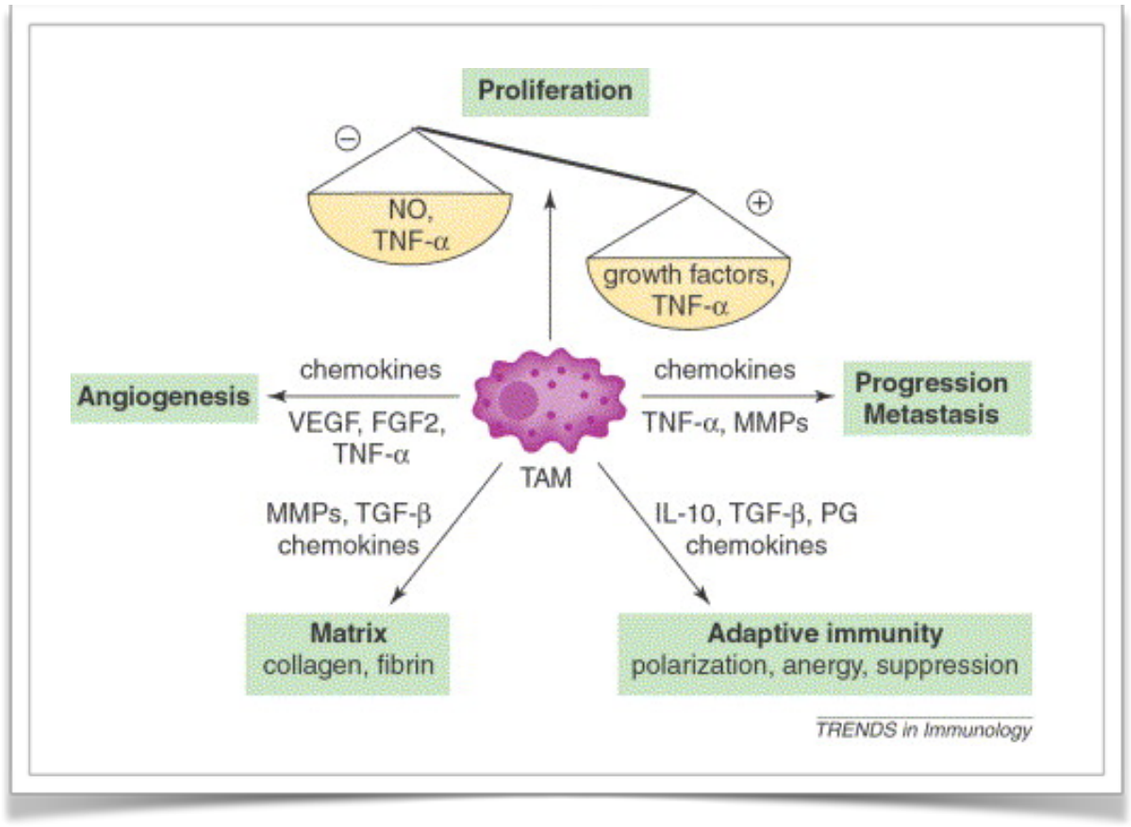
(e.g., ECM components versican TLR2 agonist)

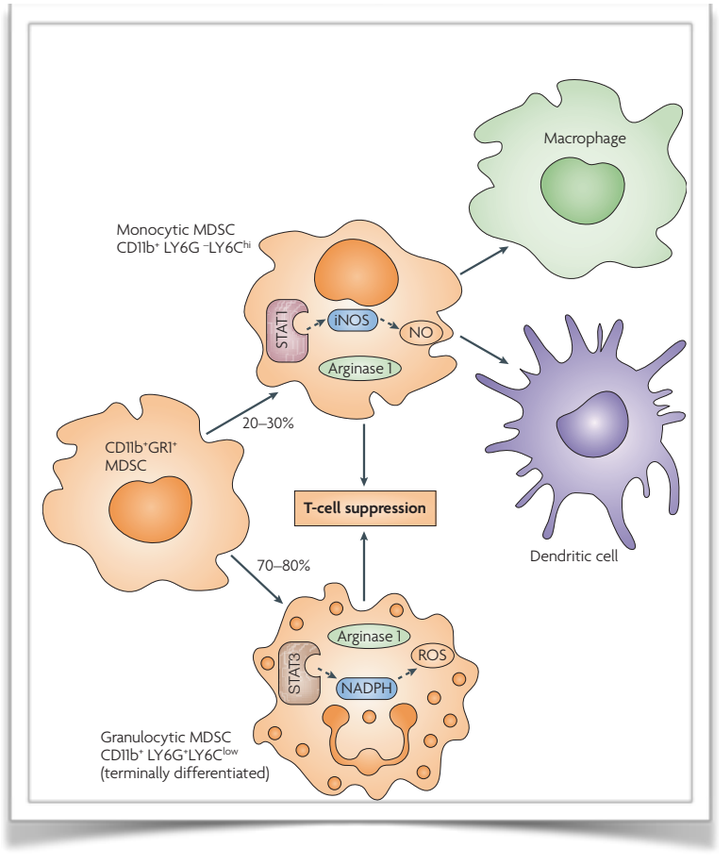
4. *Cancer therapy: radiation and chemotherapy cause massive tissue damage and cell death*

Tumors = „wounds that do not heal“

Immune cells contributing…

- TAMs: tumor associated macrophages (M2 type)
- T cells: Tregs, CD4+ TH2 cells
- Myeloid-derived suppressor cells





Anti-inflammatory drugs in cancer therapy?

Tumor-promoting inflammation can be targeted by several distinct ways

1) Inhibition of cytokine signaling pathways
   - Nf-kB inhibitors/ in combination
   - Jak2/STAT3 inhibitors
2) Sequestration of key inflammatory cytokines or chemokines
   - Cytokine inhibitors (Anti-IL6/TNFa), but effects are modest
3) Reducing/augmenting the inflammatory response after treatment
4) Depletion of immune cells that promote tumor growth

Harnessing the immune system against cancer

Immune checkpoint therapy

Adoptive T cell transfer therapy

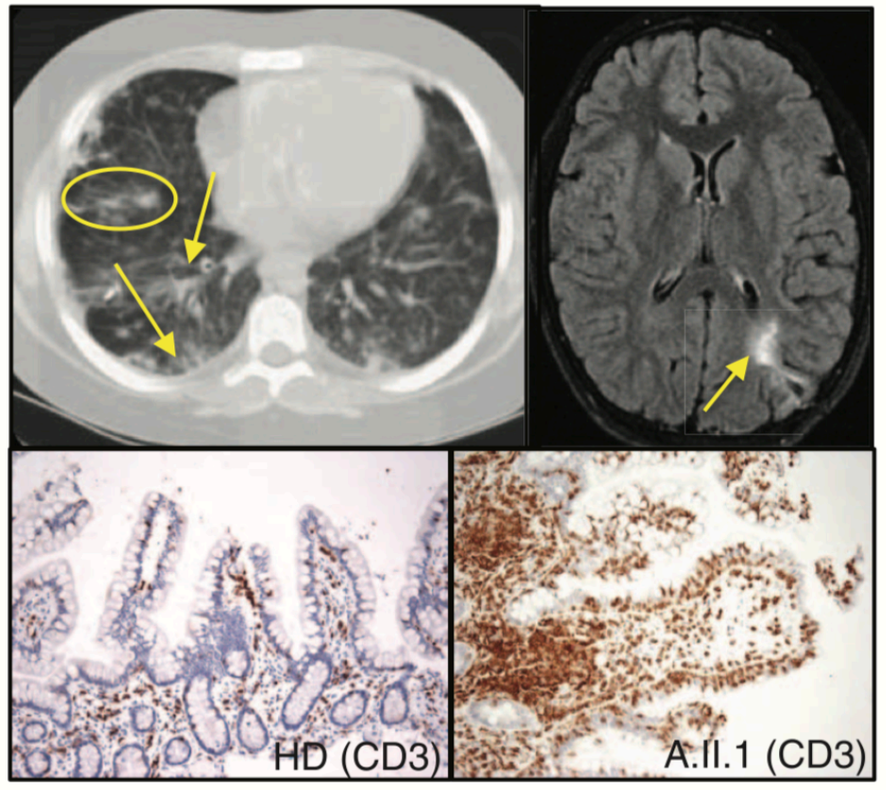
Cancer vaccines

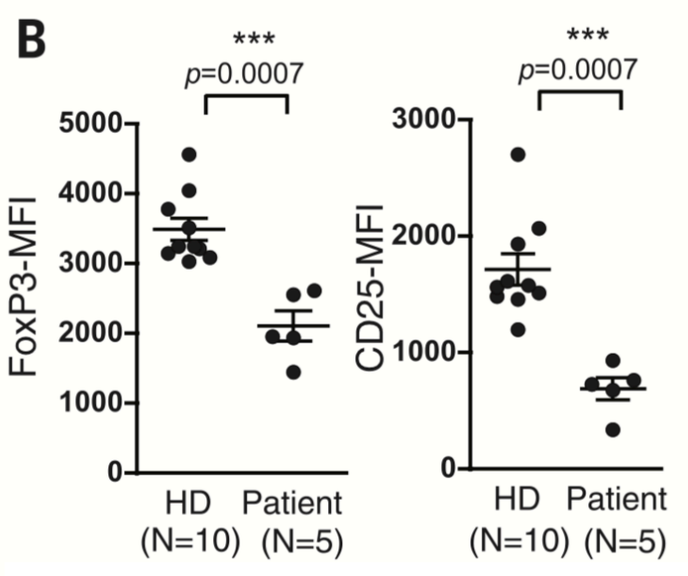
Biological functions of CTLA4

- CTLA4 is structurally related to CD28 and a member of the immunoglobulin superfamily
- CTLA4 is expressed at low basal level in conventional T cells, whereas Treg cells express CTLA4 constitutively
- Structurally, CTLA4/CD28 form membrane-bound heterodimers comprising a Ig-like extracellular domain, a TM domains, and a cytoplasmic tail domain
- The sequence similarity between CD28 and CTLA4 is highest in their extracellular binding domain - which interacts with B7 molecules. However, CTLA4 has a greater affinity and avidity than CD28 for B7 ligands

Immunoregulatory functions of CTLA4

- CTLA4 inhibits T cell activation and proliferation
- *Ctla4*-knockout mice develop T cell-mediated lymphoproliferative autoimmune disease
- In humans, haploinsufficiency of *CTLA4* lead to a severe autoimmune disease, which can be treated with abatacept (CTLA4Ig = soluble recombinant CTLA4 fused to Fc domain that competes with endogenous CD28 for its ligands)

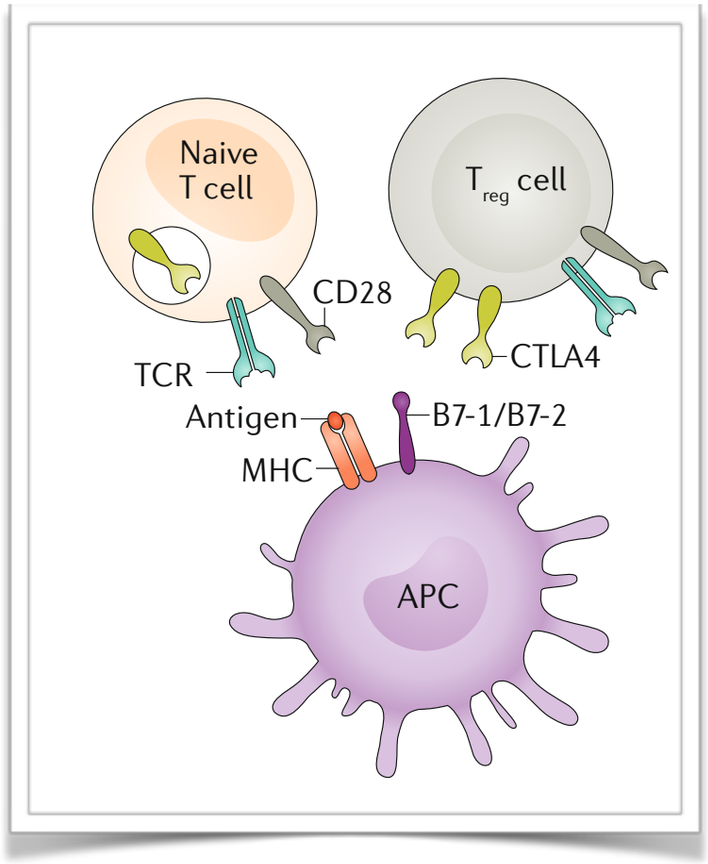




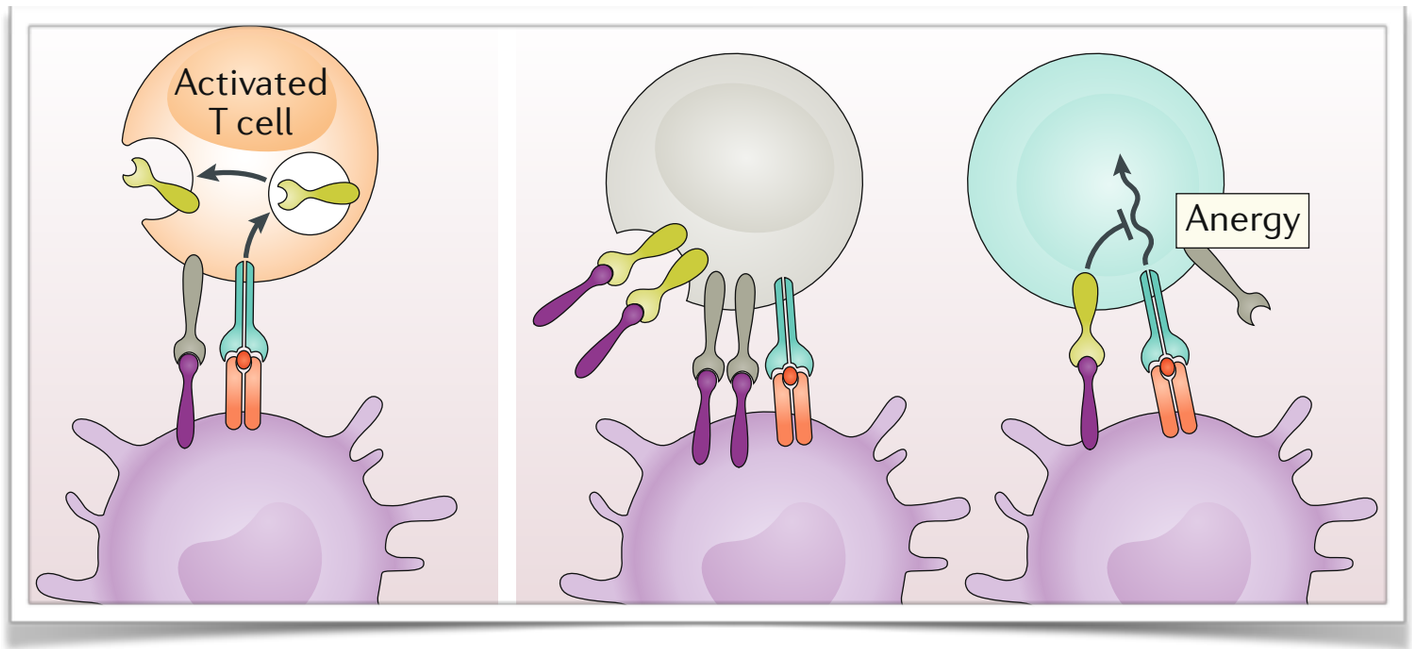
Kuehn et al., Science 2014

Mechanism of CTLA4 inhibition

- Direct antagonism with CD28
- Competing for immunostimulatory ligands (internalization of CD28)
- Recruiting inhibitory effectors (SH2 and inhibits CD3-ζ signalling)
- Preventing *immune-conjugate* formation (recruited to the immunological synapse and interferes with cytoskeleton)

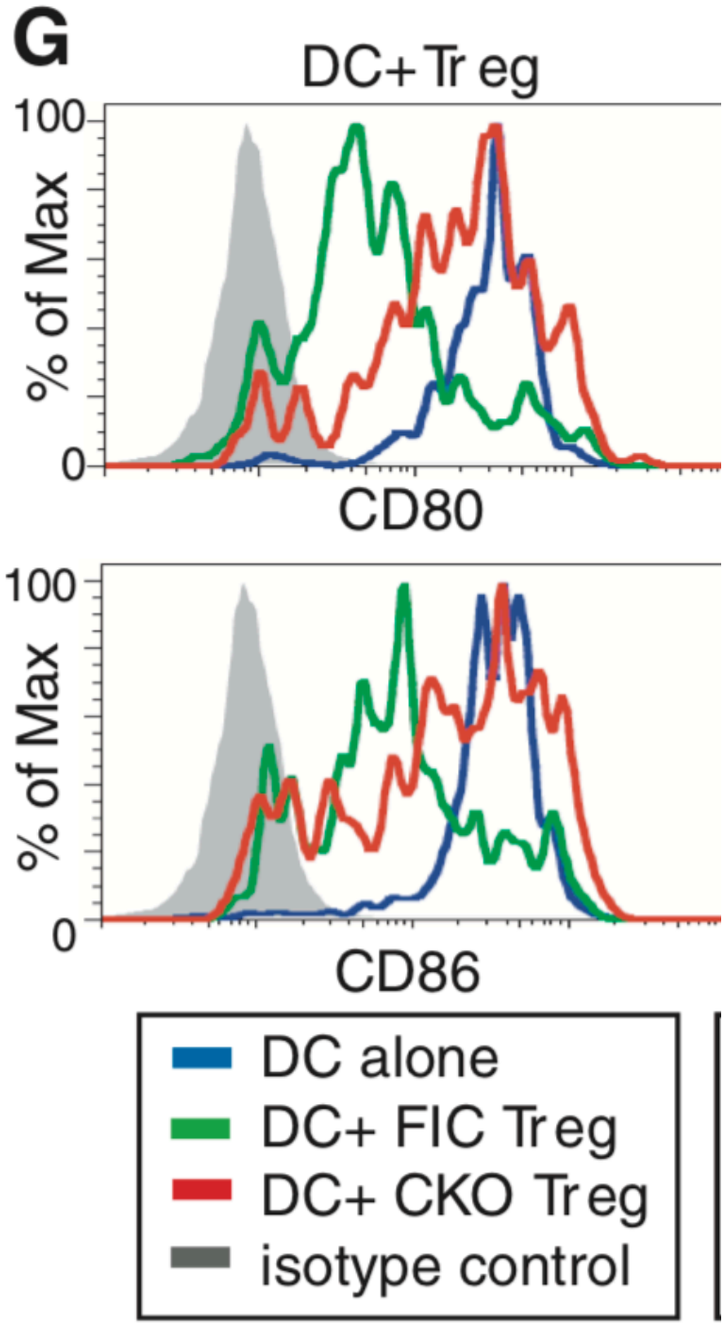


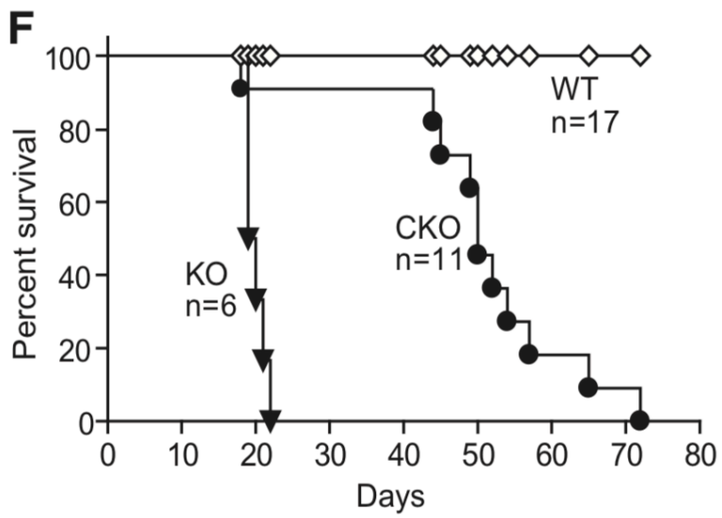
>>> Antagonism of AP-1, NF-κB, and NFAT activation
>>> Anergic reprogramming of T cells



Effect of CTLA4 on Treg cells

- Required for anti-inflammatory properties of Treg cells
- Adoptively transferred *Ctla4*+ Treg cells can revert the autoimmune phenotype induced by *Ctla4*- T cells
- CTLA4 on Treg can mediated inhibition by transcytosis of CD28

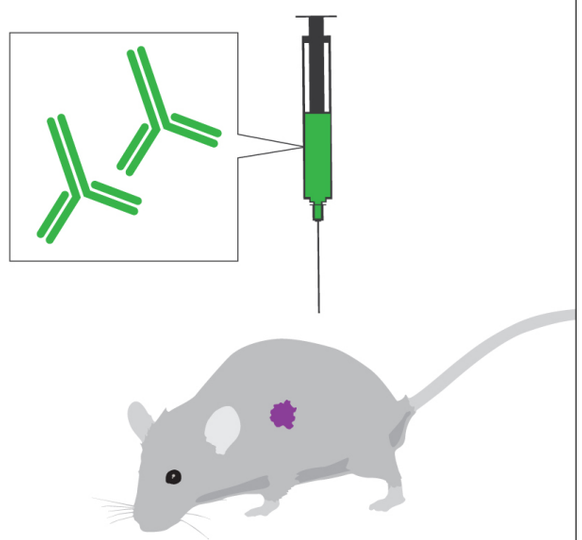


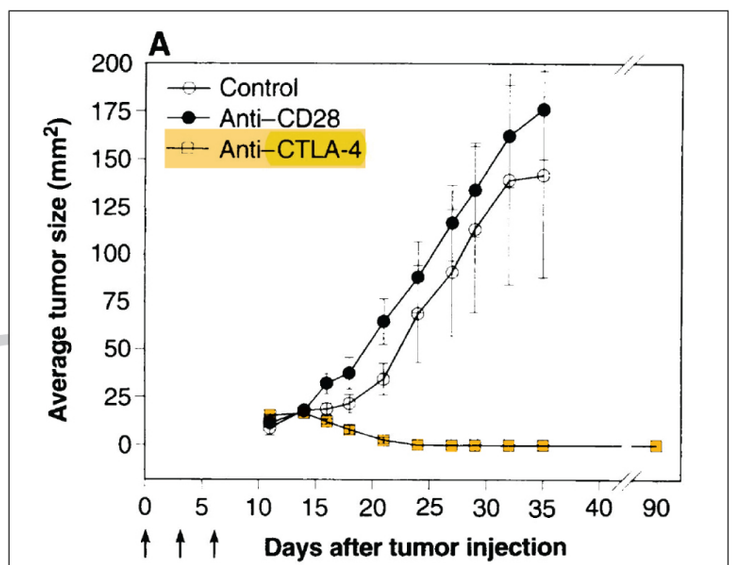


Wing et al., Science 2008

CTLA4 blockade in cancer (I)

Anti-CTLA-4 against cancer

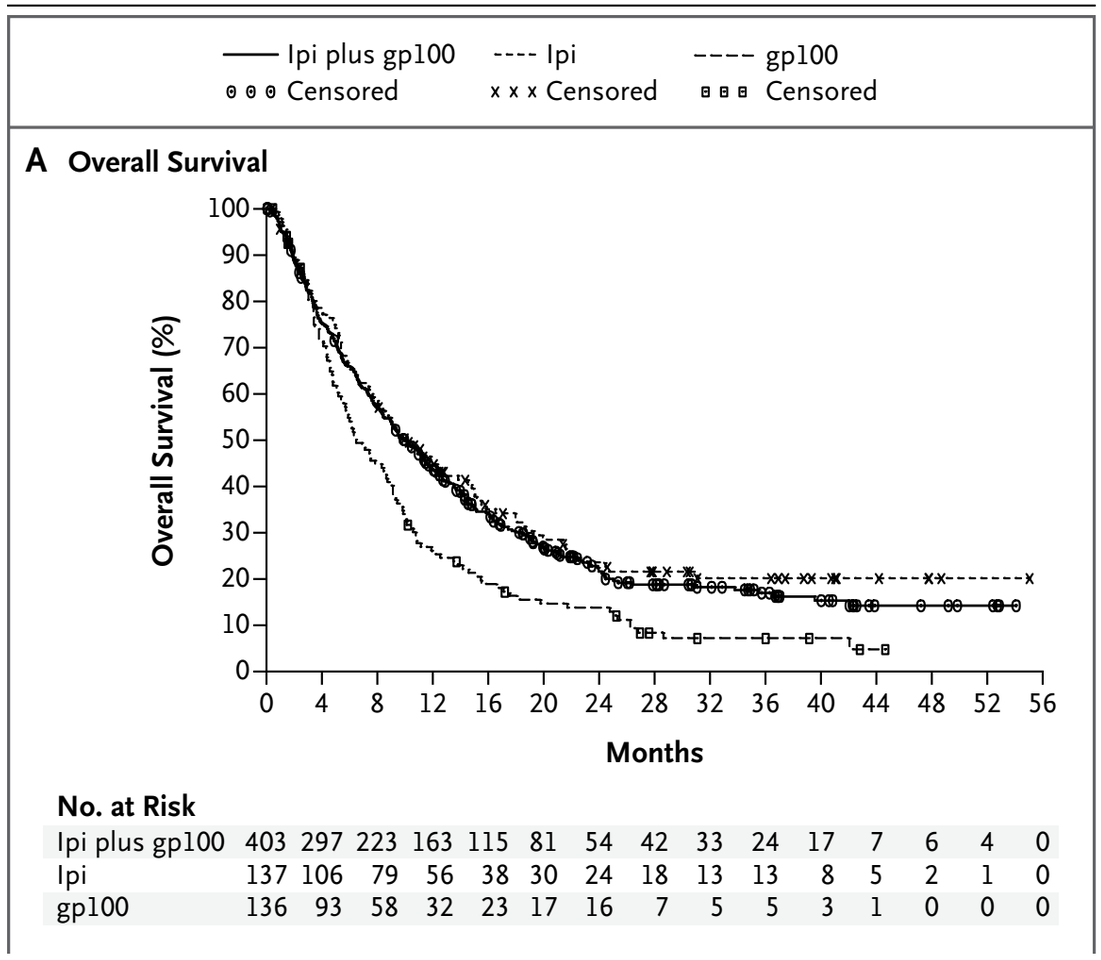




Leach et al., Science, 1996

- No requirement to understand “tumor antigen”, instead a universal strategy
- Treatment strengthened anti-tumor responses even in established tumors
- Durable response
- No favorable response in less immunogenic tumors (B16 melanoma)
- Greater tumor burden (= enhanced immunosuppressive environment) less efficacy

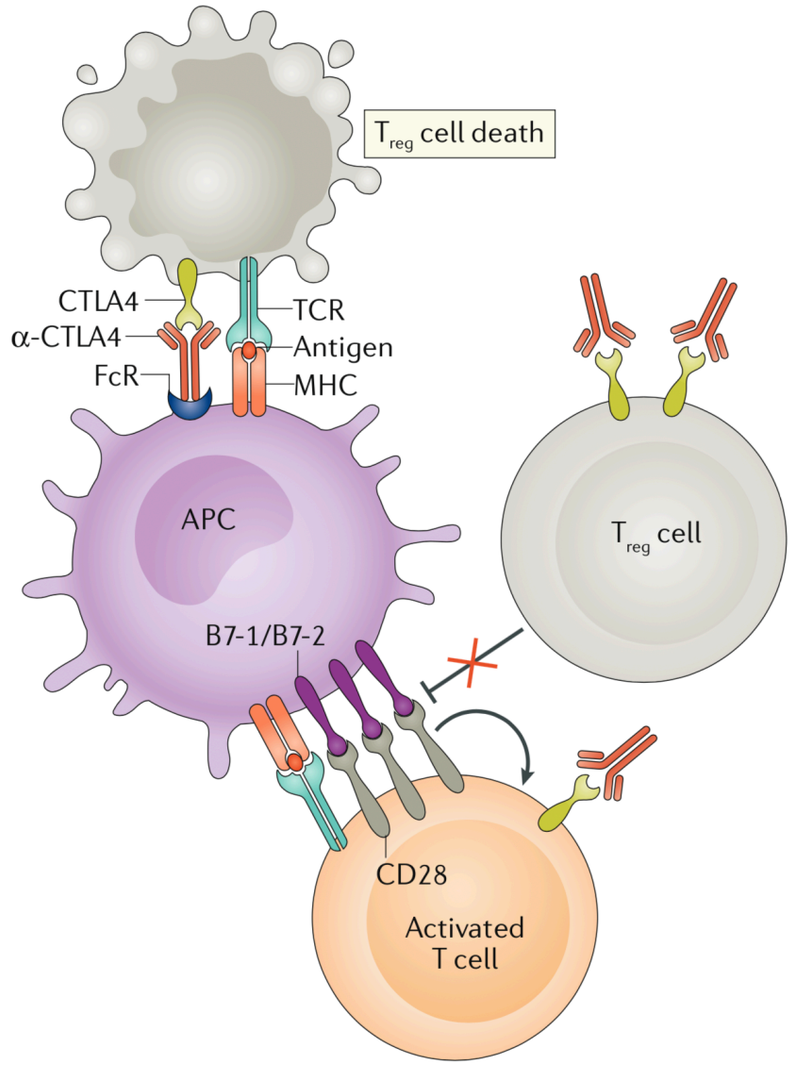
CTLA4 blockade in cancer (II)



- Clinical trial in unresectable, advanced stage melanoma
- Approval of ipilimumab in 2011
- Tremelimumab, IgG2 isotype of anti-CTLA4, awaits approval

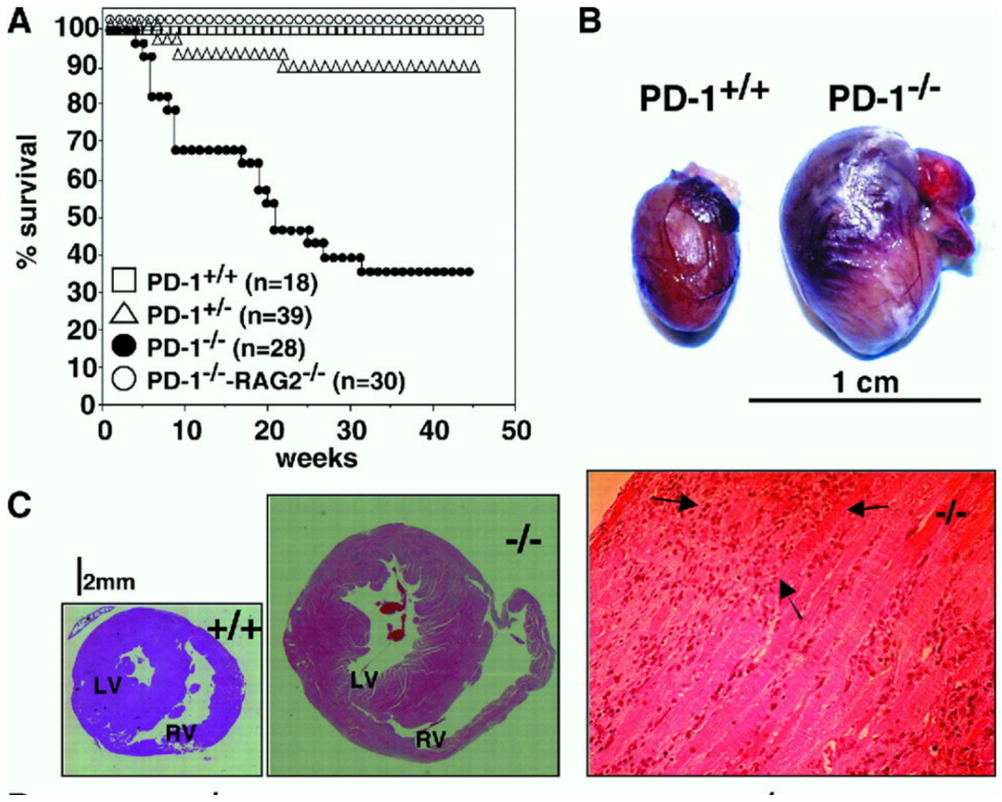
Mechanism of antitumor activity of anti-CTLA4

- Depend on T cells - as T cell depletion abolishes the effect in animal models
- Enhances polyclonal T cell responses to tumor-associated neoantigens; higher neoantigen burden portends a favorable response to anti-CTLA4 therapy
- Depletion of local, intratumoral Treg cells through ADCC
- Inhibition of Treg action in tumors
- Promoting of conventional T cell responses through blocking CTLA4 on their surface



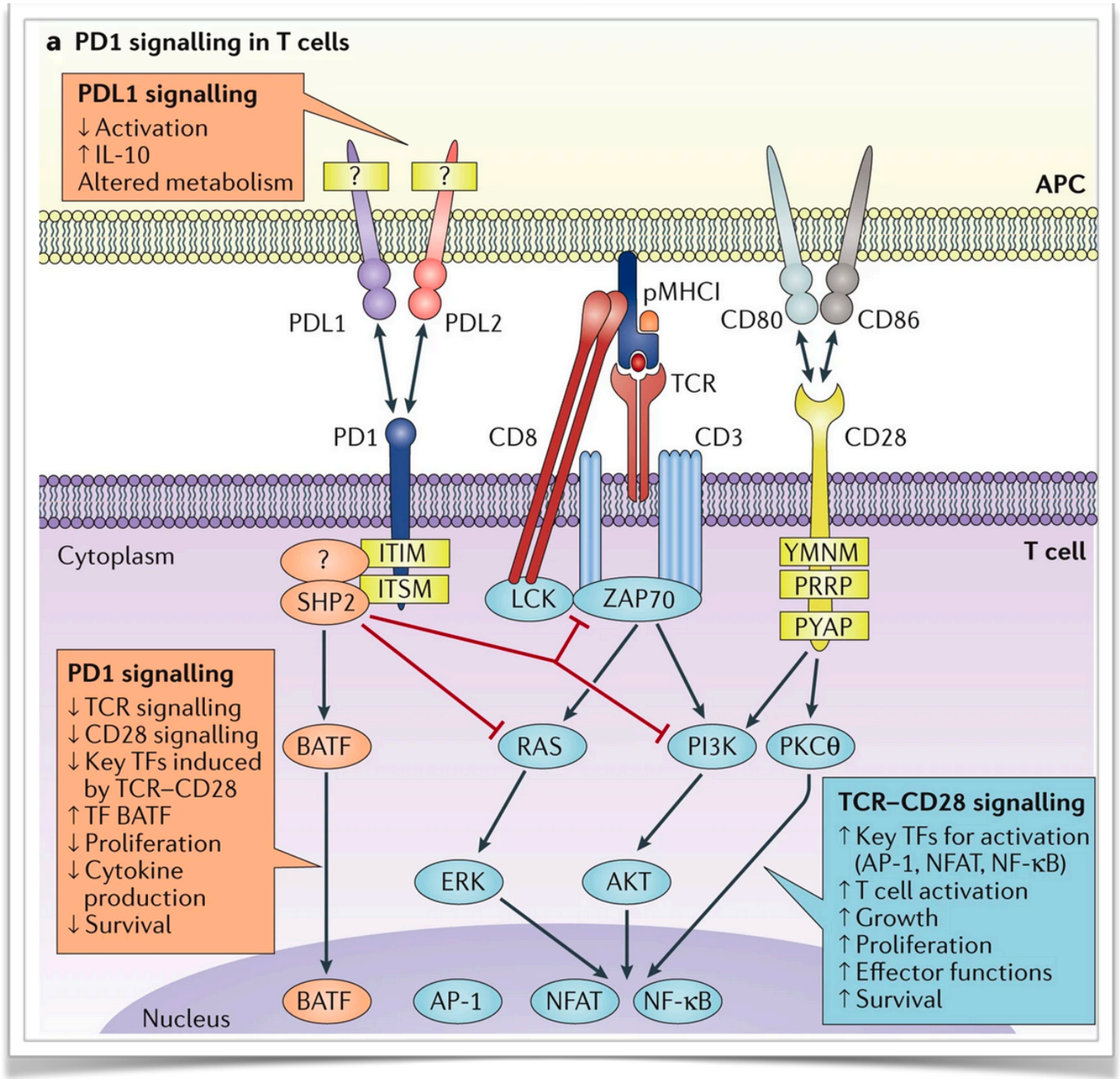
PD1/PDL1 biological function

- Type I transmembrane glycoprotein of the Ig superfamily
- PD1 is expressed on T cells after TCR stimulation and bind the B7 homologues PDL1/2
- PDL1/2 expressed on APCs and can be induced in non-hematopoietic cells through inflammatory cytokines
- Loss of PD1 in mice (*Pdcd1*) causes autoimmunity in vivo (rather late onset compared to CTLA4)
- PD1 acts later and in the periphery >>>plays a unique role in maintaining tolerance of T cells

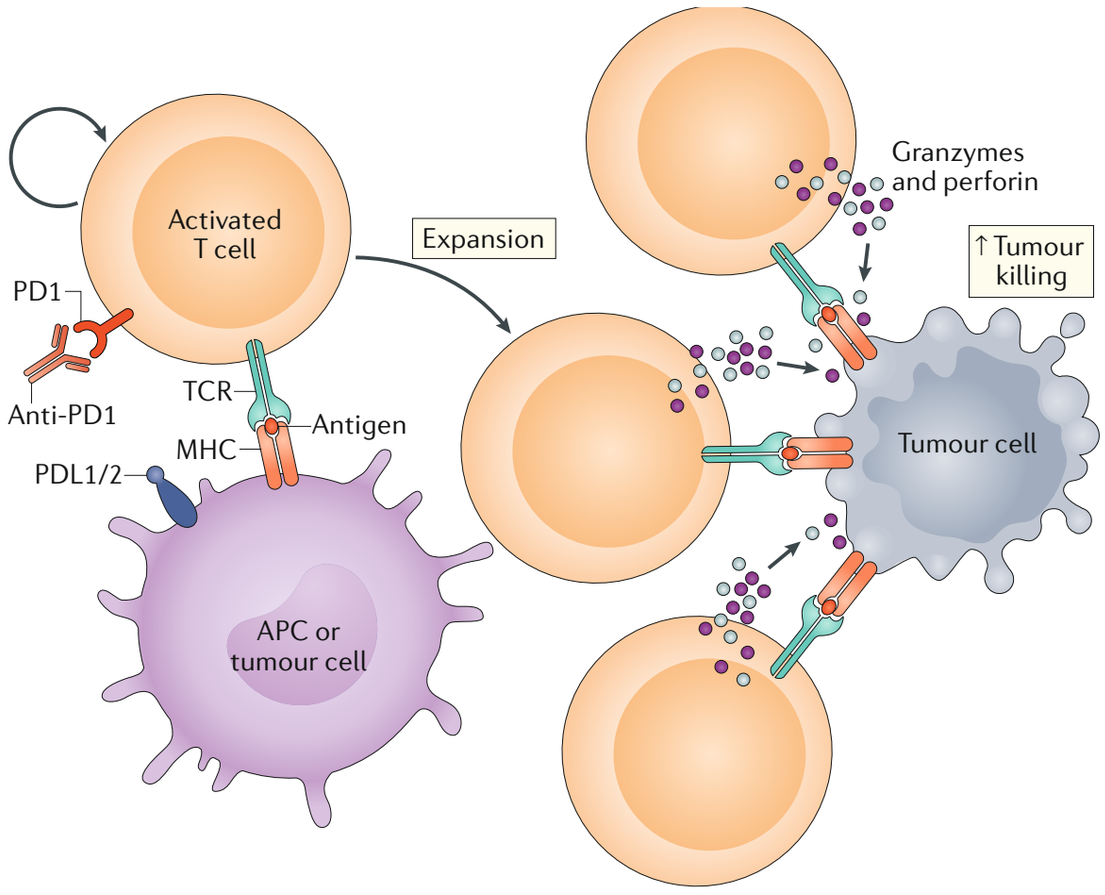


Mechanism of inhibition by PD1

- Engagement of inhibitory signaling pathways in T cells
- Can trigger T cell exhaustion
- Can promote the differentiation of T reg cells



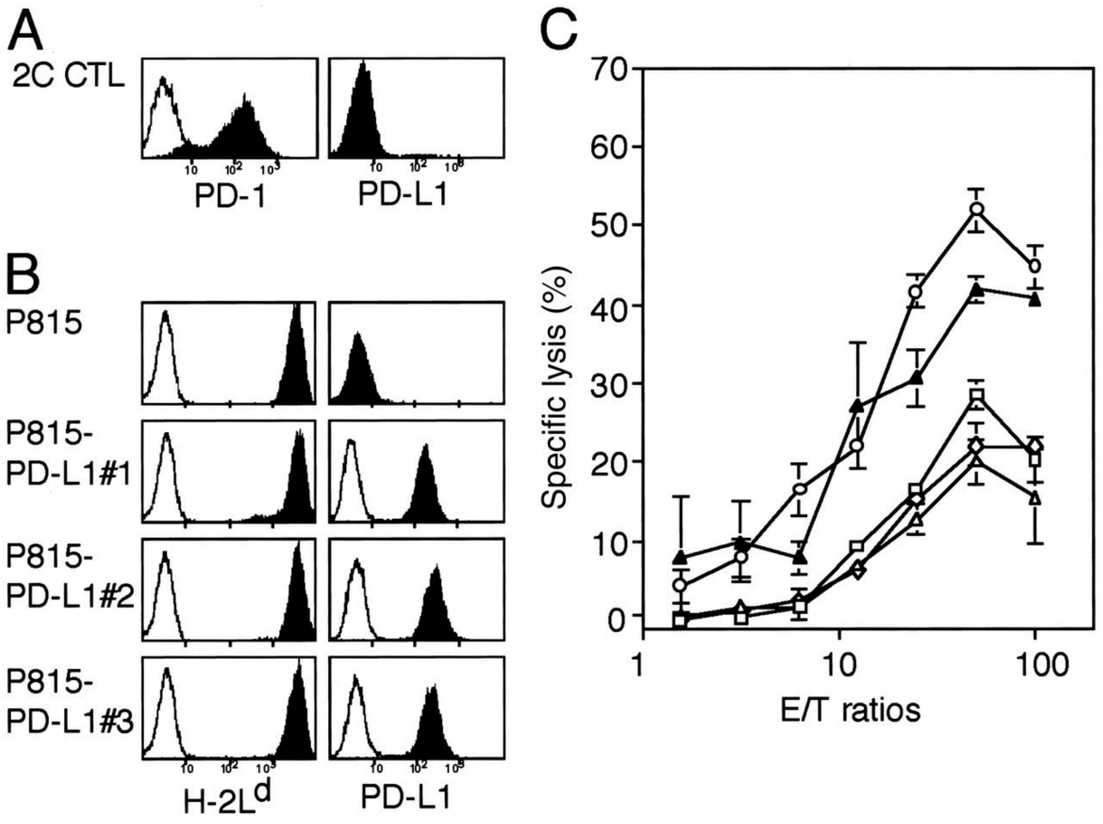
Mechanisms of PD1 axis inhibition



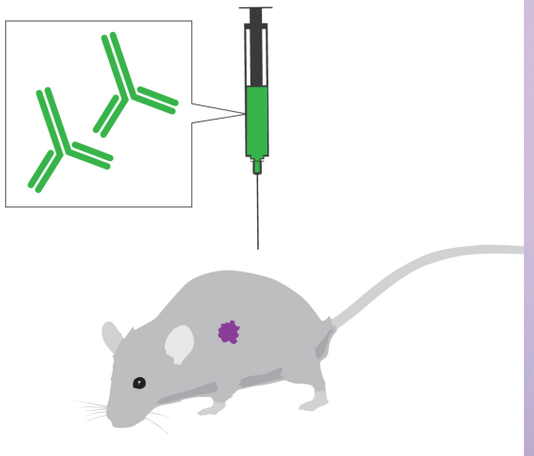
Activated T cells express PD1, which engages PDL1/2. Inhibition of this axis, promotes increased T cell activation and proliferation by enhancing effector functions and formation of memory cells.

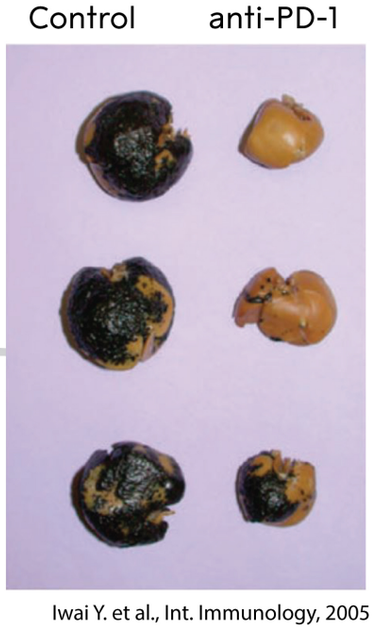
PD1/PDL1 blockade in cancer

- Expression of PD-L1 on tumor cells protects against CTL-mediated killing
- Anti-PD-1 promotes tumor cell clearance



Anti-PD-1 against cancer





Iwai Y. et al., Int. Immunology, 2005

Antitumor effect of PD1/PDL1 blockade

- PD1 blockade can also limit metastasis
- Induction of anti-tumor immune responses also against tumors that did not express PDL1.
- PD1 expression on T cells and PDL1 expression on tumor cells serves as a biomarker for poor prognosis
- In 2014 anti-PD1 pembrolizumab and nivolumab became first FDA-approved PD1-targeted therapeutics for melanoma
- In 2016, PDL1-targeted antibody (atezolizumab) was approved.
- Long-lasting immune-mediated survival benefit following PD1 blockade
- More efficient that CTL4-blockade (PD1 axis is co-opted by tumors; less severe autoimmune side effects)

Adverse effect of checkpoint blockade

- Checkpoint blockade leads to autoimmune toxicities with a therapy-specific patterns of organ involvement
- Immune-related side effects occur in 15-90% of patients. Severe side effects occur in 30% of patients on anti-CTLA4 and in 15% of patients on anti-PD1 therapy.
- Often due to loss of naive T cells and overactive memory T cells, that invade peripheral organs (GI tract, lungs).
- Treatment of side-effects is stage-specific, including discontinuation of the ICB drugs, glucocorticoids, infliximab treatment
- Nevertheless, overall toxicities due to ICB appears less than those after chemotherapy
- To address side effects, research aims at interrogating predictive markers of organ-specific cytotoxicity and local delivery of antibodies.

Adoptive T cell transfer therapy

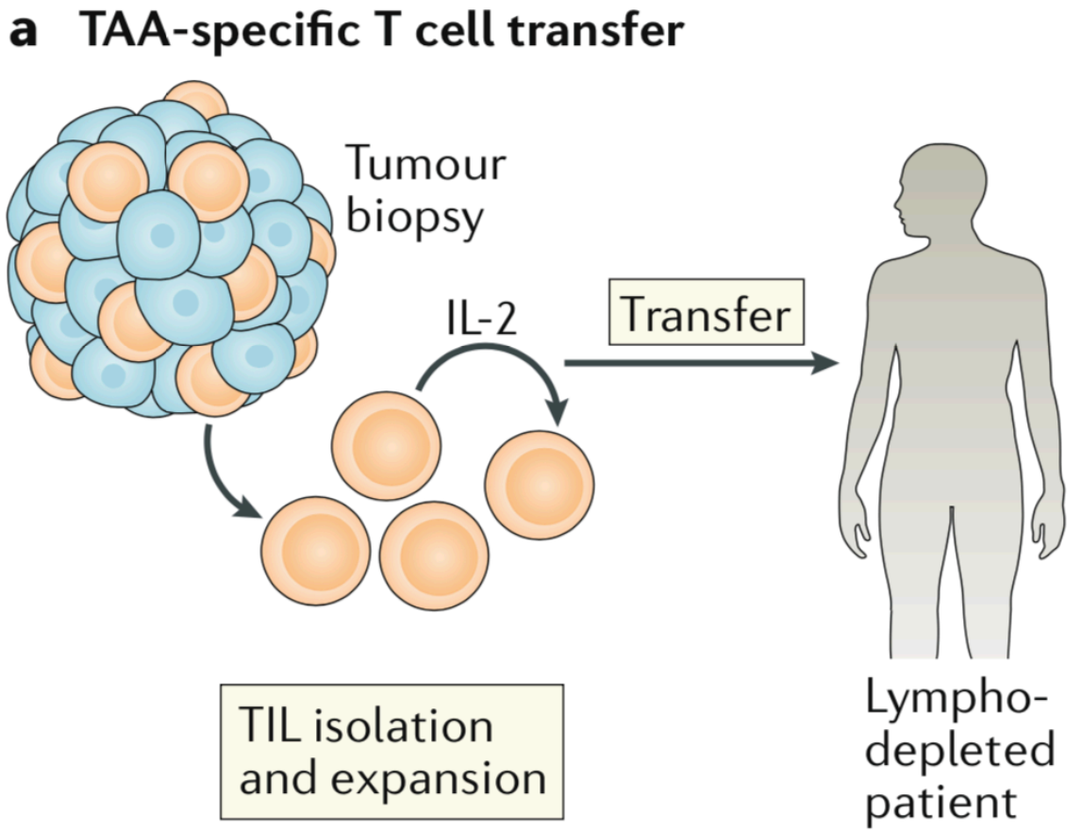
Infusion of autologous or allogenic T cells in patients with cancer

- *"…they suggest that the leukocytes (? lymphocytes) of cancer patients have a specific inhibitory effect on the growth of the cancer cells of that individual."* Southam et al., Cancer 1966

- Allogenic HSC transplantation exerts potent antitumor effects through T cell graft versus tumor responses

ATC with TILs

- Tumor-infiltrating lymphocytes (TILs) are collected in re-infused
- Durable response varies/is low
- Prerequisite for TIL-based ATC therapy is that effector T cells must be present in the tumor, which is not the case for many tumors.

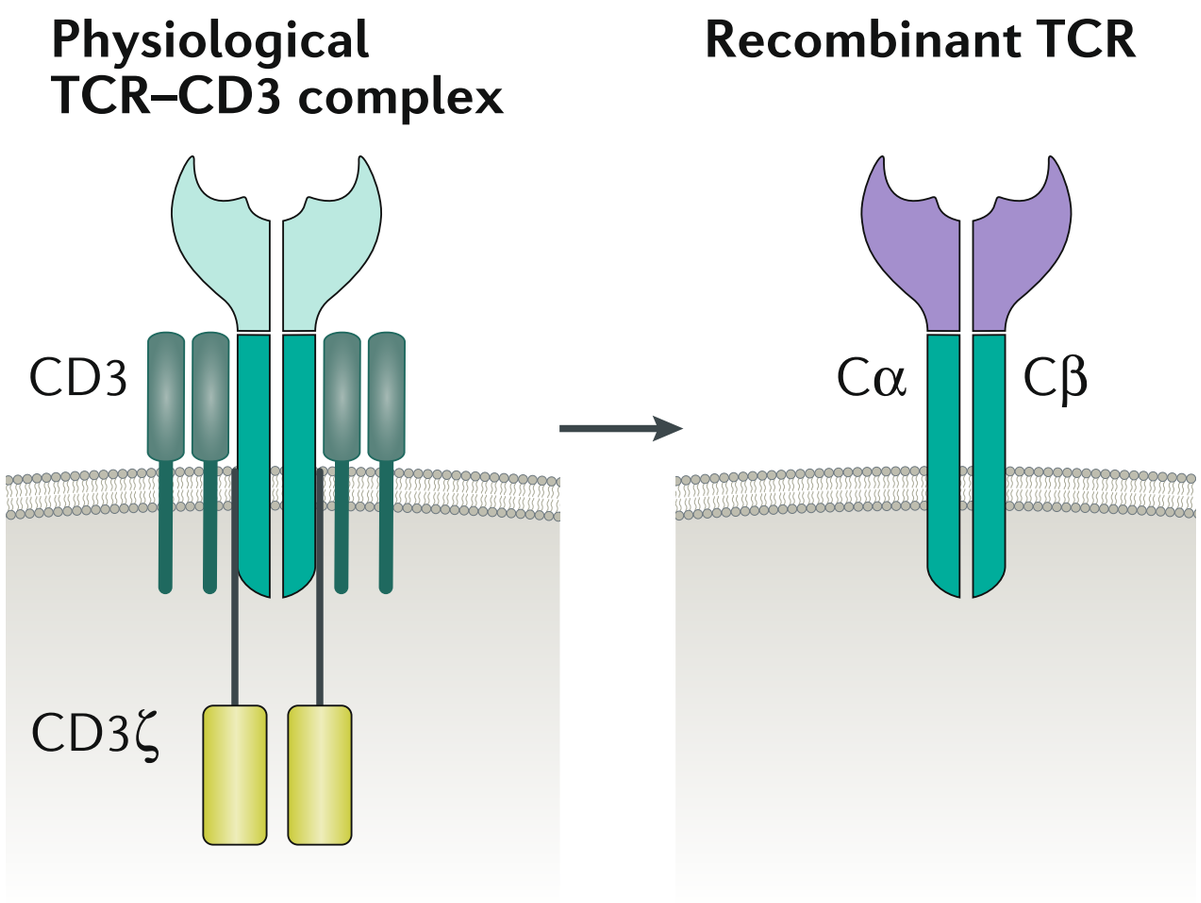


Engineering lymphocytes for ATC

- Bioengineering of the TCR α- and β-glycoprotein antigen-binding domain, while preserving the conserved domains, allows for the development and expansion of T cells with specificity to tumor antigens

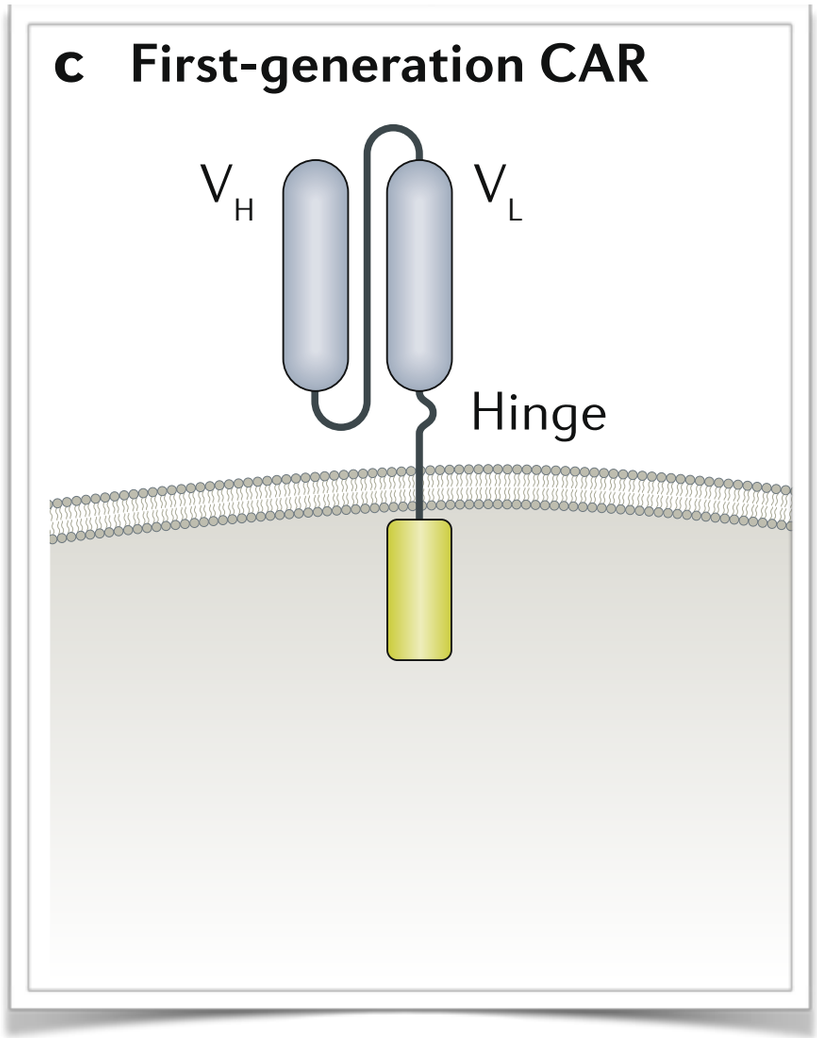
- Limited responses, however, due to MHC restriction

- Tumor cells of down regulated MHC expression



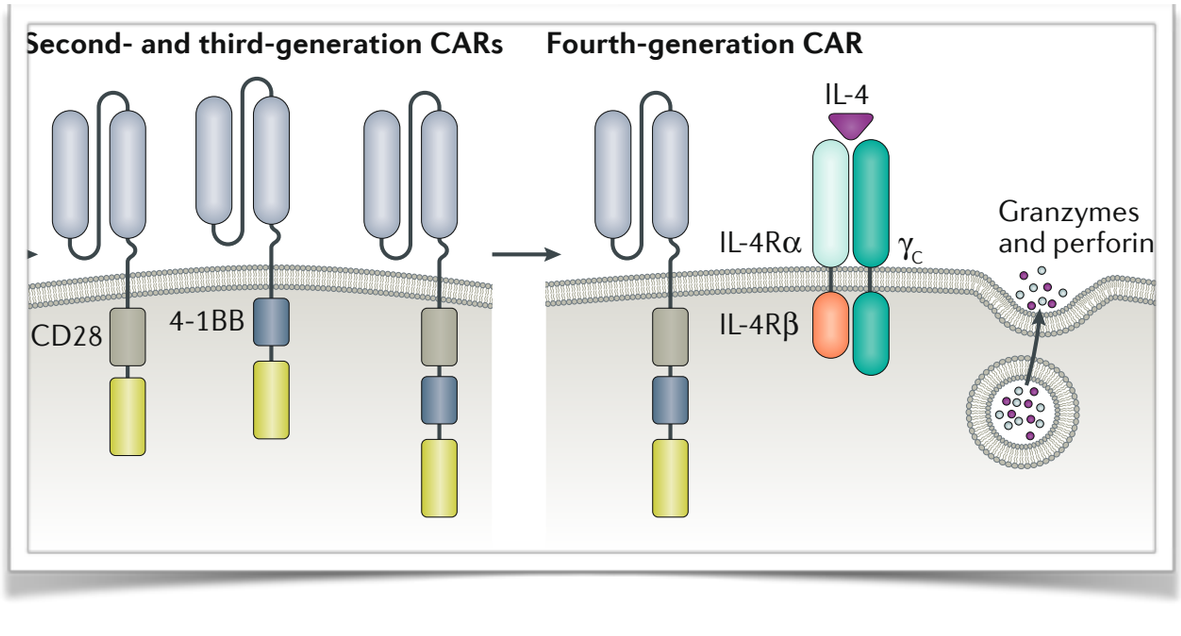
Chimeric antigen receptors (CARs)

- Antigen-binding domain (mostly from variable regions of antibodies) linked to signalling domains of the TCR (and co-stimulatory molecules)
- Isolation of T cells from the patient (or allogeneic donors), genetic modification to allow CAR expression, expansion and reperfusion



Chimeric antigen receptors (CARs)

- Next-generation CARs incorporate various signalling domains (e.g., from CD28, CD40 ligand, PD1 blocker or cytokine receptors) and are more potent to promote T cell proliferation and cytokine production in vivo.



- Powerful results in haematopoietic malignancies: First CAR cell therapy approved for a therapy targeting CD19 in patients with diffuse B cell lymphoma, B cell acute lymphoblastic leukaemia , and chronic lymphocytic leukemia (e.g., for ALL trials showed 83% complete remission in 29-months follow-up).

Limitations/adverse effects

- Only modestly successful for solid tumors
- Quest for ideal “target” with high on-tumor and low off-tumor, on-target cytotoxicity
- Side effects: cytokine release syndrome (CRS) and neurotoxicity ranging from mild symptoms to severe/detrimental outcomes
- Research aims at designing CARs with reduced affinity receptors or with additional safeguards, such as transient expression (e.g., suicide cassettes).
- Broad application is limited by high costs (~ 400.000 USD/treatment (ICB: 12.000 USD/months)), limited accessibility to treatment centres, and variability in the generation of effective CARs

Cancer vaccines

- Cancer vaccines activate the immune to respond to cancer
- *Prophylactic vaccines* prevent infection by oncogenic viruses (e.g., hepatitis B or papillomavirus agains hepatocellular carcinoma and cervical cancer, respectively.)
- *Therapeutic vaccines* aim at harnessing the immune system to already existing neoplastic cells (First example, BCG as a primitive therapeutic vaccine in bladder cancer)
- Vaccines to elicit tumor-specific responses require an antigen or multiple antigens that act as target, appropriate formulation (e.g., peptide, RNA or DNA vaccine), and an adjuvant

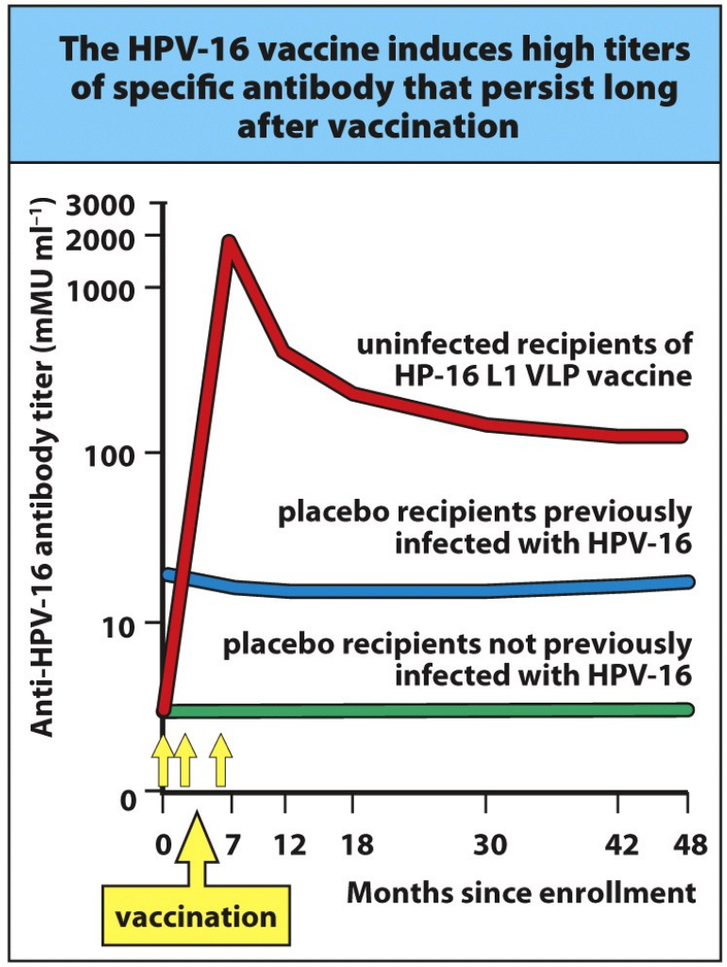
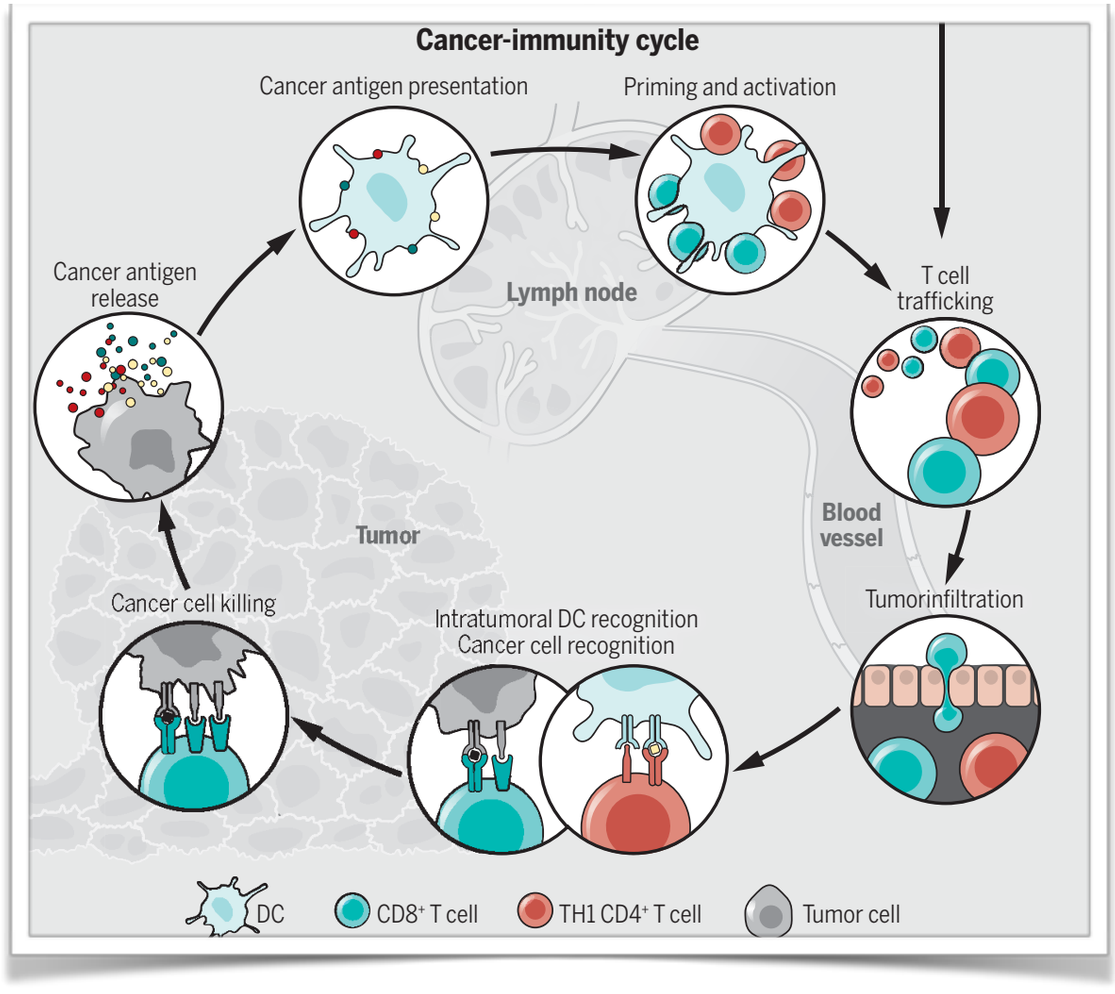


Figure 16.20 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

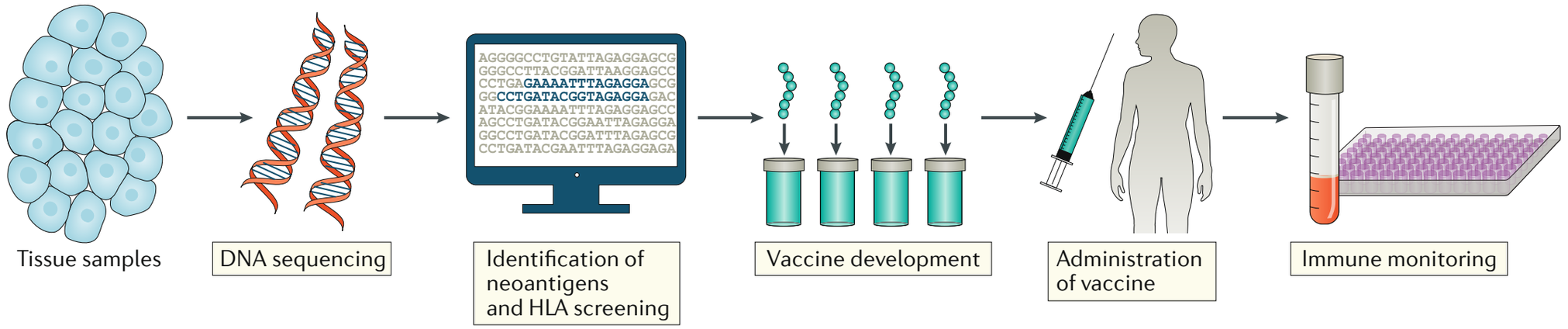
Autologous tumor vaccines

- Early approaches used patient-derived cancer cells together with an adjuvant or virus to induce polyclonal T cell approaches

- A key limitation is to obtain patient-derived tumor cells for certain tumors



Personalized cancer vaccines



- Tumors are collected and DNA sequencing allows the identification of neoantigens, which are then assessed by an algorithm to predict those antigens best suited to be presented by the patient's MHC molecules

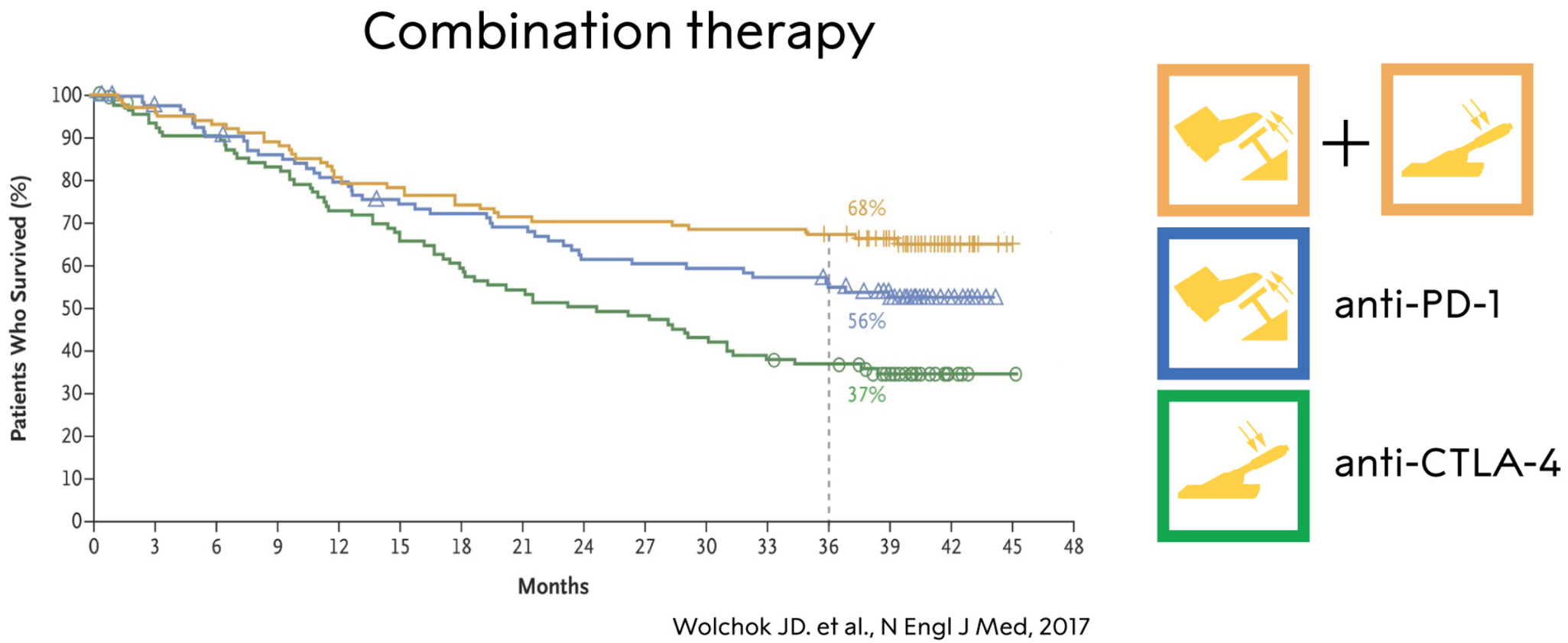
- A mixture of most suitable neoantigens is delivered as synthetic peptides, mRNAs, or DNA

- Immune monitoring and cancer monitoring assess therapy efficacy

Pitfalls and challenges

- Prediction of neoantigen binding to more diverse MHC class II molecules remains challenging
- Costs
- Time of personalized vaccine generation (currently ~ 4 months)

Combination therapy



Wolchok JD. et al., N Engl J Med, 2017

- Combination with radiation therapy can boost responses in conjunction with checkpoint blockade for many tumors

New targets for checkpoint blockade

- New negative regulators of T cell activation
- Stimulation of T cell co-stimulatory receptors, including ICOS, TNFRSF4/9

