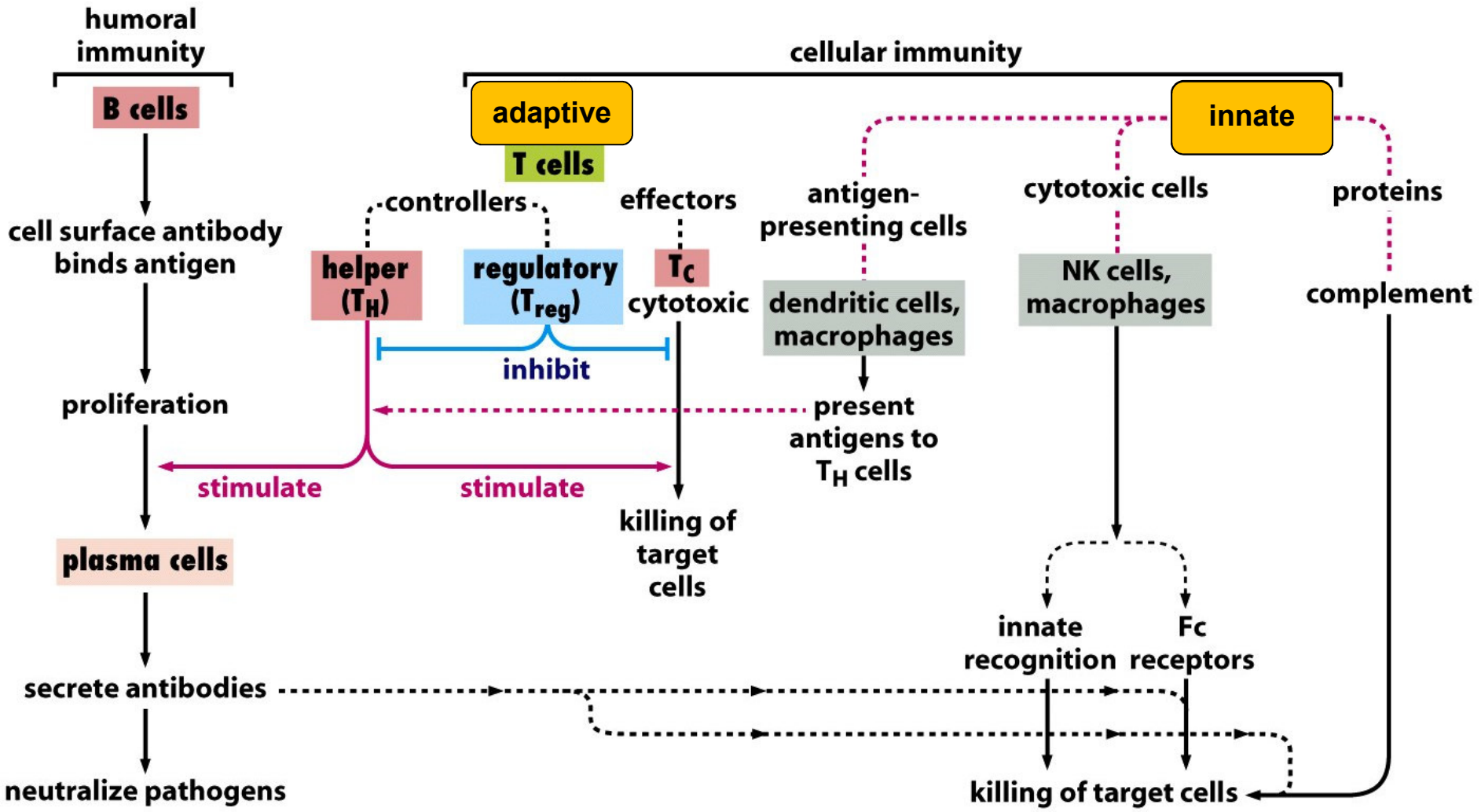
# Adaptive immunity and immunotherapy

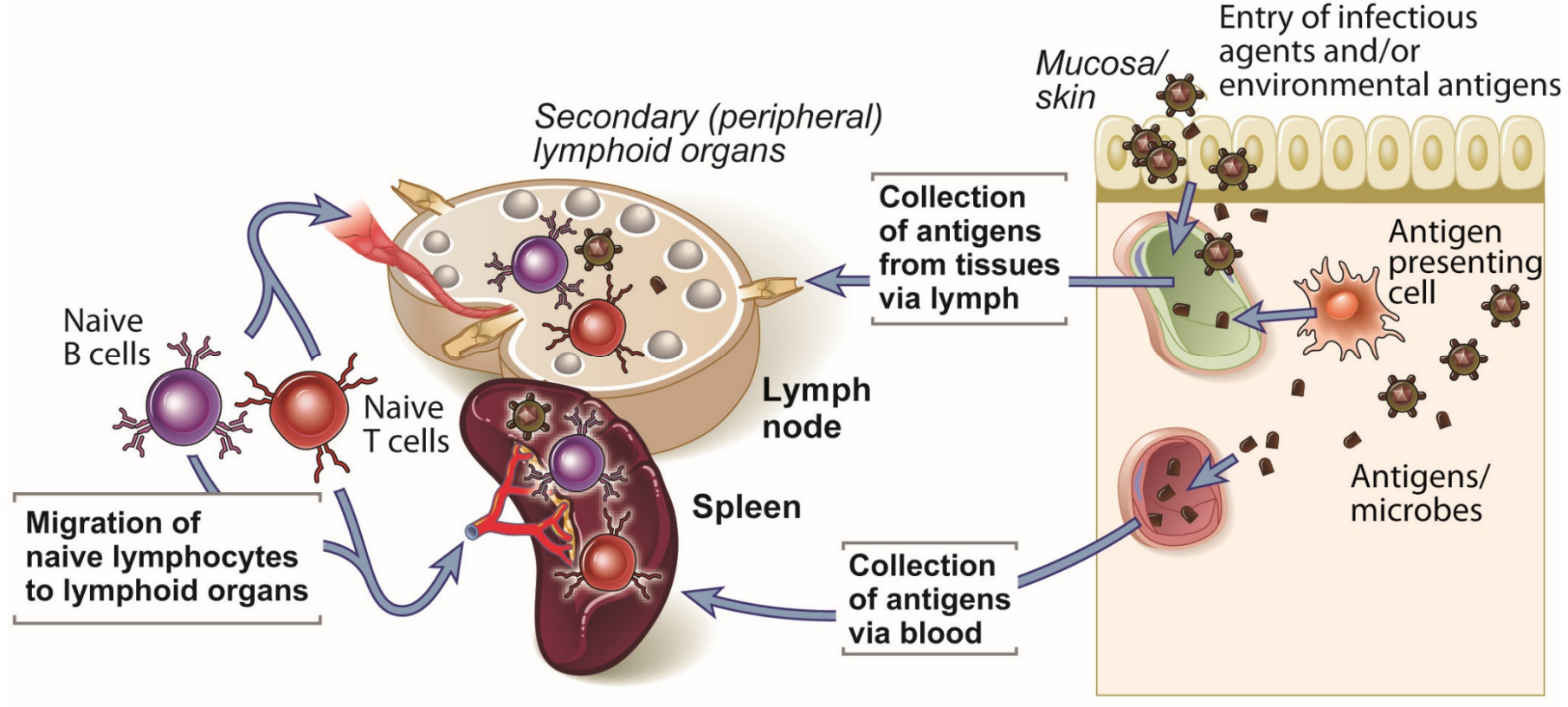
## Learning objectives

- Basic mechanisms of immune activation and regulation
- Immune surveillance and escape mechanisms
- Cellular and molecular components of tolerance and immune suppression
- Types of immune therapies and differences to classical therapies
- Prospects of immune engineering

# Summary: regulatory network of the immune system



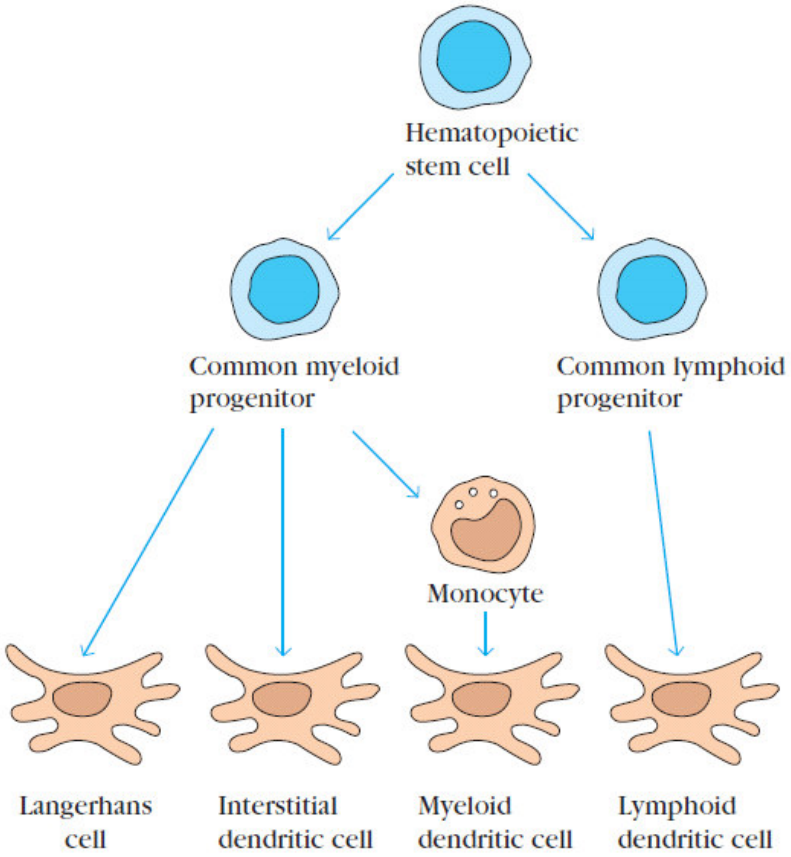
# Secondary lymphoid organs for maturation of naïve B and T cells




- professional antigen presenting cells (**APC**) collect/process antigens and then migrate to secondary lymphoid organs to present them via MHCII and/or MHCI to naïve CD4 and/or CD8 T cells in the spleen (blood-borne antigens) or the lymph node (tissue antigens)
- APCs link responses of the innate immune system to the adaptive immune system
- APCs are: **dendritic cells** (**DC**), **phagocytic macrophages** (**MΘ**) or **B cells**
- these cells are either tissue-resident (DC) or are recruited and activated (**IFNγ**) during inflammation (MΘ) and after antigen capture migrate into secondary lymphoid organs

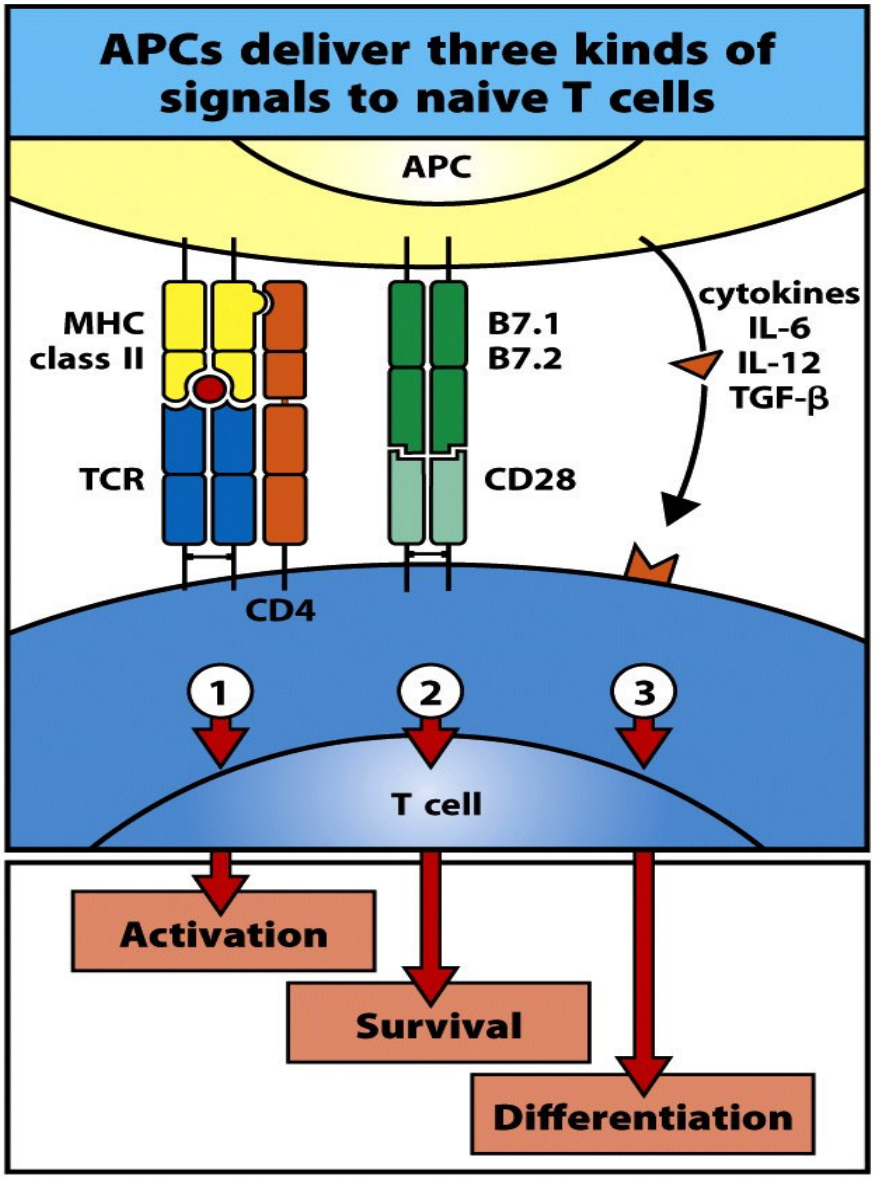
# Phenotypic alterations during activation of dendritic cells

| | Immature dendritic cell | Mature dendritic cell |
|---|---|---|
| Principal function | Antigen capture | Antigen presentation to T cells |
| Expression of Fc receptors, mannose receptors | ++ | – |
| Expression of molecules involved in T cell activation: B7, ICAM-1, IL-12 | – or low | ++ |
| Class II MHC molecules Half-life | ~10 hr | >100 hr |
| Number of surface molecules | ~$10^6$ | ~$7 \times 10^6$ |




- Fc receptors (antibody binding) and mannose receptors (bind bacteria) allow antigen collection
- APCs travel after antigen capture to present to T cells in the lymph node or in the spleen

# Safety first:
# Co-stimulatory signals are required to activate naïve T cells

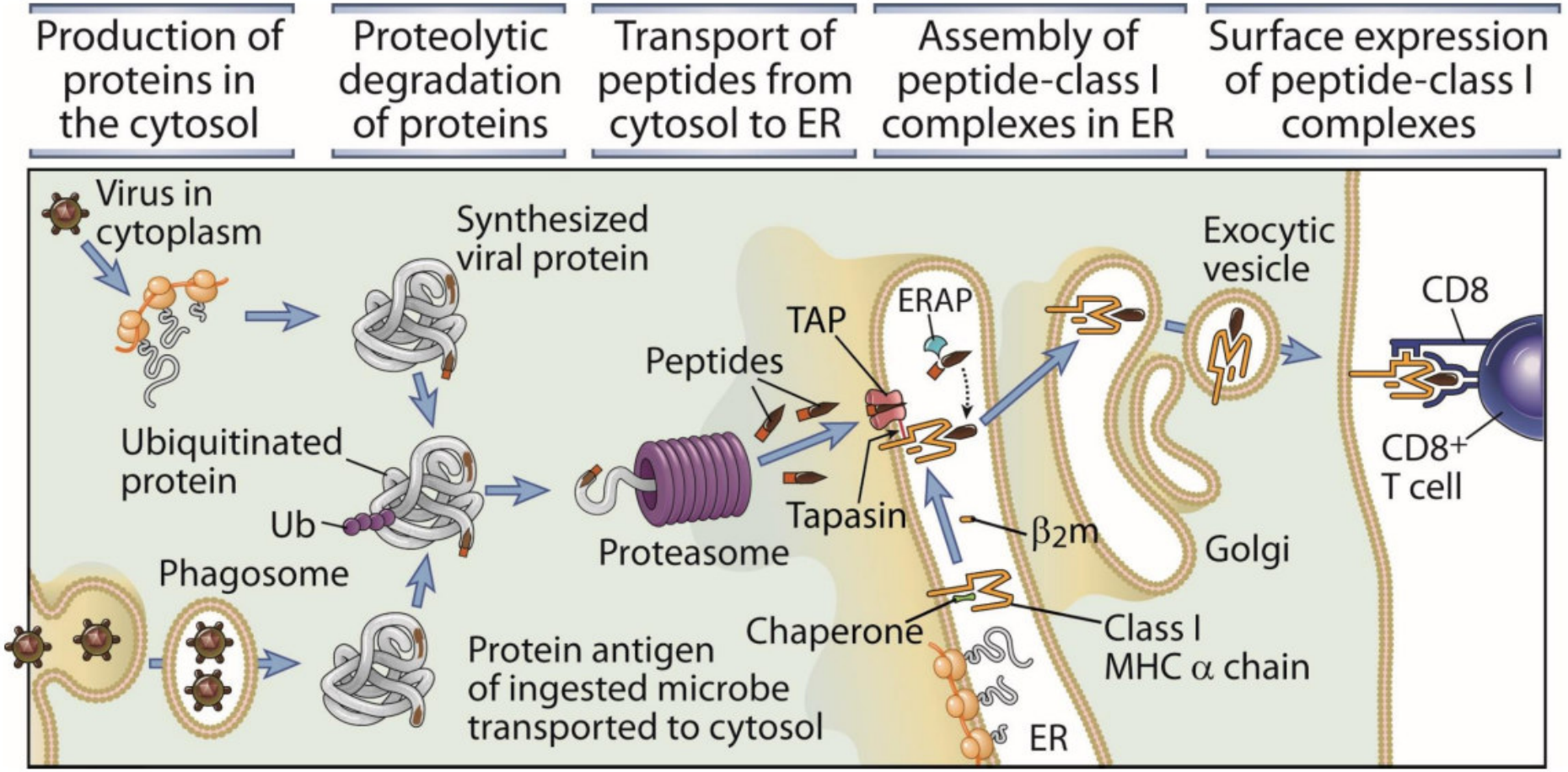



activation of **naïve T cells** (T cells which have not encountered their antigenic peptide before) by APCs typically requires **3 signals**:

1) **TCR and MHC** interaction
   => activation of CD3 complex
2) interaction of co-stimulatory (**CD28**, **CD137=4-1BB, CD134=OX40R** (only late)**, ICOS**) receptors on the T-cell and surface molecules on the APC (**CD80/86** (=**B7.1/B7.2**)**, CD137L, OX40L, CD275**)
   => survival signaling
3) activating cytokines from APC
   => differentiation
4) APC also provide amino acids essential for T cells (arginine, cysteine)

this is relevant for naïve CD8 CTLs responding to MHC I presentation and naïve CD4 $T_H$ cells responding to MHC II

# MHC loading



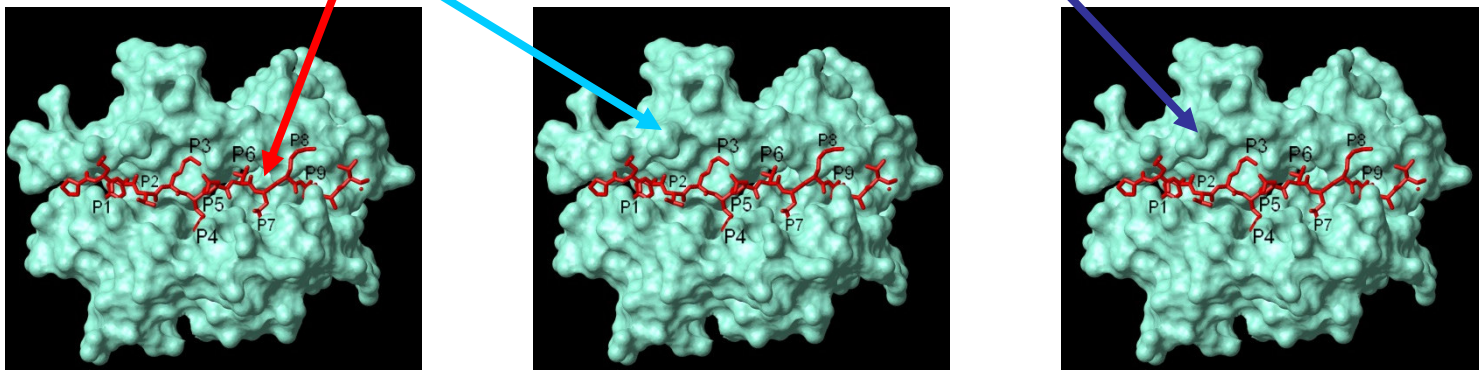

- proteasome degradation generates small peptides of self or foreign proteins
- TAP (transporter associated with antigen processing) transports these peptides into the ER, where they are loaded onto the nascent MHC-I complex
- only loaded MHC complexes are transported via the Golgi to the cell surface

# Peptide/HLA restriction

…..LTLAKHTI**SSDYVIPIGTY**GQMK……EKCDICTDEYMGGQHPTN….

TLEGFASPLTGIADASQSSMHNALHIYMNGTMSQVQGSAND……

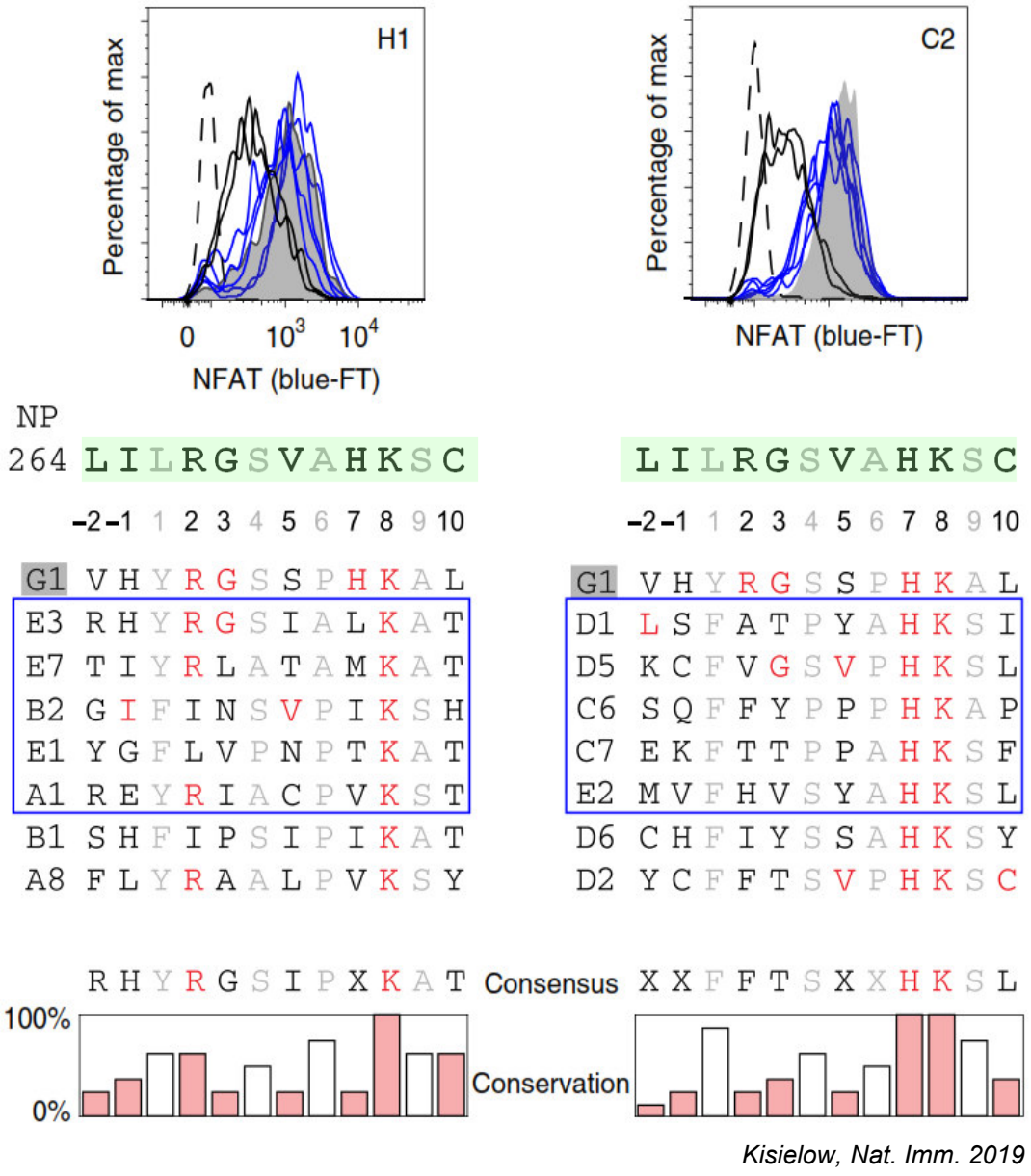
…VLTALLAGLVSLLCRHKRK…




HLA-A1 HLA-A2 HLA-A3

each individual has up to **six haplotypes** (three genes, two alleles each from mother and father) **of HLA,** more than **35,000** alleles have been discovered for the HLA system, and it is estimated that the total variation in the whole human population could reach several million alleles

# Specificity of TCR/MHC interaction

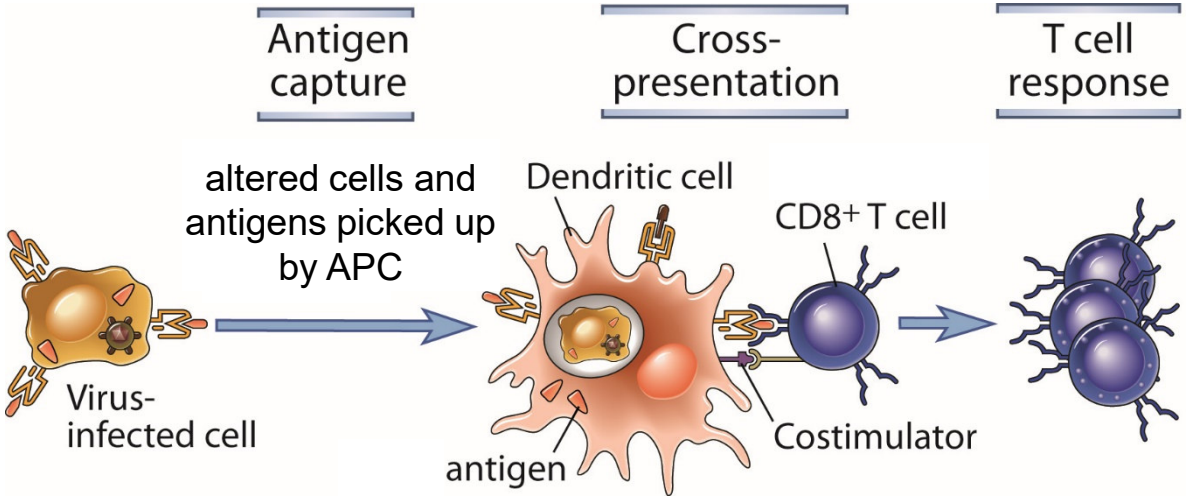


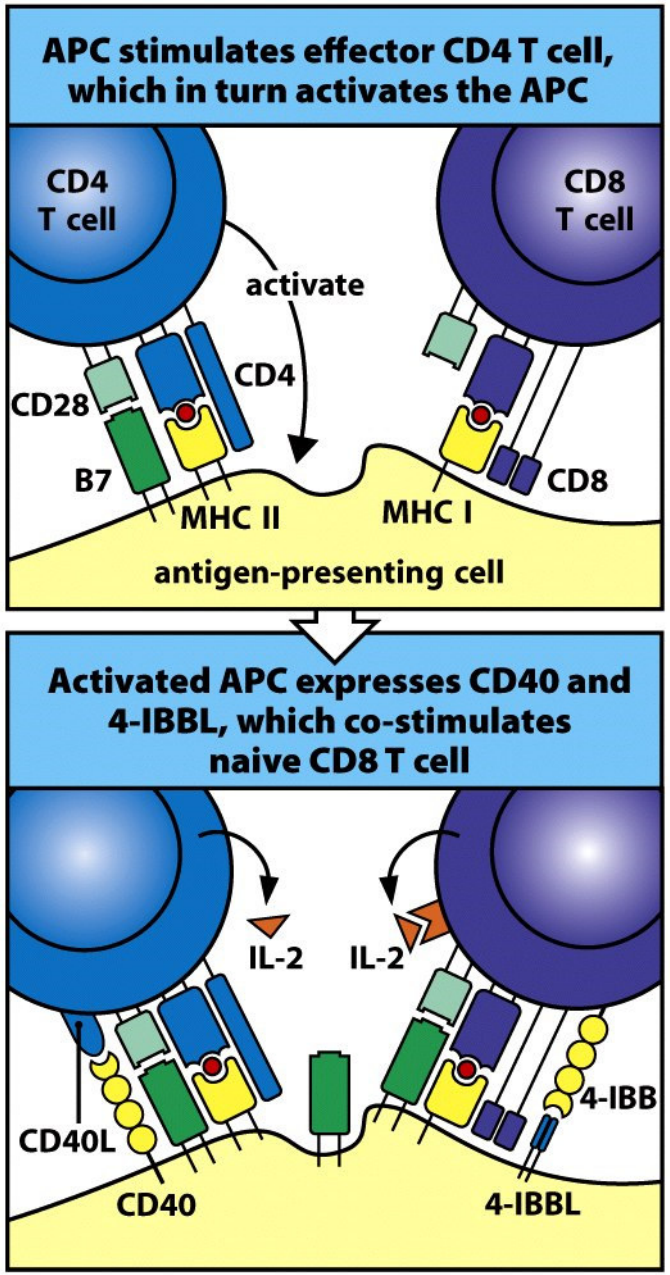

*Kisielow, Nat. Imm. 2019*

Systematic evaluation of MHC/peptide complexes has revealed a surprisingly low selectivity of TCR recognition.

Two different TCR clones reacting against the same viral epitope were screened against 5 million peptides using a NFAT-BFP reporter activated by TCR signaling. Nearly all of the strongly recognized peptides (in blue) shared only little homology to the original viral peptide (light green). Hardly any of the peptides (only G1) were recognized by both TCR clones while most of the peptides recognized by one of the TCR clones were not cross-reacting with the other TCR clone even though both TCRs were highly specific for the original viral peptide.

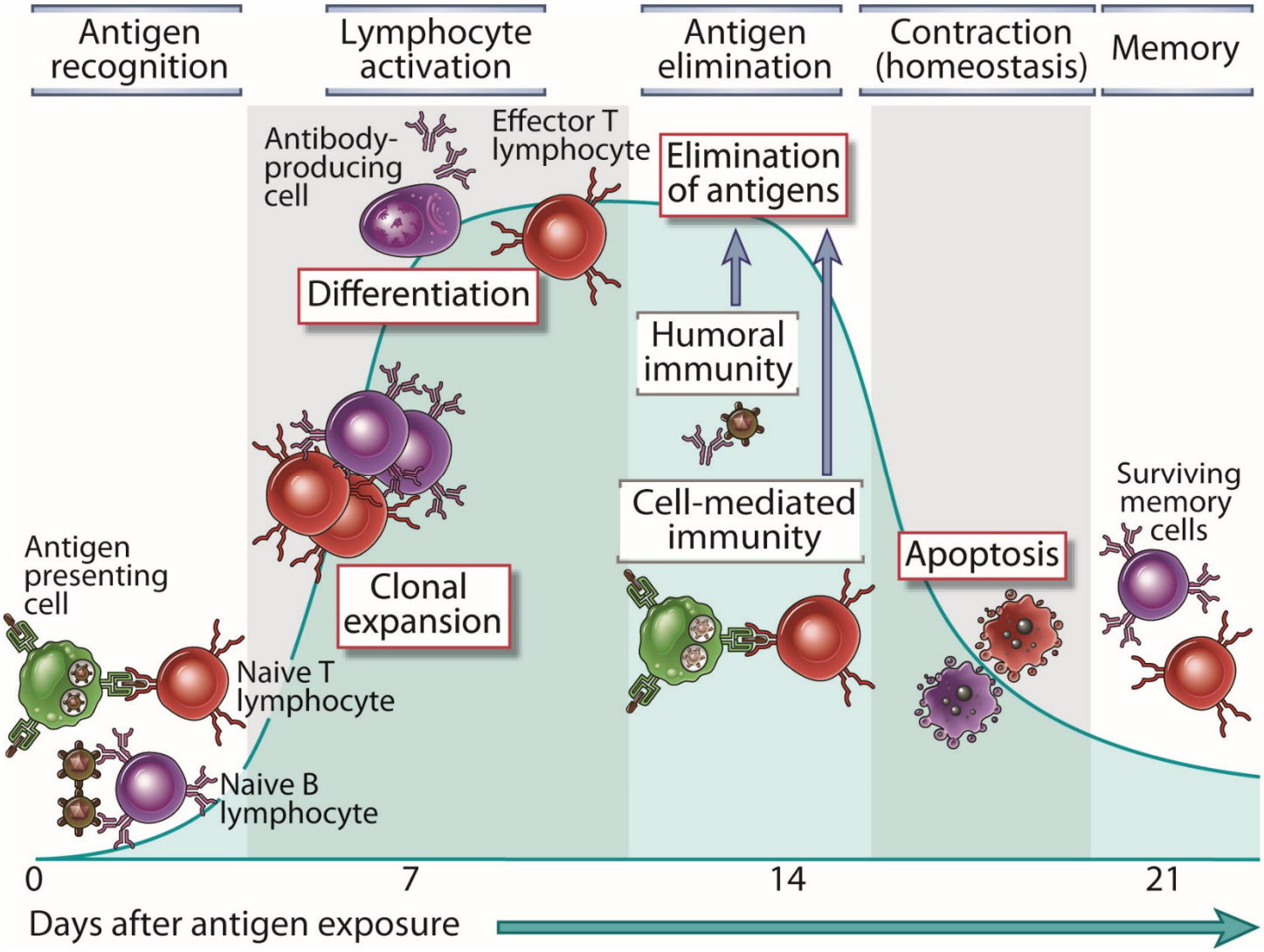
# CD4 $T_H$ cells help to activate CTL via cross-presentation of APCs



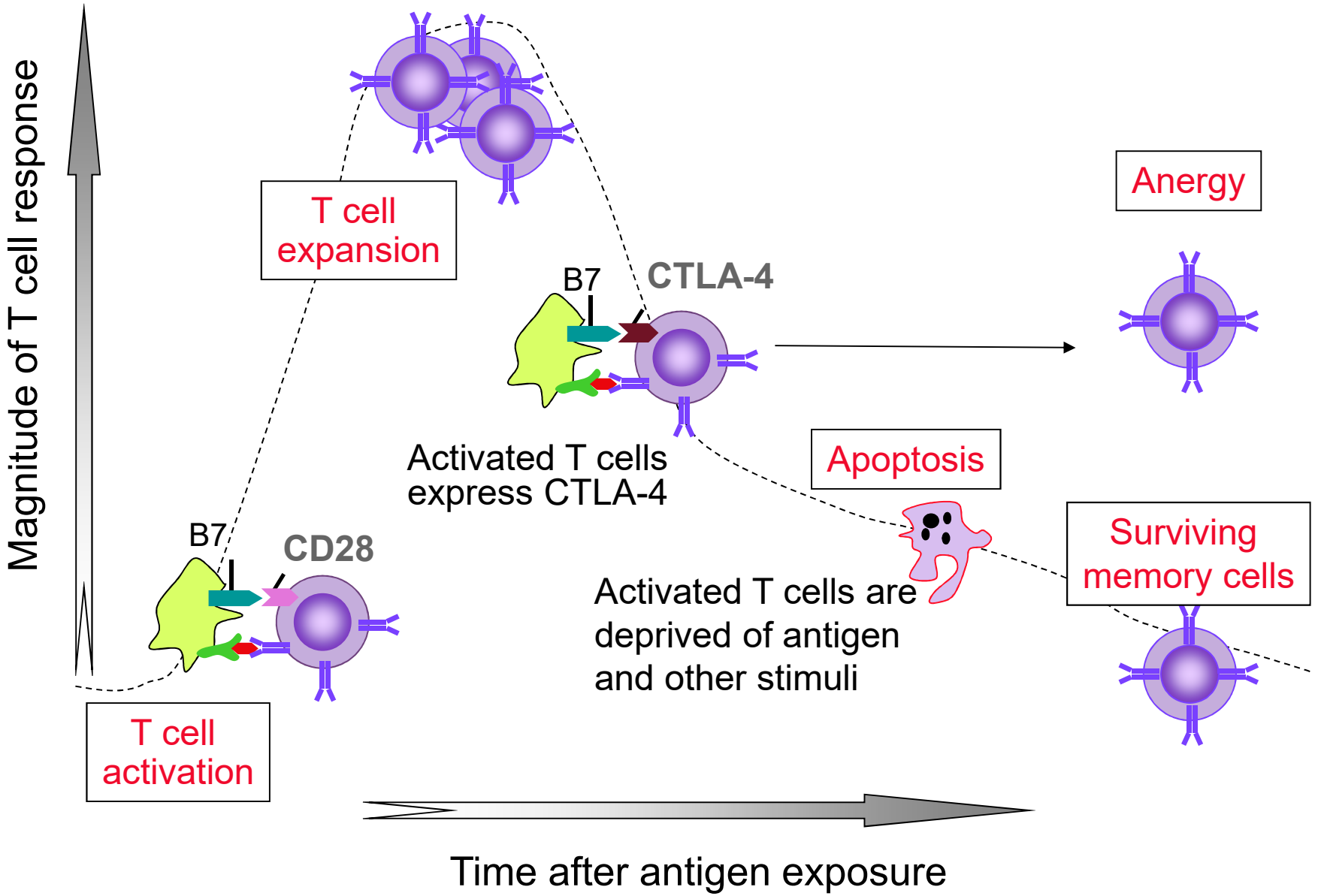




- phagocytosis of viral infected or tumor cells or uptake of particles of such dying cells allows APCs to **cross-present** antigenic peptides to CD8 T cells via MHC1
- often help by CD4 cells is required to fully activate CTLs
- CD4 $T_H$ cells recognize <u>another</u> antigenic peptide derived from the altered cell and presented via MHC2
- **APC licensing**: CD4 T cells activate APCs via CD40/CD40L to express ligands CD80/86 and CD137L
- T cells receive co-stimulatory signals from the APC to produce IL2 for their differentiation and expansion

# Clonal selection, expansion and memory allow the immune system to form an adaptive response to any type of threat

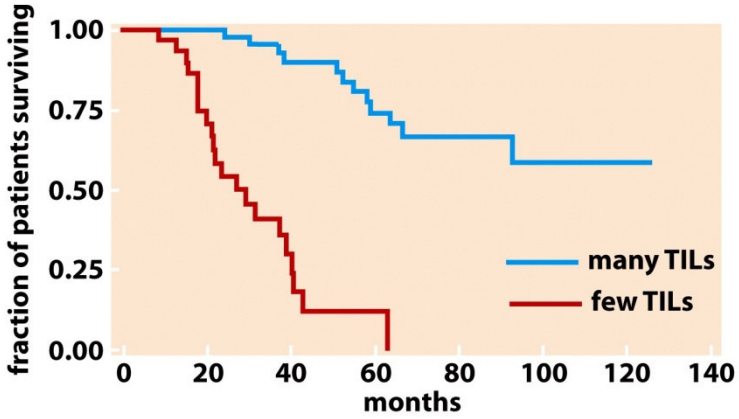


# Regulation of T cell homeostasis during immune responses



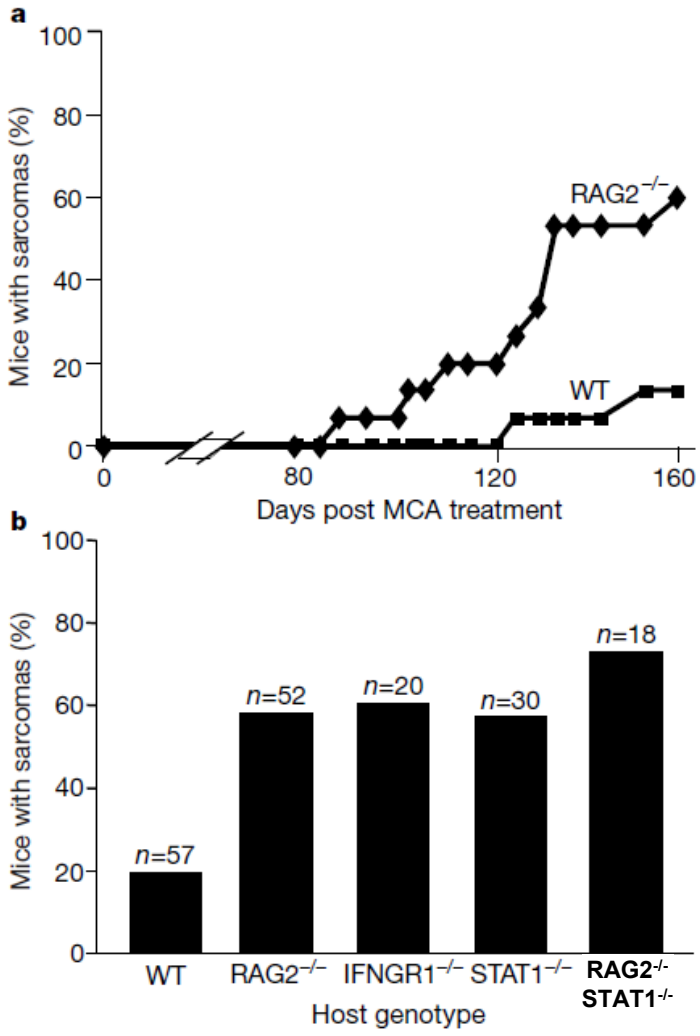
# Impact of the immune system on cancer formation in patients

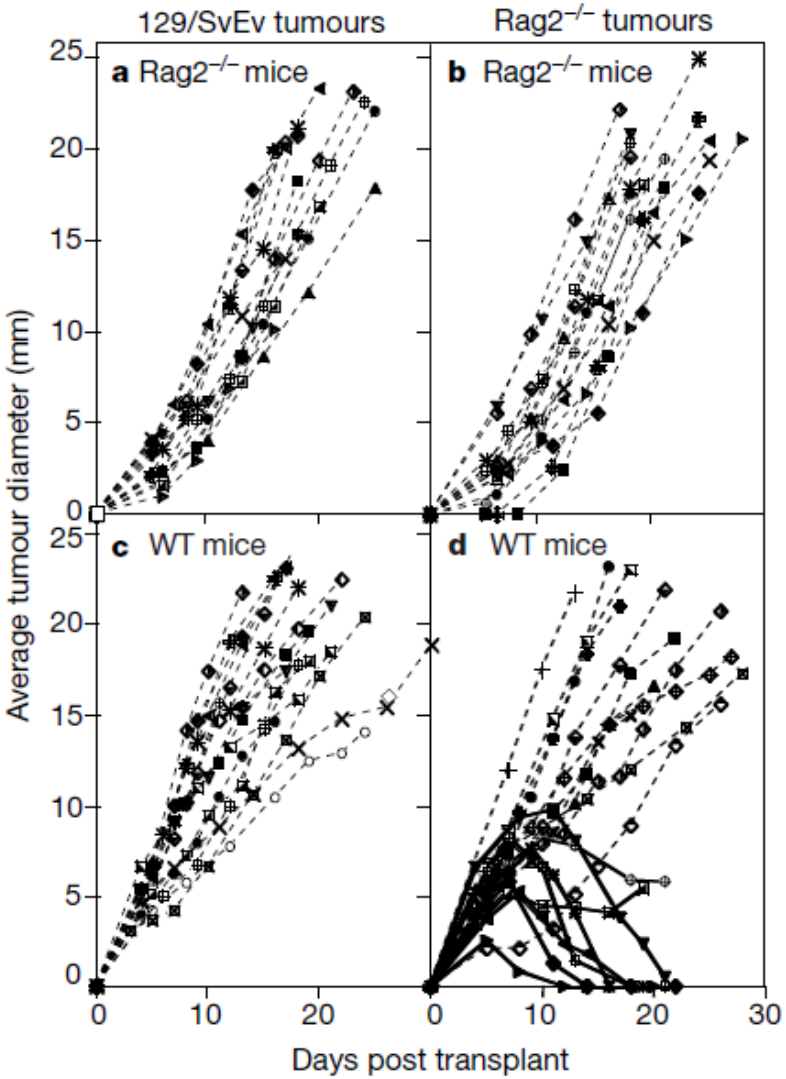
clinical epidemiology increasingly supports the existence of anti-tumor immune responses in human cancers:




- abundance of **tumor infiltrating lymphocytes** ***(TILs)*** correlates with **favorable prognosis**
- abundance of **infiltrating, innate immune cells** *(e.g. macrophages, mast cells, and neutrophils)* correlates with **increased angiogenesis** and **poor prognosis**
- long-term use of anti-inflammatory drugs *(non-steroidal and selective cyclooxygenase 2 (COX2) inhibitors)* reduces cancer incidence
- immunosuppressed organ transplant recipients have been observed to develop donor-derived cancers, suggesting that in the tumor-free donors the cancer cells were held in check in a dormant state by a fully functional immune system

# IFNγ and lymphocytes shape tumor immunogenicity

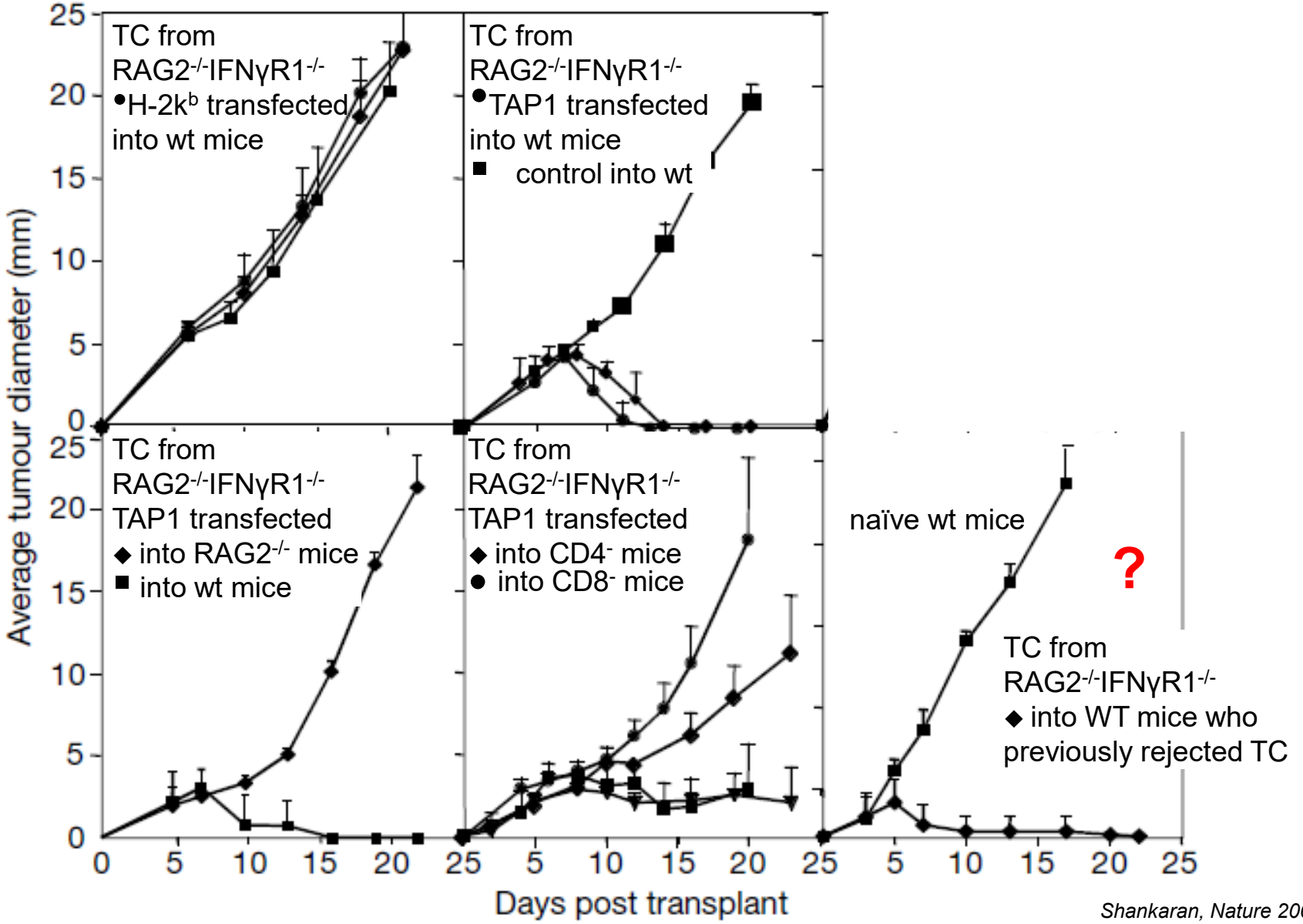



sarcoma formation after chemical carcinogenesis (MCA)

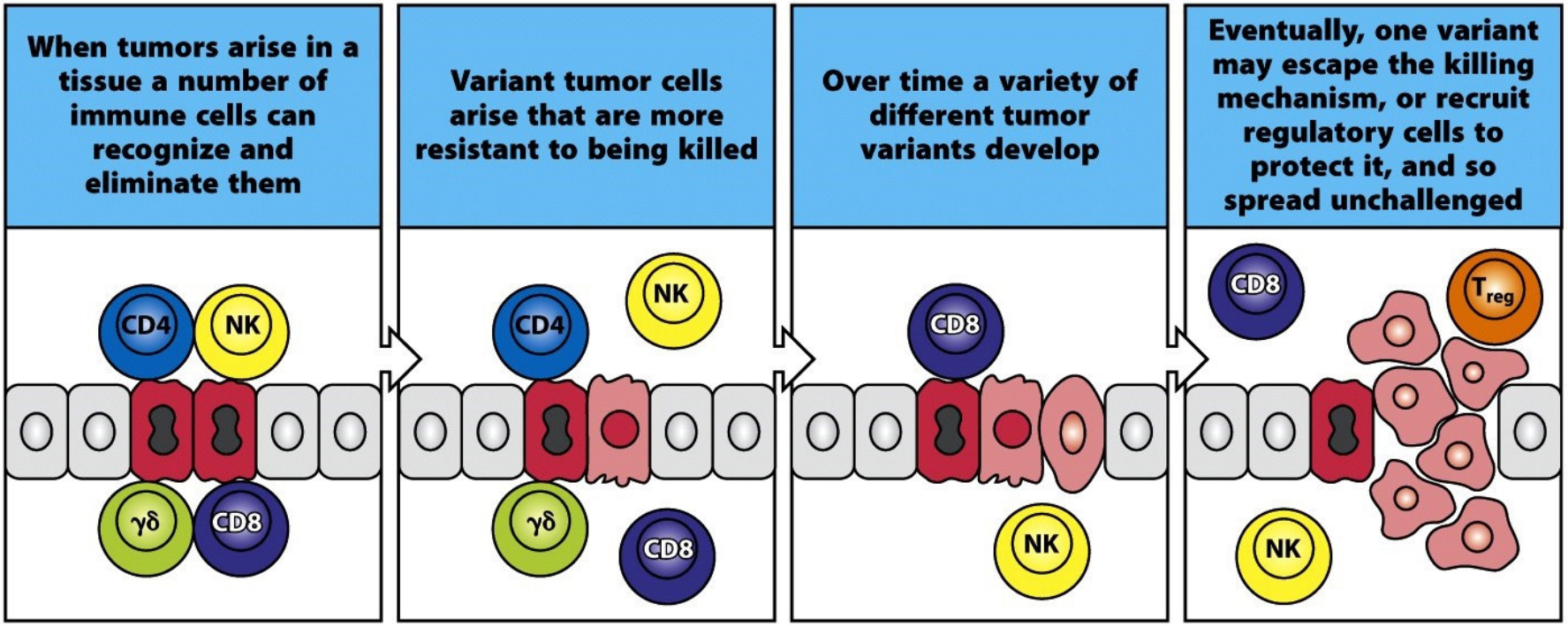
*Shankaran, Nature 2001*

# Insufficient antigen presentation by the tumor prevents the host immune reaction




*Shankaran, Nature 2001*

# Immunosurveillance



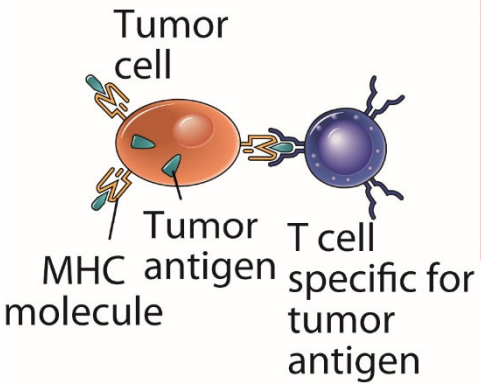

immunosurveillance - the tumour-suppressing role of the immune system

the concept of immunosurveillance is considered to encompass three phases:

1. "Elimination phase" - recognition and destruction of tumor cells
2. "Equilibrium phase" – occurs if elimination is not successful, tumor cells adapt to immune mediated selection pressure and undergo changes in a process called immunoediting
3. "Escape phase"- tumour cells evolved enough to grow unimpeded and form a large tumor mass

# Immune evasion mechanisms in cancer

## Anti-tumor immunity




**T cell recognition of tumor antigen leading to T cell activation**

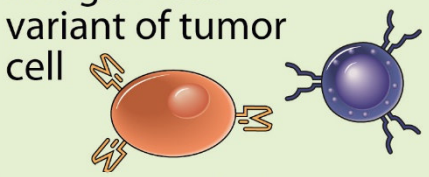
**Attraction/reprogramming of other immune cells**

regulatory T cells (Treg)
tolerizing dendritic cells
MDSCs, type2 macrophages/neutrophils

**Inhibition of T cell activation**

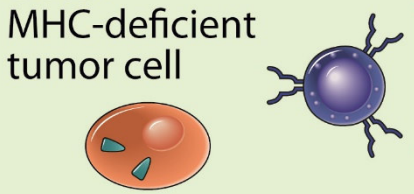
## Immune evasion by tumors

**Failure to produce tumor antigen**



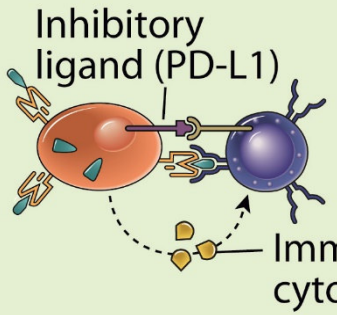

**Lack of T cell recognition of tumor**

**Mutations in MHC genes or genes needed for antigen processing**



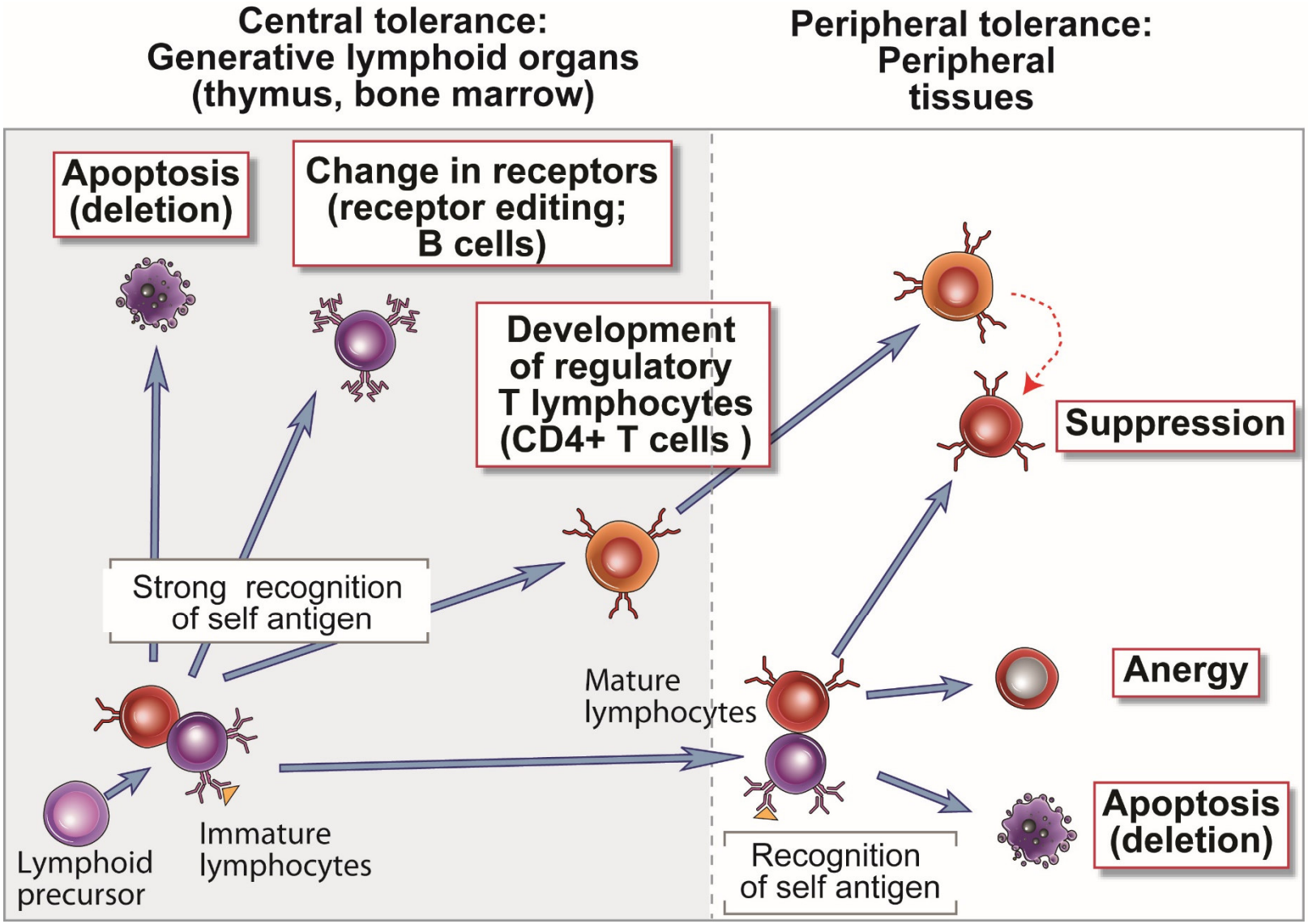

**Lack of T cell recognition of tumor**

**Production of immuno-suppressive proteins**

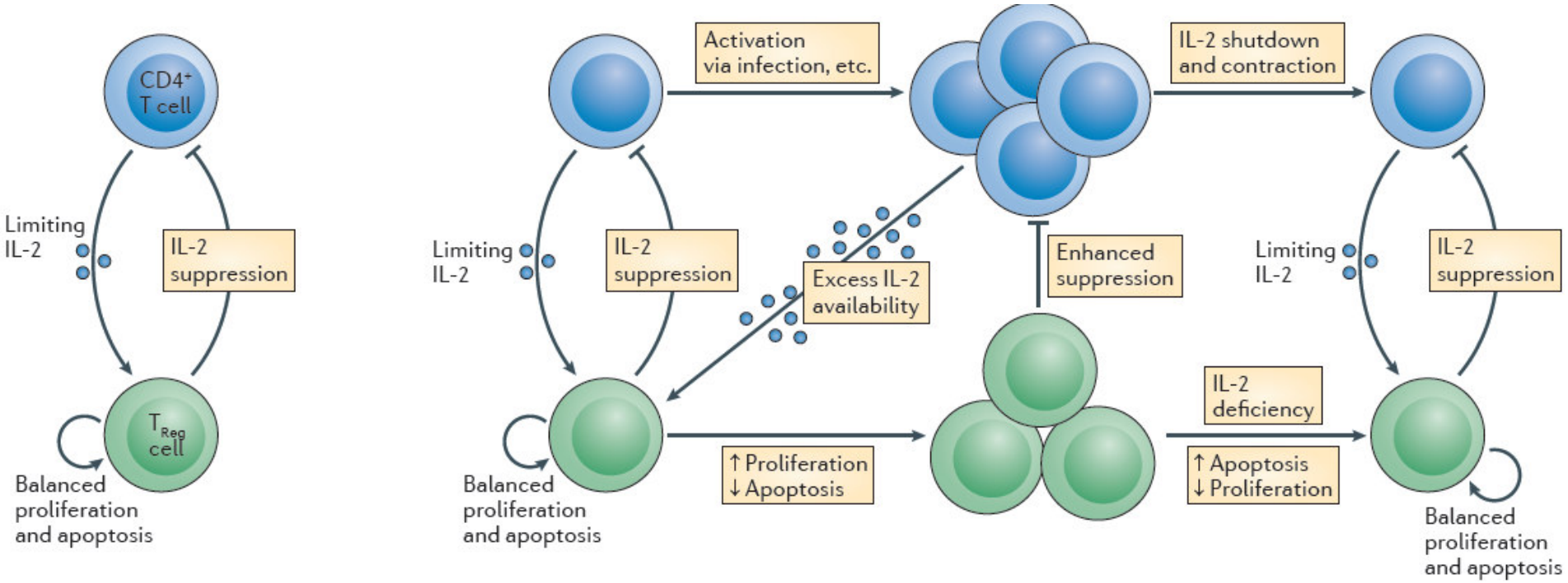



**Inhibition of T cell activation**

# Central and peripheral tolerance

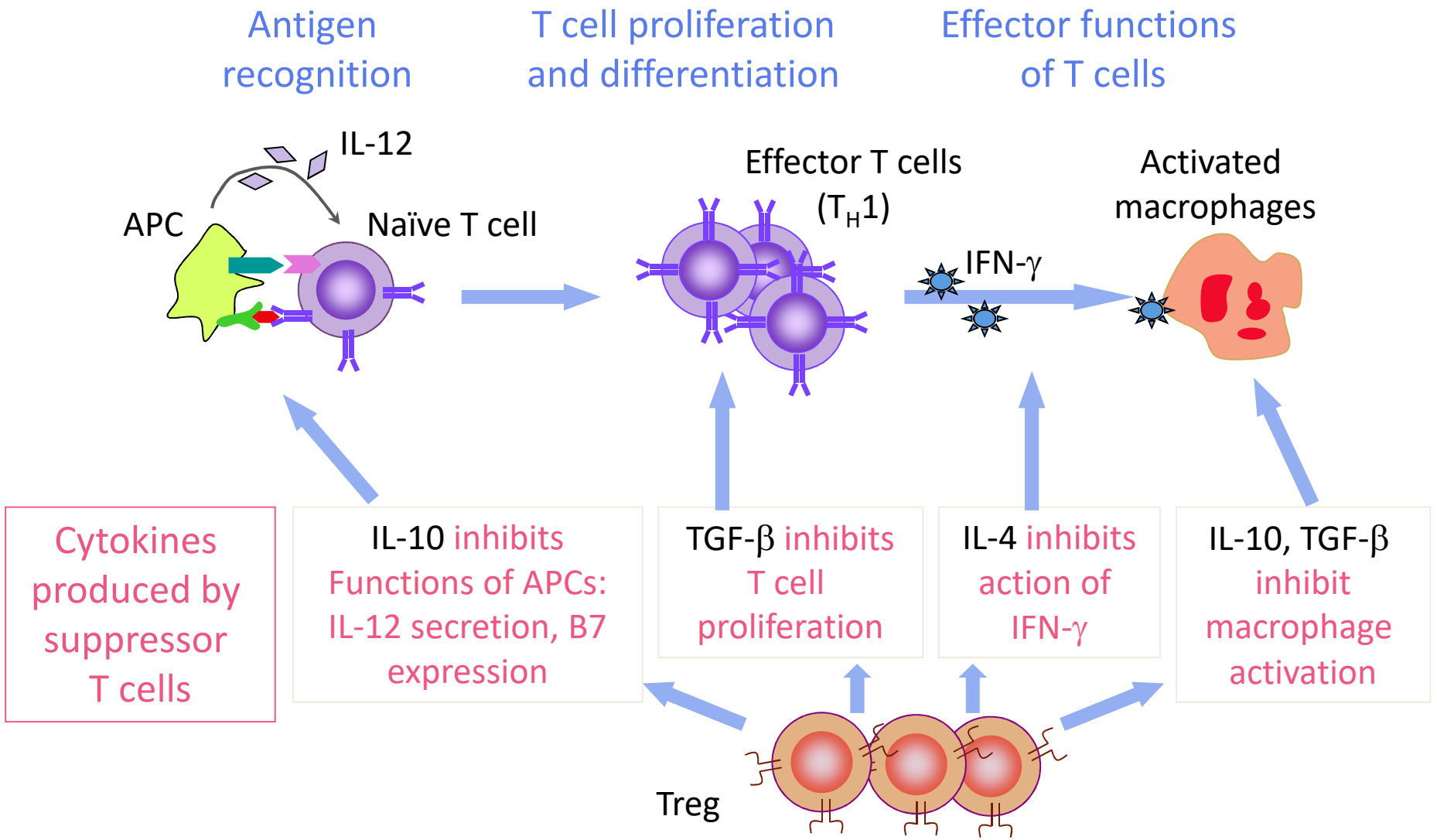


# Competition for IL2 creates homeostasis between pro- and anti-immune functions

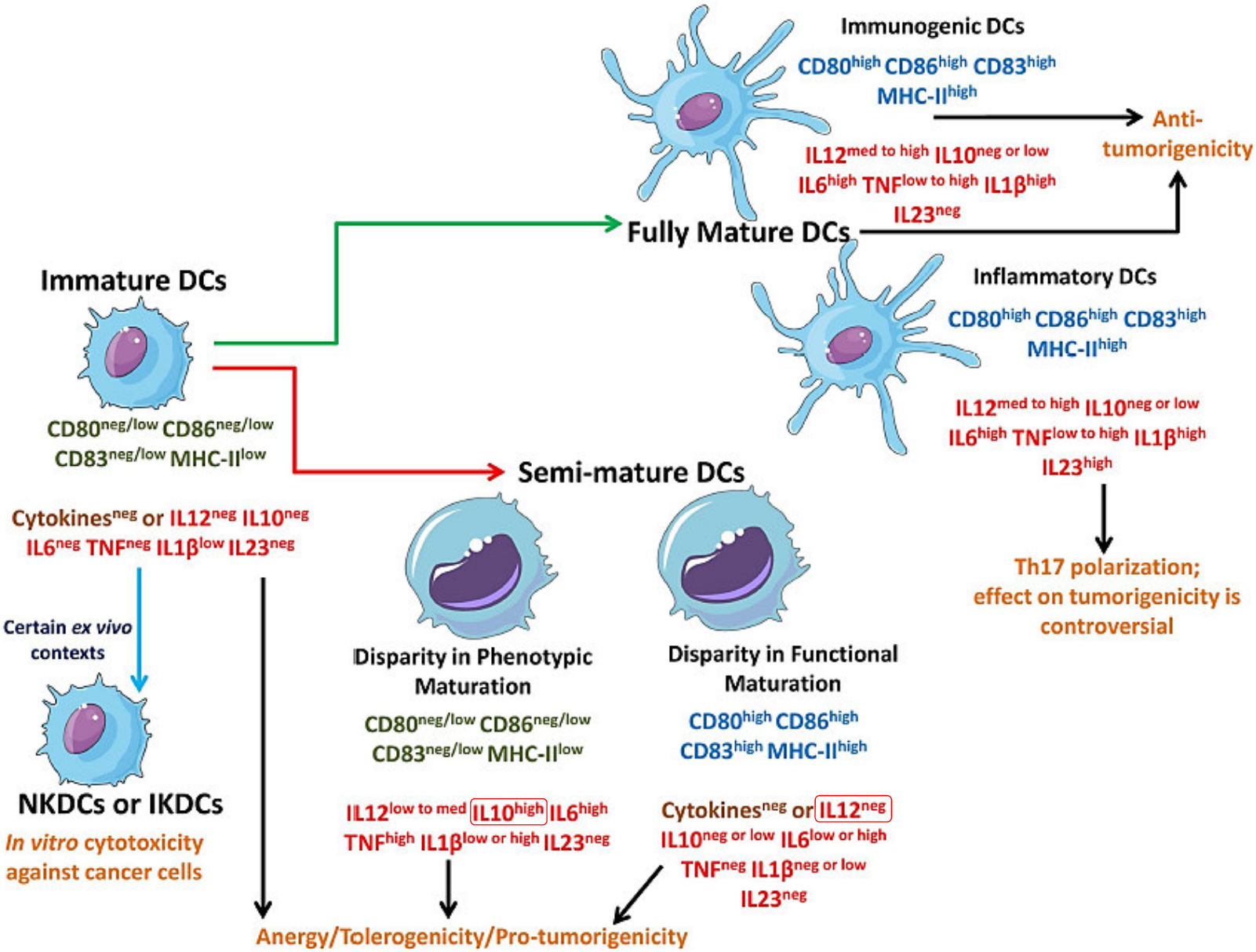



In addition, balance between Treg and activated T cells can also be mediated by direct killing of Treg cells by FASL expression on CTLs

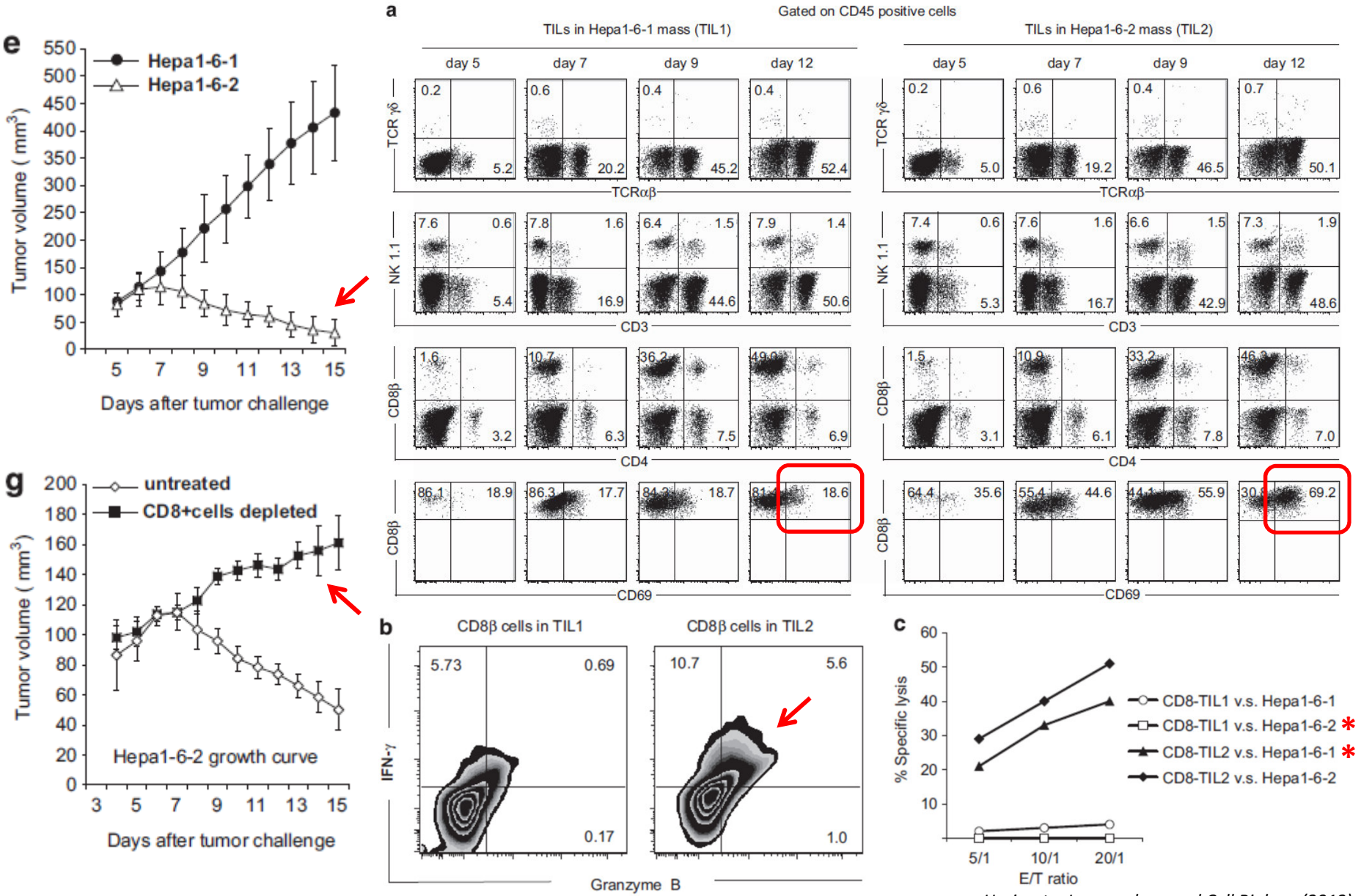
# Role of Treg derived cytokines in suppression of cell-mediated immune responses



# Different DC phenotypes in the tumor setting



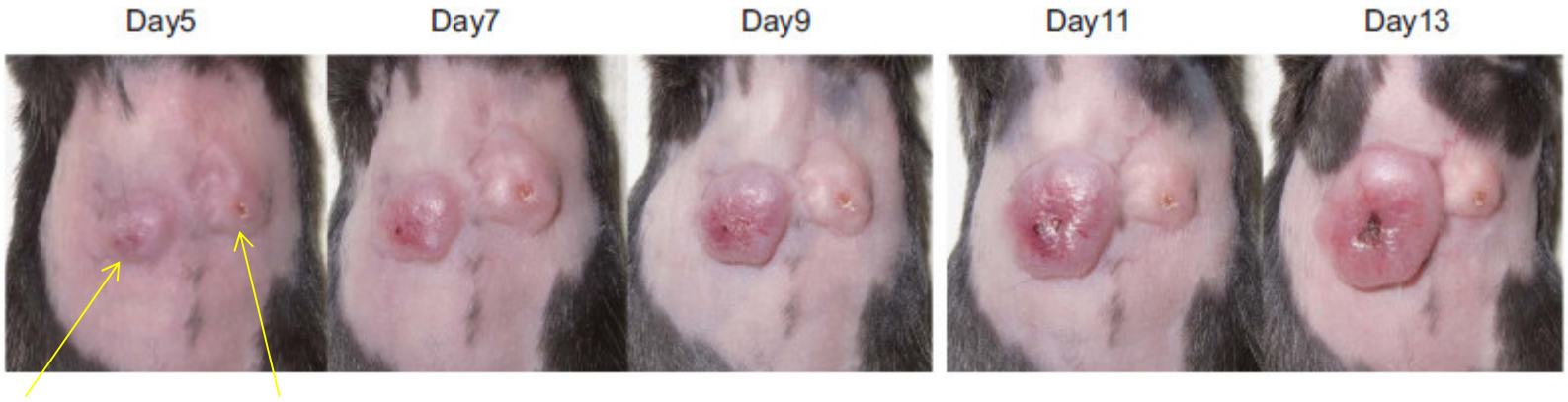
# Tolerogenic DCs can enhance tumor growth



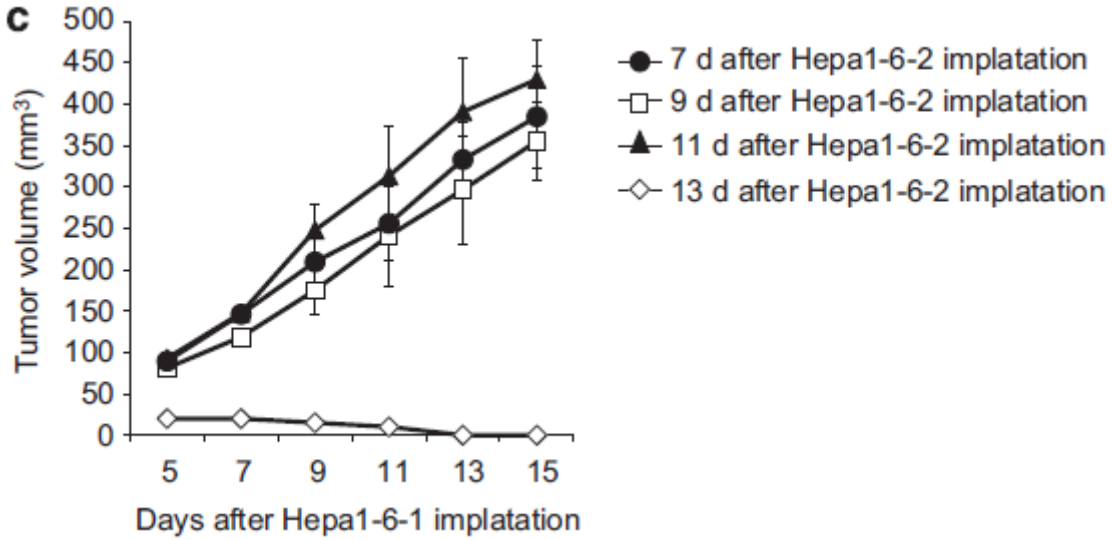

*Harimoto, Immunology and Cell Biology (2013)*

# Co-implantation provides first hints at tolerizing environment

simultaneous implantation

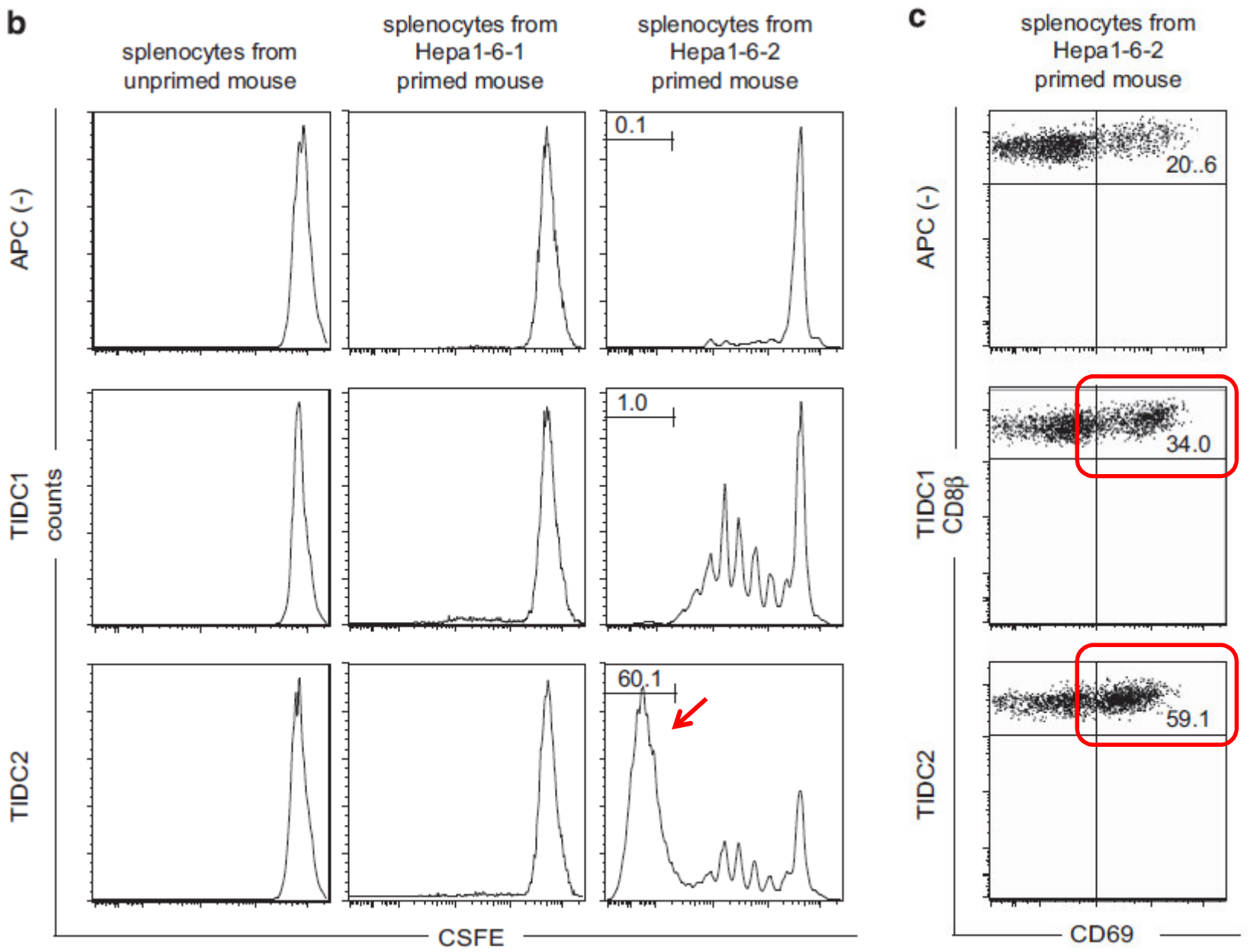



successive implantation, separated by 7, 9, 11, 13d

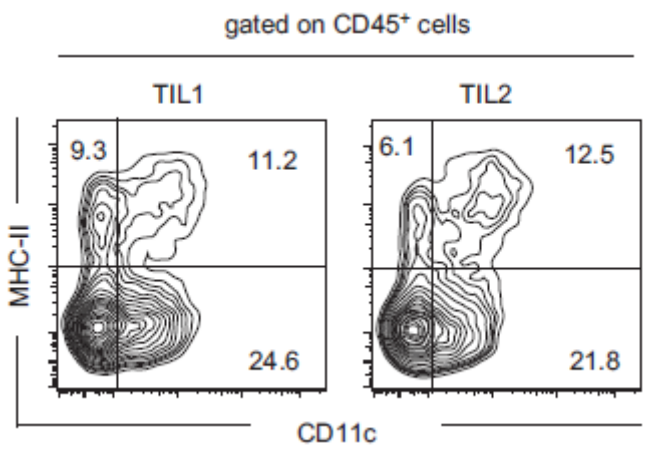


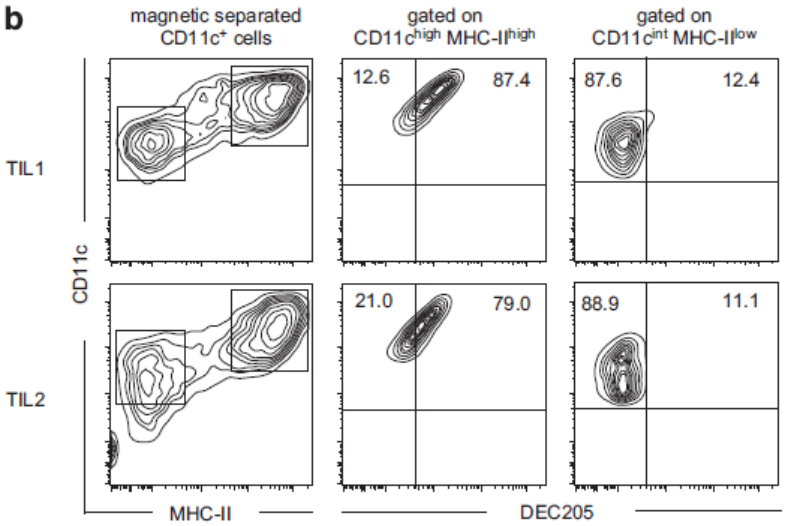

*Harimoto, Immunology and Cell Biology (2013)*

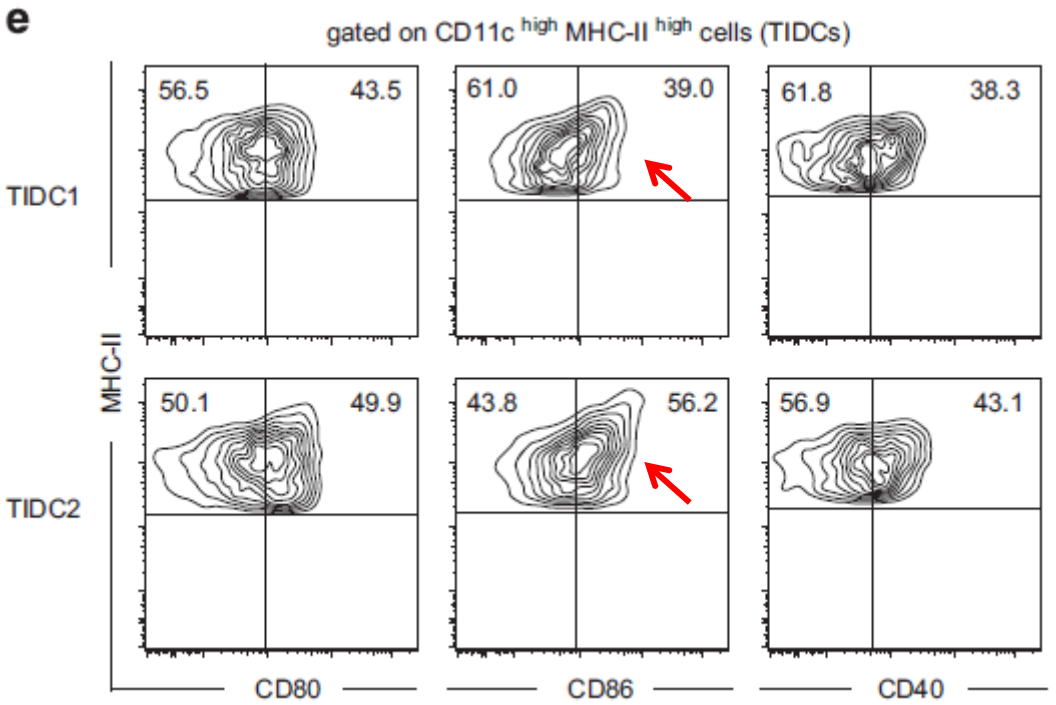
# Tolerizing TIDCs fail to stimulate tumor primed T cells

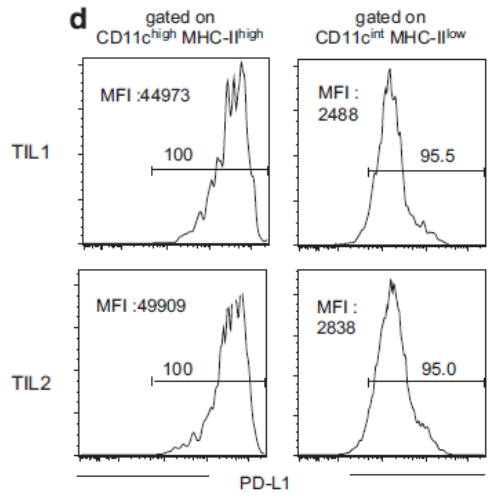


# Tolerizing TIDCs lack co-stimulatory markers



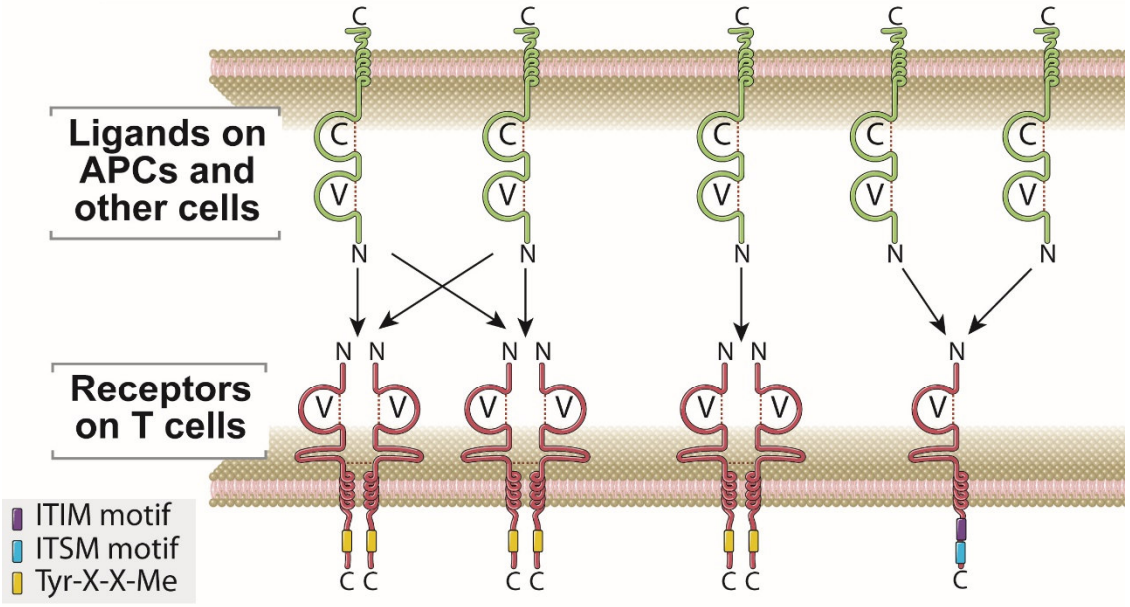



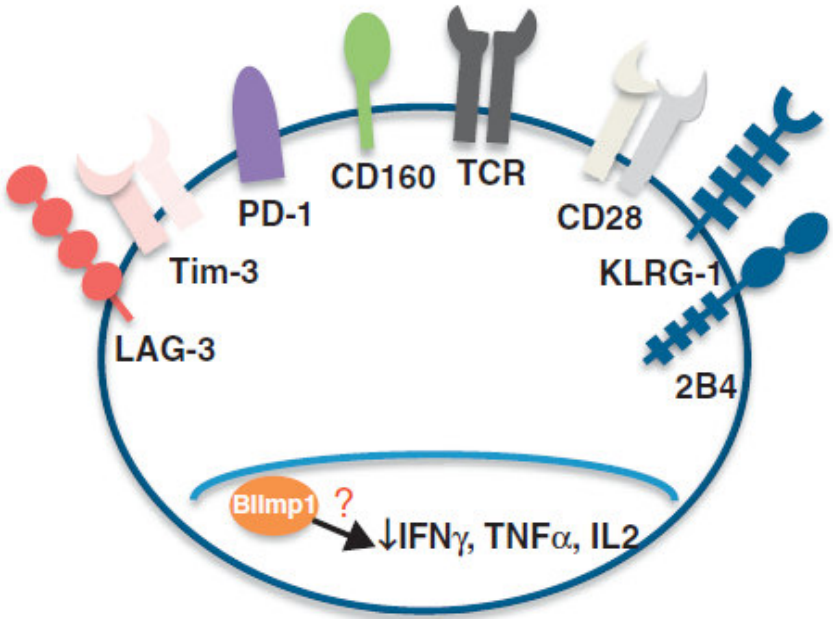
# Activating and inhibitory receptors on T cells

| | | | | | |
|---|---|---|---|---|---|
| Expression | DCs, Macs, B cells | DCs, Mas, B cells | DCs; Macs, B cells | Many cell types | DCs; Macs, B cells |
| Name | **B7-1 (CD80)** | **B7-2 (CD86)** | **ICOS-L (CD275)** | **PD-L1 (B7-H1, CD274)** | **PD-L2 (B7-DC, CD273)** |




| Name | **CD28** | **CTLA-4** | **ICOS** | **PD-1** |
|---|---|---|---|---|
| Expression | T cells | T cells; (activated) | T cells; (activated) | T cells, B cells, myeloid cells; (activated) |
| Major function | Costimulation of naive T cells; generation of Treg | Neg. regulation of immune responses; self-tolerance | Costimulation of effector T cells and Treg; generation of $T_{FH}$ | Neg. regulation of T cells |

# Failure of T cell function: exhaustion




**Exhausted T Cells**

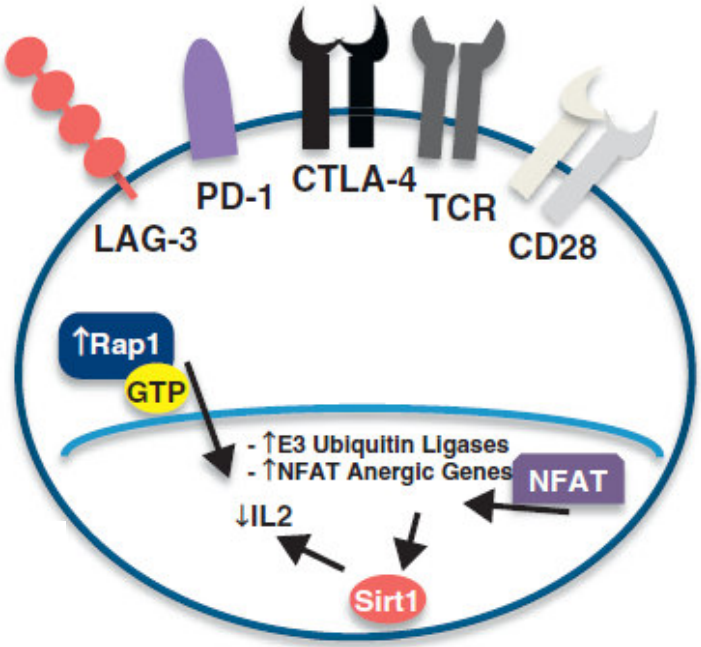
-Unresponsive state-loss of effector functions.
-Long-lived and cell cycle arrested.
-Accumulate due to chronic infection or disease.
-Stable expression of inhibitory receptors.
-Layered co-inhibition (in function of repeated-activation).

Although the underlying mechanisms causing T cell anergy, exhaustion and senescence are not well defined, compelling evidence indicate that dysfunctional T cells express in different degrees 'inhibitory' molecules such as **PD-1, Tim-3, LAG-3, 2B4, CD160**, and **KLGR-1**. It suggests that different categories of T cell abnormalities may be mechanistically intertwined.

# Failure of T cell function: anergy/senescence

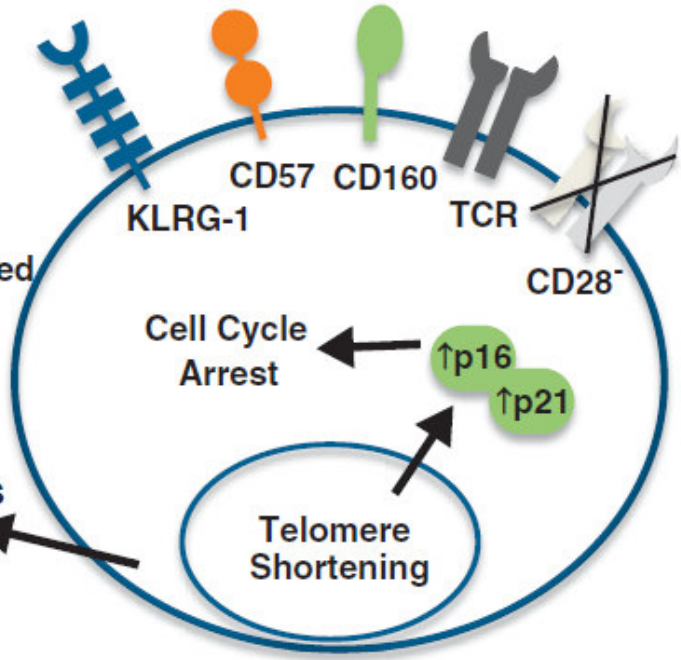
## Anergic T Cells

-Induced non-responsive state as part of peripheral tolerance.
-Low IL-2 production.
-Long-lived cells (immunosuppressive role?).



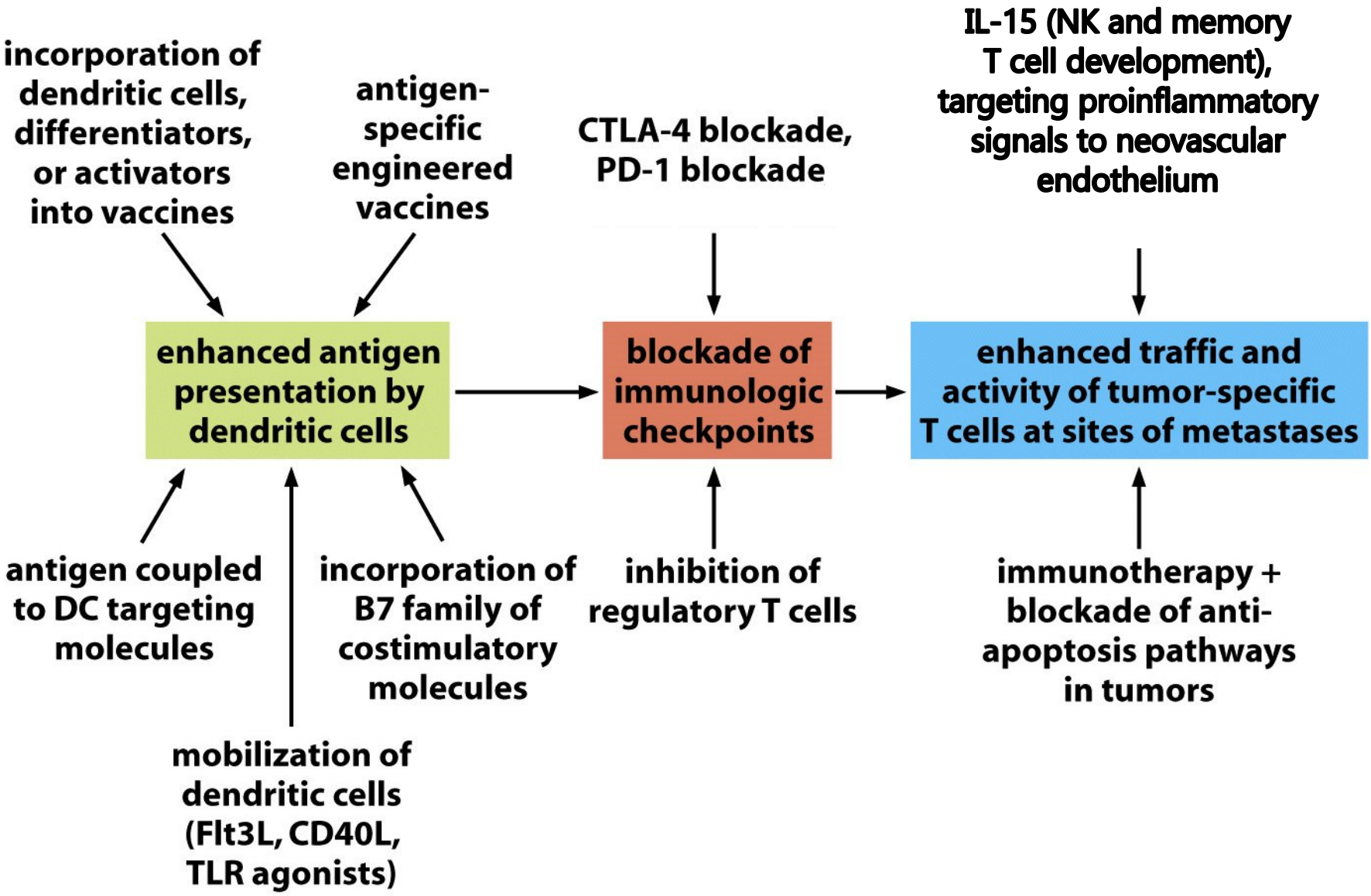

## Senescent T Cells

-Accumulate in an oligoclonal-manner.
-Occurs as a results of ageing and/or chronic infection.
-Cell cycle arrest may be induced because of damaged DNA.

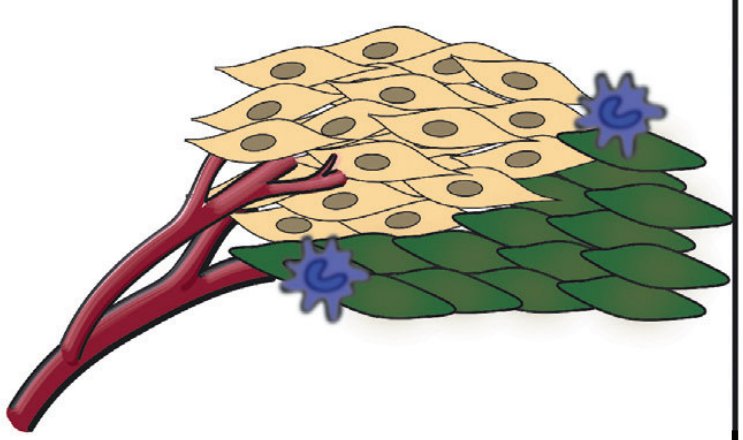
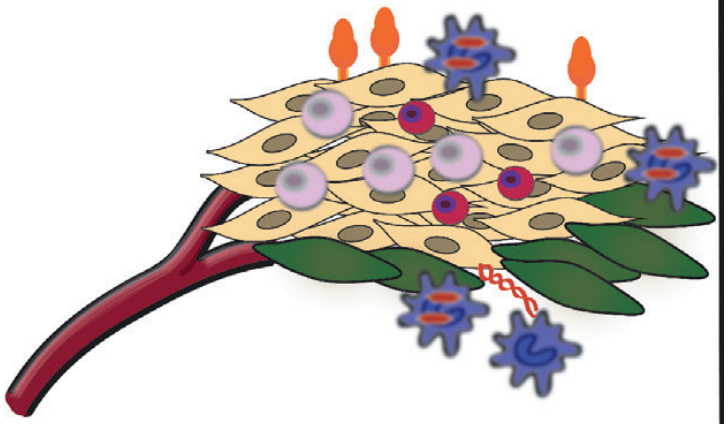



Immunosuppressive roles (?)

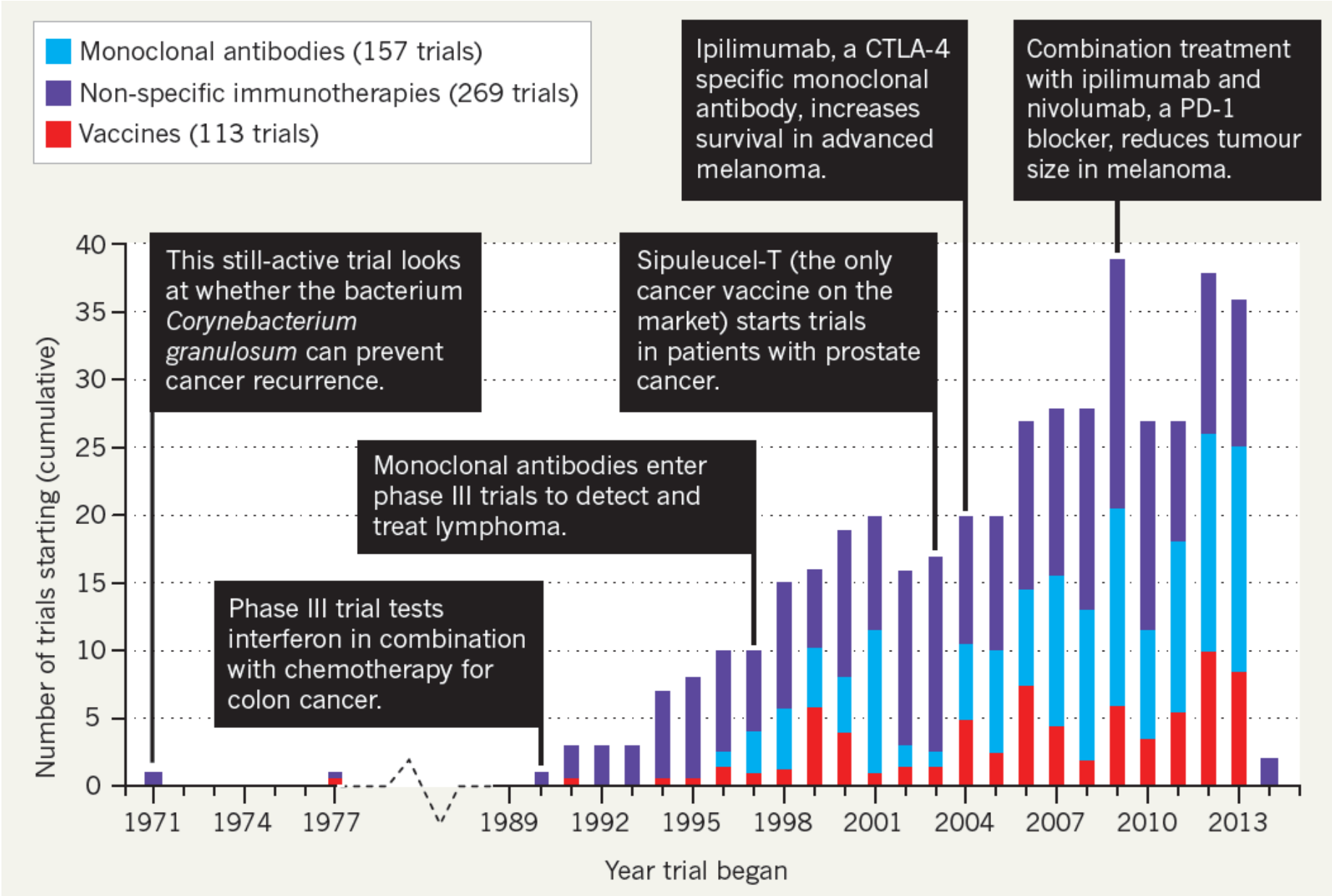
# Current strategies to enforce the immune system to fight cancer



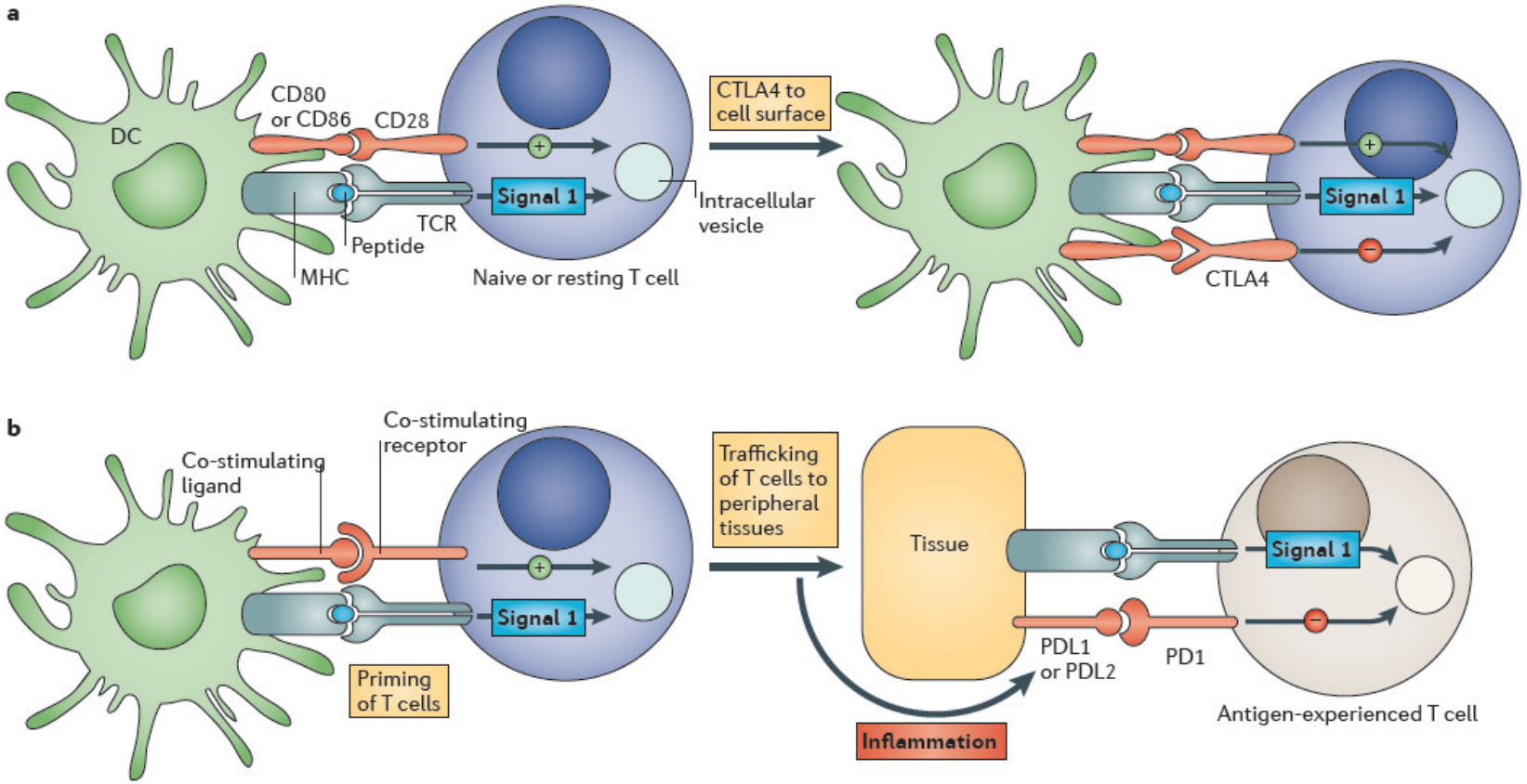
# Mechanisms of immune evasion

| T cell-poor tumor | T cell-inflamed tumor |
|---|---|
|  |  |
| • Lack of antigen or antigen presentation<br>• insufficient T cell activation by tolerizing dendritic cells<br>• Lack of T cell attraction by chemokines or dense stroma<br>• Lack of innate immune activation | • Expression of inhibitory factors<br>• T cell anergy/exhaustion<br>• Presence of regulatory immune cells |
| **Therapeutic Interventions** | |
| Innate immune activation<br>Stroma disruption<br>Manipulation of oncogene pathway | α-PD1 / PDL1<br>Treg depletion<br>IDO inhibition<br>Homeostatic cytokines |

# Trials in immunotherapy



# Immune checkpoint blockade

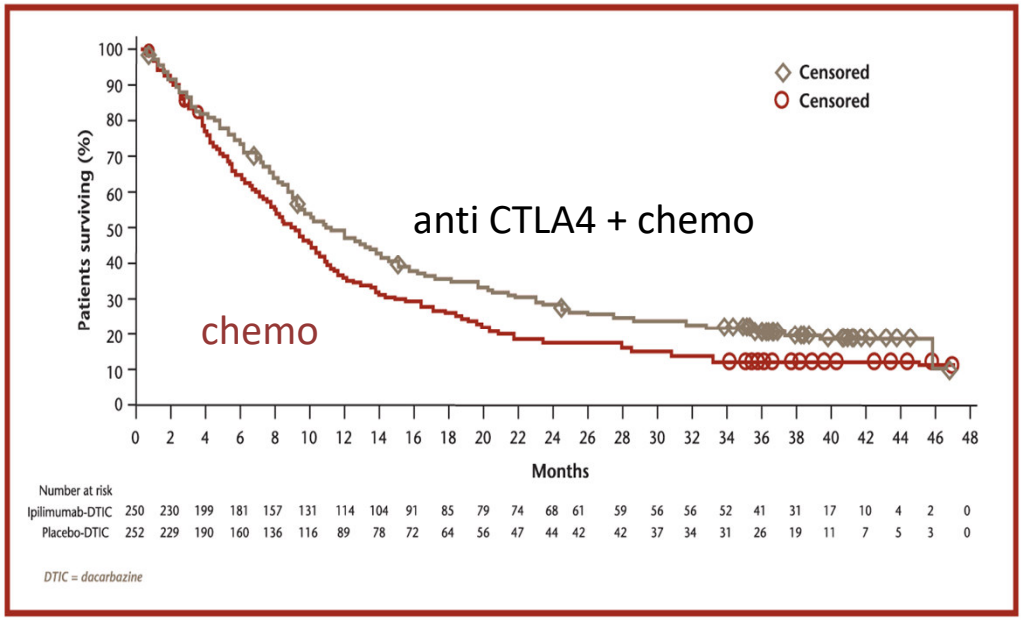



**CTLA4** is induced in T cells at the time of APC licensing with the level of CTLA4 depending on the amplitude of TCR-mediated signalling (naive and memory T cells do not express CTLA4). CTLA4 functions as a signal dampener to maintain a consistent level of T cell activation in the face of widely varying concentrations and affinities of ligand for the TCR.

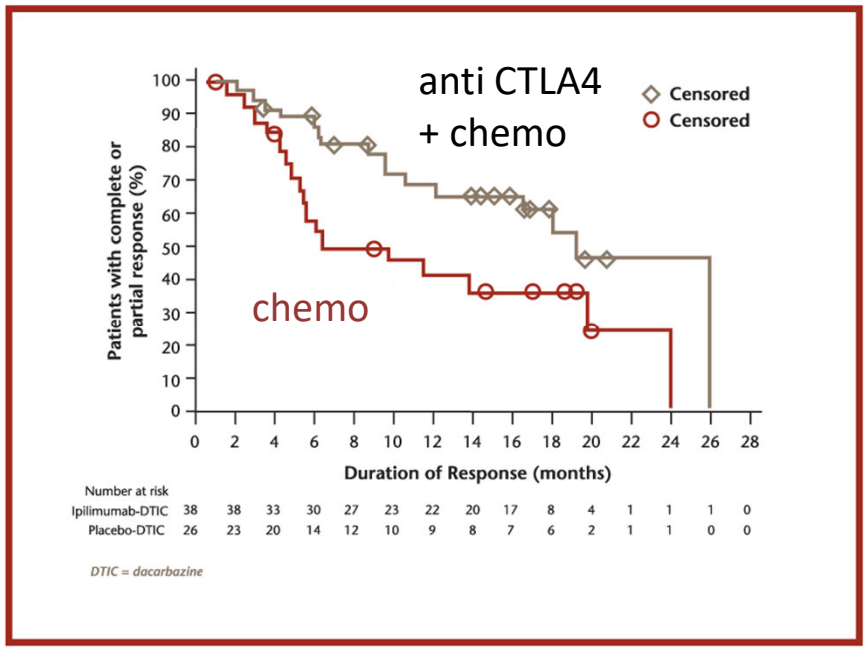
Activated T cells upregulate **PD1** and continue to express it in tissues. Inflammatory signals in the tissues induce the expression of PD1 ligands, which downregulate the activity of T cells and thus limit collateral tissue damage. Excessive induction of PD1 on T cells in the setting of chronic antigen exposure can induce an exhausted or anergic state in T cells.

# CTLA-4 treatment of metastatic melanoma

overall survival



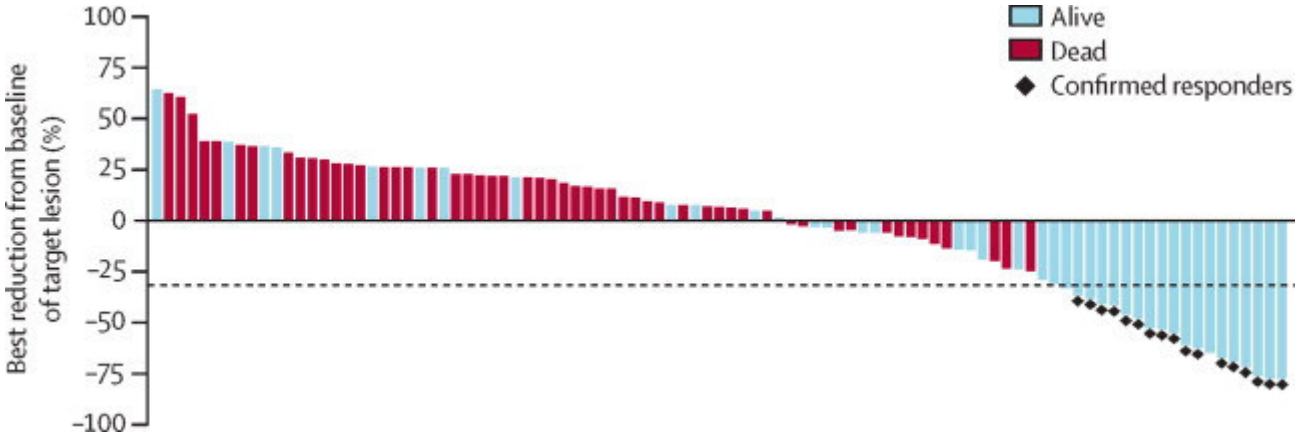

complete or partial response



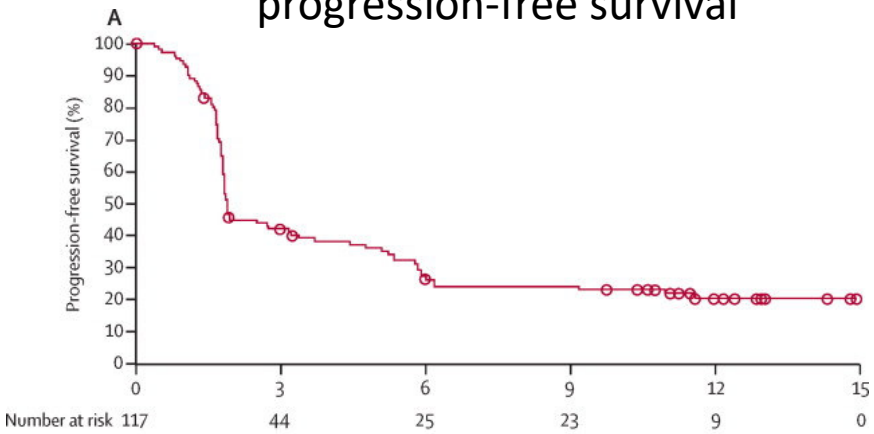

*Petrella et al., NE Onc. 2012*

# Anti PD-1 for advanced, refractory, squamous NSCLC

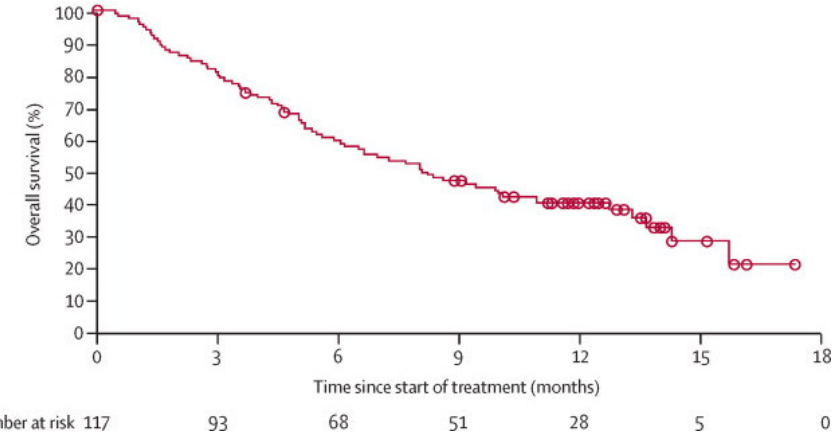
effect on tumor size




progression-free survival



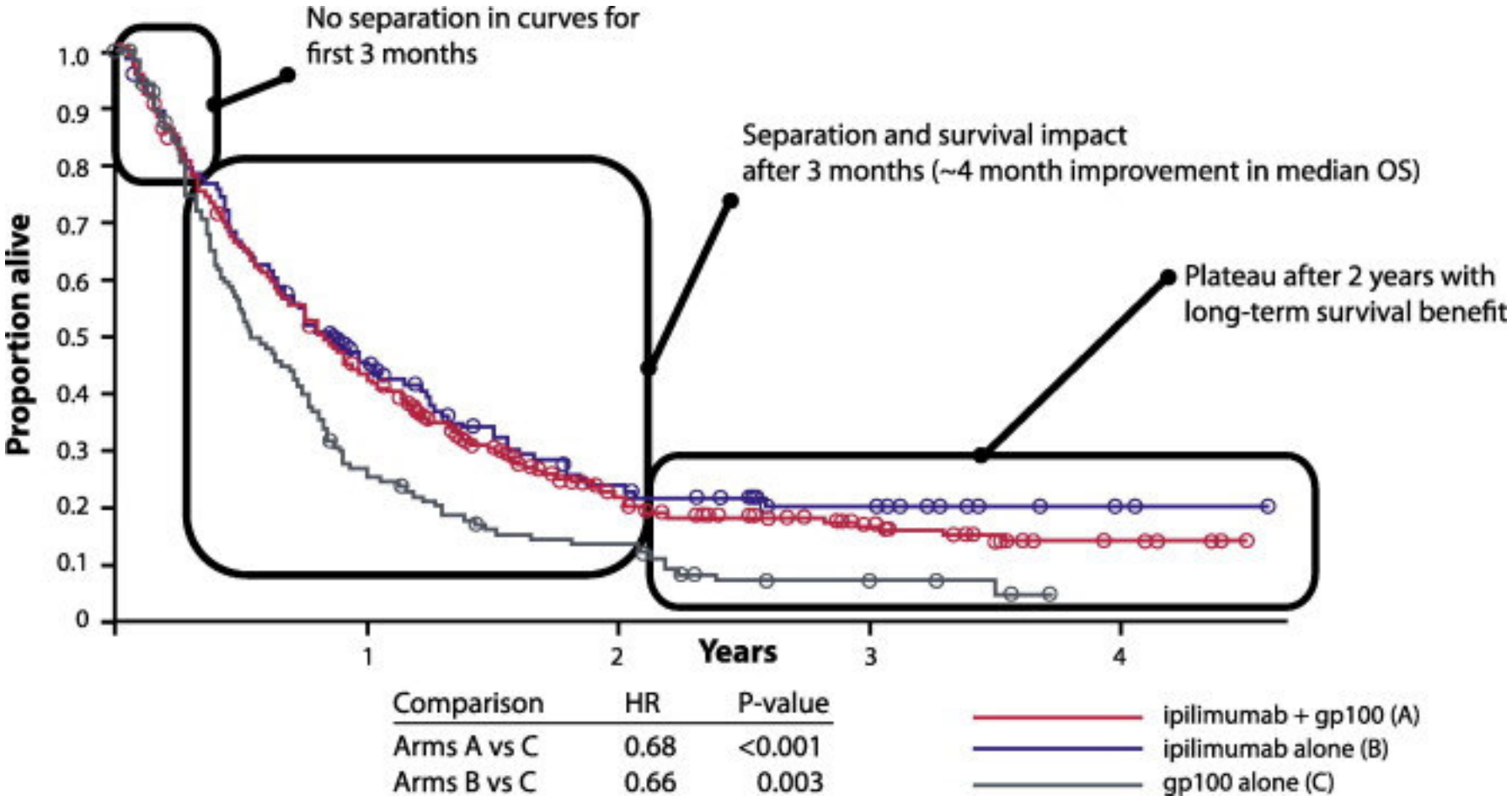

overall survival




*Rizvi et al. Lancet Onc. 2015*

# Typical response for checkpoint blockade

**CTLA-4 treatment of metastatic melanoma**

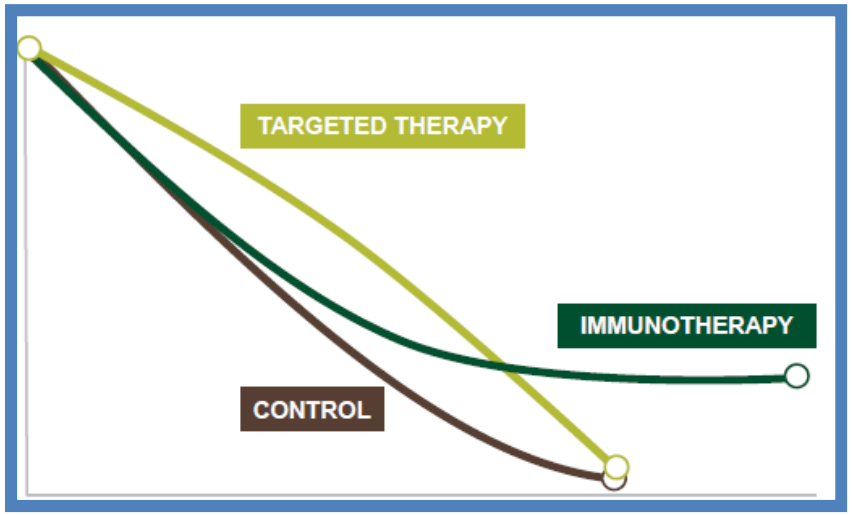



*McDermott et al., Canc. Treat. Rev. 2014*

# Immunotherapy of cancer: NOVEL ASPECTS

- **Immune-related response criteria**
- **Endpoints**
- **The immune-related toxicities**

**All differ considerably from conventional cytotoxic agents and targeted therapies**



# RESPONSE EVALUATION FOR IMMUNOTHERAPY

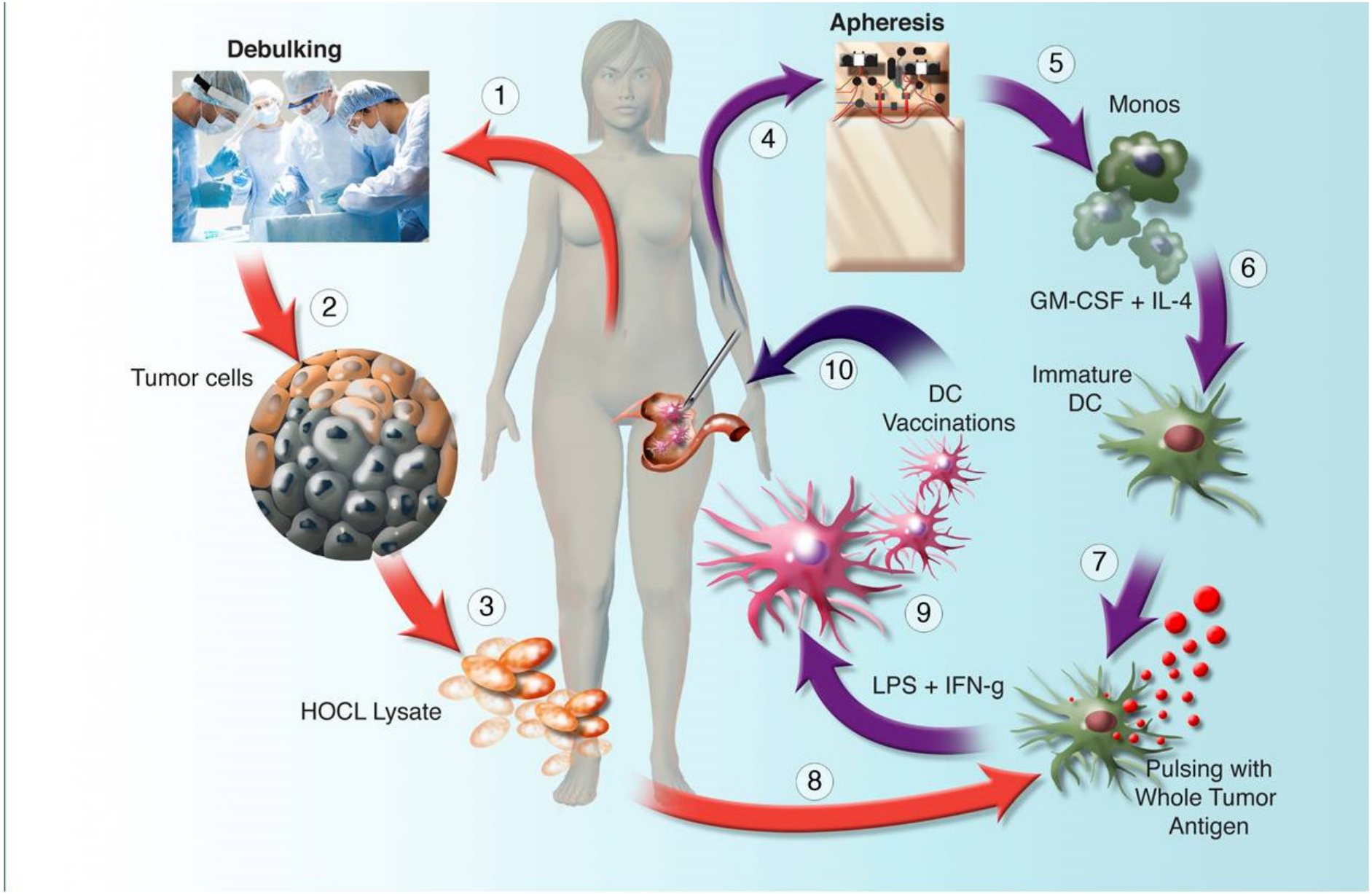
- **Permissive not restrictive**
- **R**esponse **E**valuation **C**riteria **I**n **S**olid **T**umors (**RECIST)/mWHO modified by immunological criteria**
- **First radiological examination the most critical (pseudo-progressions)**
- **irResponders with new lesions also but decrease in baseline lesions**
- **PD should be confirmed after 4 w**

**TABLE 2. Immune-Related Response Criteria Defined[28]**

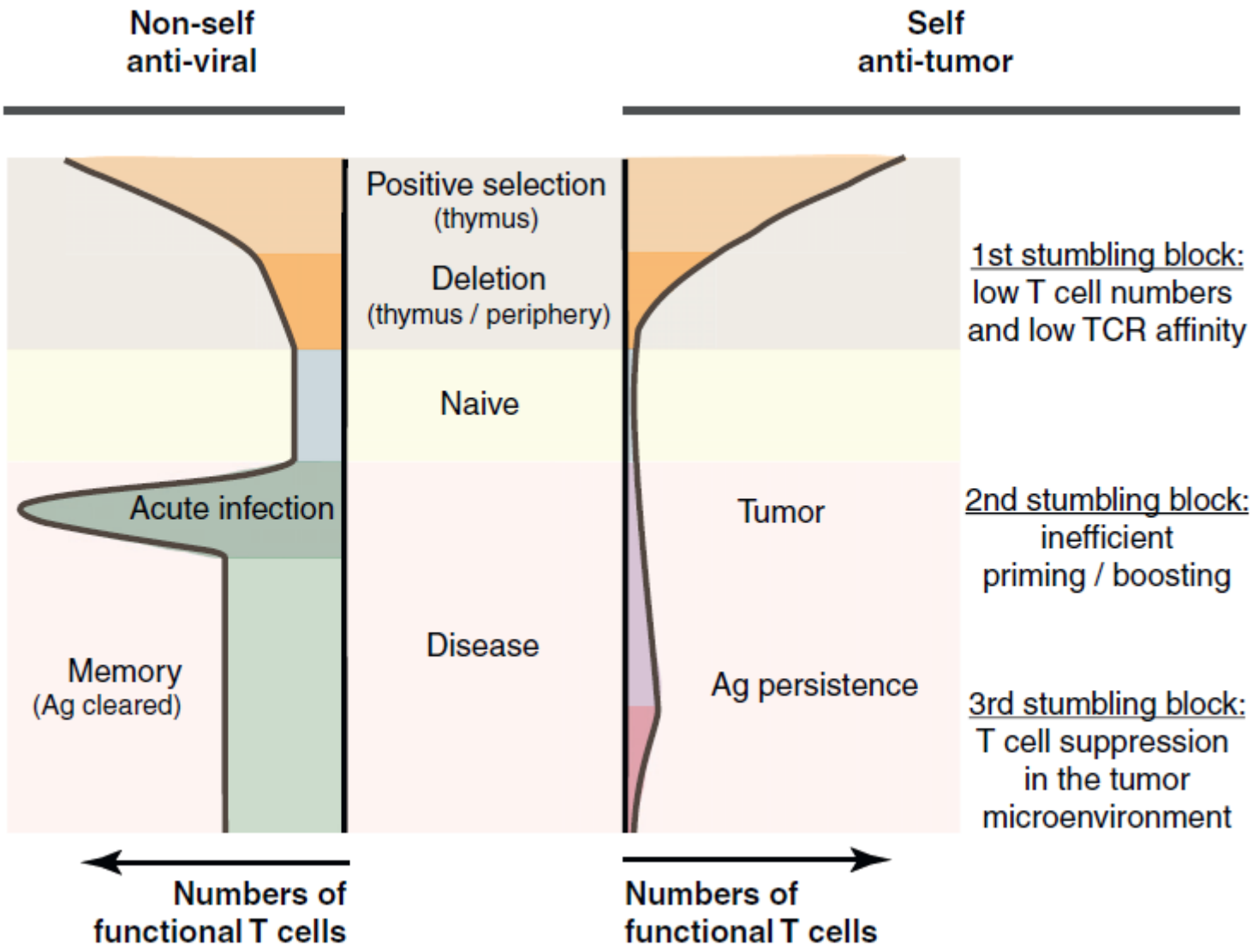
| | |
|---|---|
| Immune-related complete response (irCR) | Complete disappearance of all lesions (whether measurable or not, and no new lesions) |
| | Confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented |
| Immune-related partial response (irPR) | Decrease in tumor burden ≥ 50% relative to baseline |
| | Confirmed by a consecutive assessment at least 4 weeks after first documentation |
| Immune-related stable disease (irSD) | Not meeting criteria for irCR or irPR, in the absence of irPD |
| Immune-related progressive disease (irPD) | Increase in tumor burden ≥ 25% relative to nadir (minimum recorded tumor burden) |
| | Confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented |

Wolchok JD, et al Clin Cancer Res 2009; 15:7412-7420.

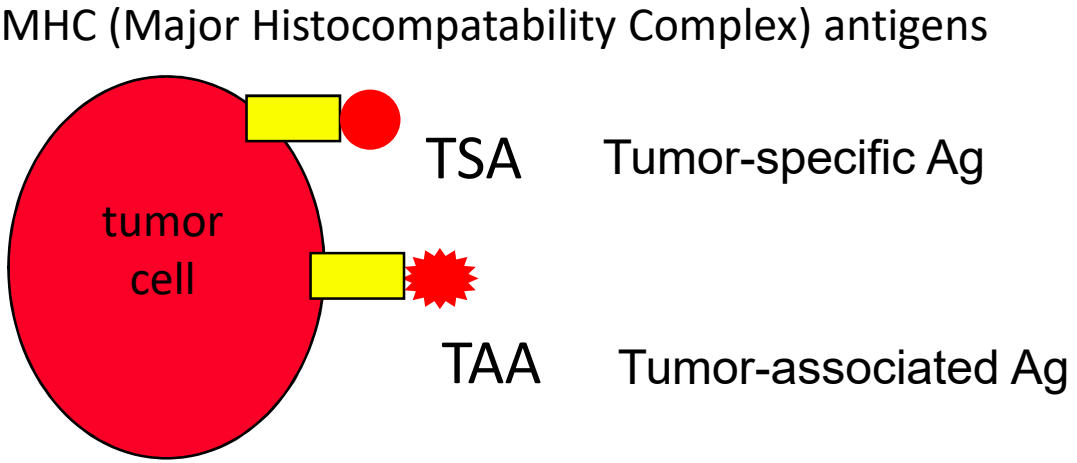
# Adoptive DC vaccination therapy



# Functional T cells: comparison infection vs. tumor



# “Novel” Antigens expressed on tumour cells




TSA: Tumor-Specific Antigen; unique to tumor

- play an important role in tumor rejection, e.g. product of mutant genes, chromosomal rearrangements etc.

TAA: Tumor-Associated Antigen; shared by normal and tumor cells

- oncofetal Ag: found in cancer cells and during development, used in diagnosis, e.g. Alpha-FetoProtein (AFP) for hepatocellular cancer, Carcinoembryonic Ag (CEA) for intestinal cancer
- cancer-testis Ag: expressed in tumor but silent in most other tissues except testis and placenta, e.g. MAGE for melanoma
- differentiation antigens: normally expressed at very low levels and therefore not tolerance inducing, e.g. MART for melanoma

# Tumor associated antigens

- more than 200 described
- majority not broadly expressed among tumors
  => patient analysis and selection
- majority not important for cancer cell function
  => immune escape by rapid selection for cell clones which lost the antigen

**ideal targets**

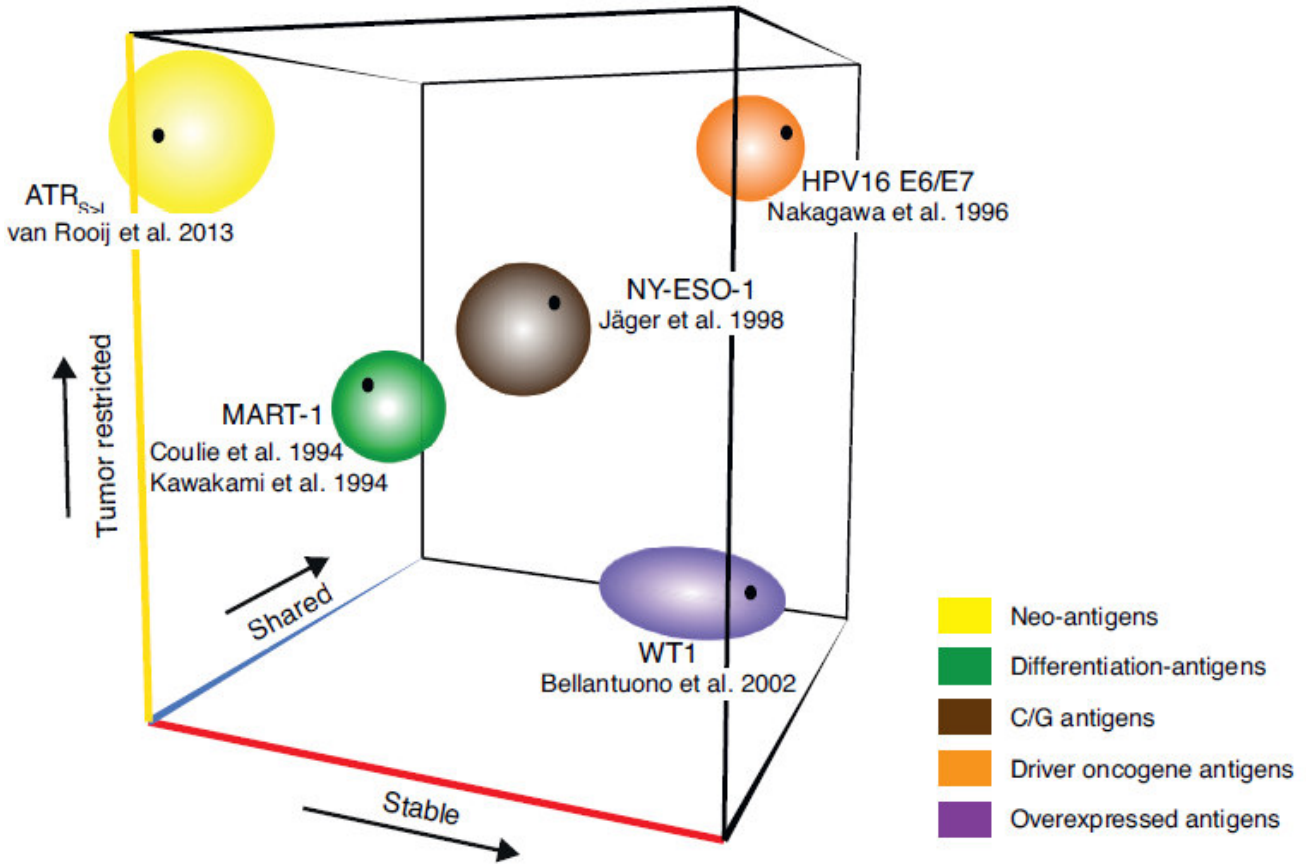
- TAA specifically and broadly expressed among tumors
- important for tumor cell growth and/or survival
- e.g. survivin
- tumor-specific antigens: mutant versions of endogenous genes which are antigenic

**highly stable epitopes should be those that can be considered essential for tumor cell survival or proliferation**
e.g. mutations in driver oncogenes such as the $BRAF^{V600E}$ mutation found in 40% of melanomas

Nevertheless, tumor cell escape through antigen loss may still occur:

- known driver mutations have recently been shown to sometimes display a heterogeneous distribution pattern in human tumors
- rapid emergence of tumor resistance upon treatment with targeted inhibitors (such as BRAF inhibitors) suggests that human tumors may perhaps rely less on specific mutations than anticipated

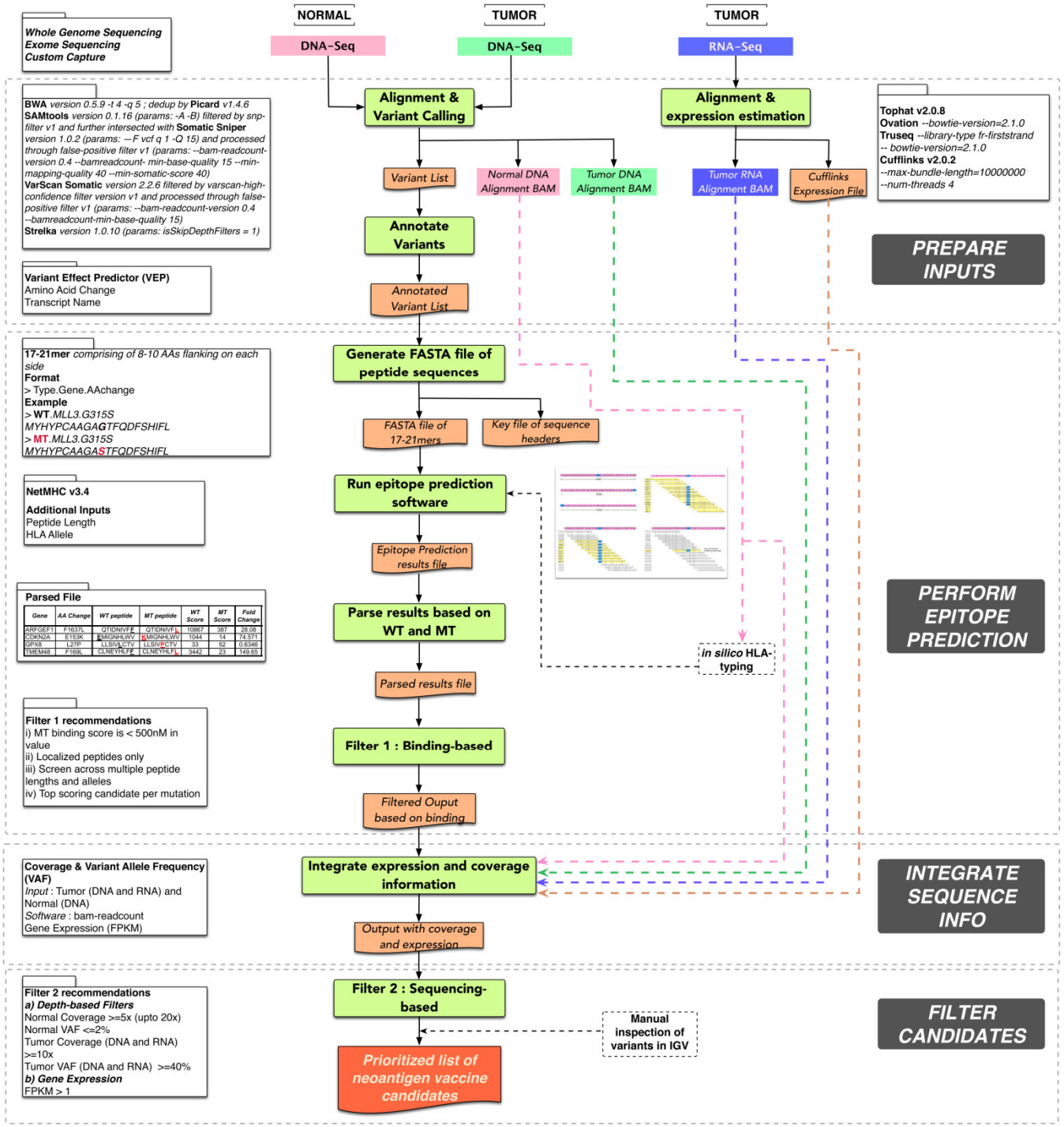
# Useful Tumor Antigens




SHARED: the extent to which these antigens are shared by larger patient groups
TUMOR RESTRICTION: the degree of tumor-restrictive expression
STABILITY: the likelihood of antigen loss the moment selection pressure is applied

# Cancer sequencing to identify neo-antigens

- identification of patient specific point mutations can be done easily by sequencing
- expression levels and mutant allele frequency are taken into account when selecting targets
- bottleneck is the prediction of antigenicity which is still not very efficient
- typically combinations of up to 20 peptides are used for validation and up to 10 for vaccination; only about 30% of those produce an immune response




*Hundal et al., Genome Med 2016*

# Types of Tumor Vaccines

| Type of vaccine | Vaccine preparation | Animal models | Clinical trials |
|---|---|---|---|
| Killed tumor vaccine | Killed tumor cells + adjuvants | Melanoma, colon cancer, others | Melanoma, colon cancer |
| | Tumor cell lysates + adjuvants | Sarcoma | Melanoma |
| Purified tumor antigens | Melanoma antigens | Melanoma | Melanoma |
| | Heat shock proteins | Various | Melanoma, renal cancer, sarcoma |
| Professional APC-based vaccines | Dendritic cells pulsed with tumor antigens | Melanoma, B cell lymphoma, sarcoma | Melanoma, non-Hodgkin's lymphoma, prostate cancer, others |
| | Dendritic cells transfected with genes encoding tumor antigens | Melanoma, colon cancer | Various carcinomas |
| Cytokine- and costimulator-enhanced vaccines | Tumor cells transfected with cytokine or B7 genes | Renal cancer, sarcoma, B cell leukemia, lung cancer | Melanoma, sarcoma, others |
| | APCs transfected with cytokine genes and pulsed with tumor antigens | | Melanoma, renal cancer, others |
| DNA vaccines | Immunization with plasmids encoding tumor antigens | Melanoma | Melanoma |
| Viral vectors | Adenovirus, vaccinia virus encoding tumor antigen +_ cytokines | Melanoma, sarcoma | Melanoma |

# Results of recent clinical trials

- **Wide variation in markers of response:**

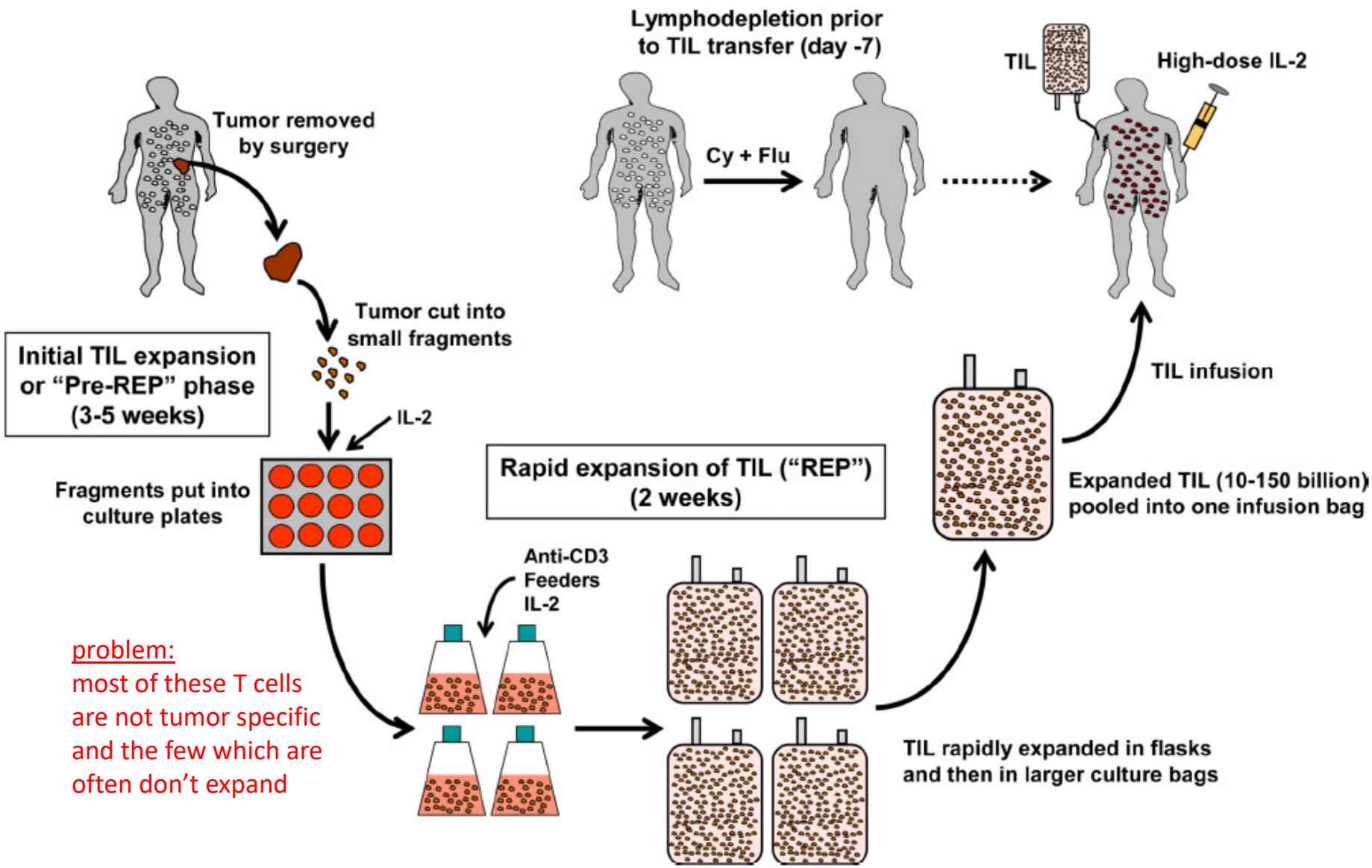
  Evidence of immune response (IR) through delayed type hypersensitivity (DTH), CD4 proliferation, isolation of tumour-specific cytotoxic T lymphocytes (CTL) in periphery and detection of tumor infiltrating lymphocytes (TIL).

- **How do these reflect true responses to therapy?**

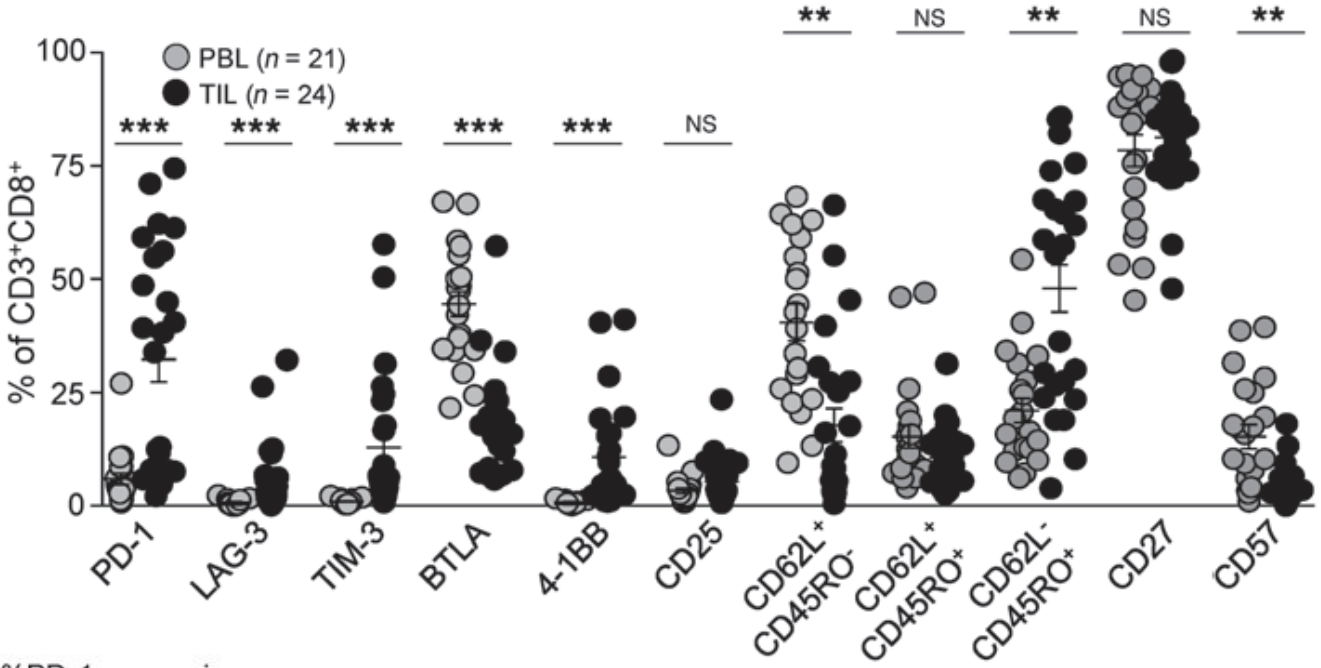
| Peptide vaccine trials | Tumour vaccine trials | DC vaccine trials |
|---|---|---|
| 175 patients total | 142 patients total | 257 patients total |
| 7 patients responded | 6 patients responded | 16 patients responded |
| (4.0%) | (4.2%) | (6.2%) |

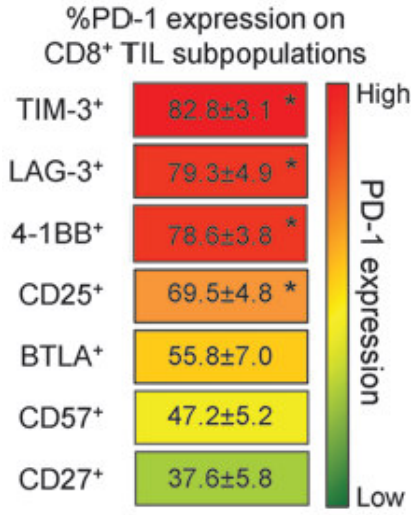
**Total for all cancer vaccine studies = 3.8%**

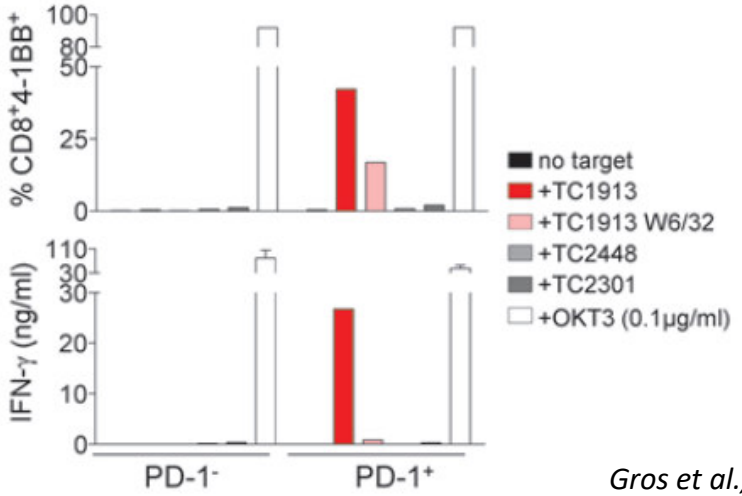
# Adoptive T cell therapy



# How to isolate tumor specific CTLs?
# Identification of surface markers

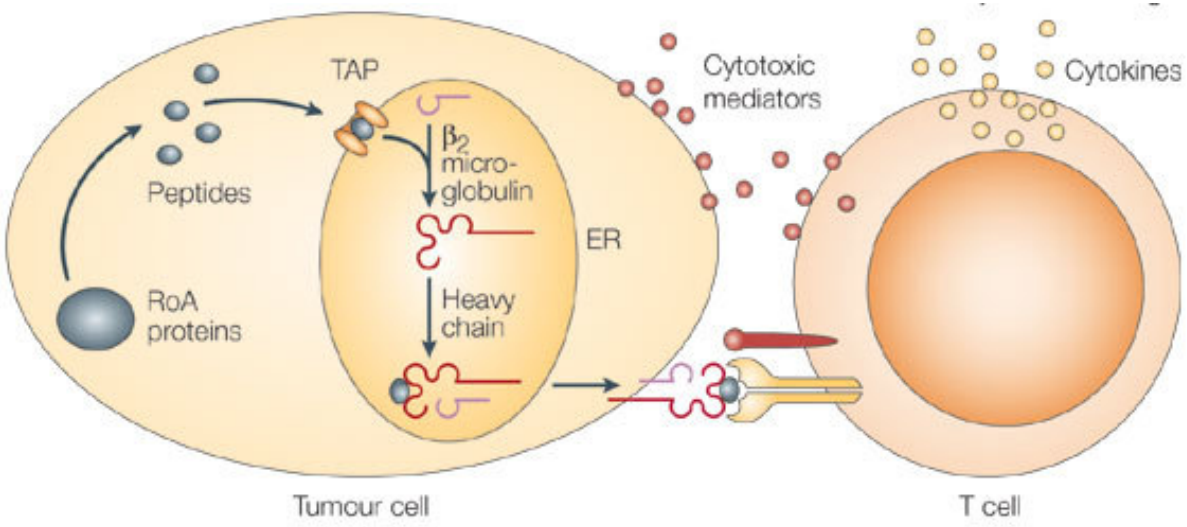


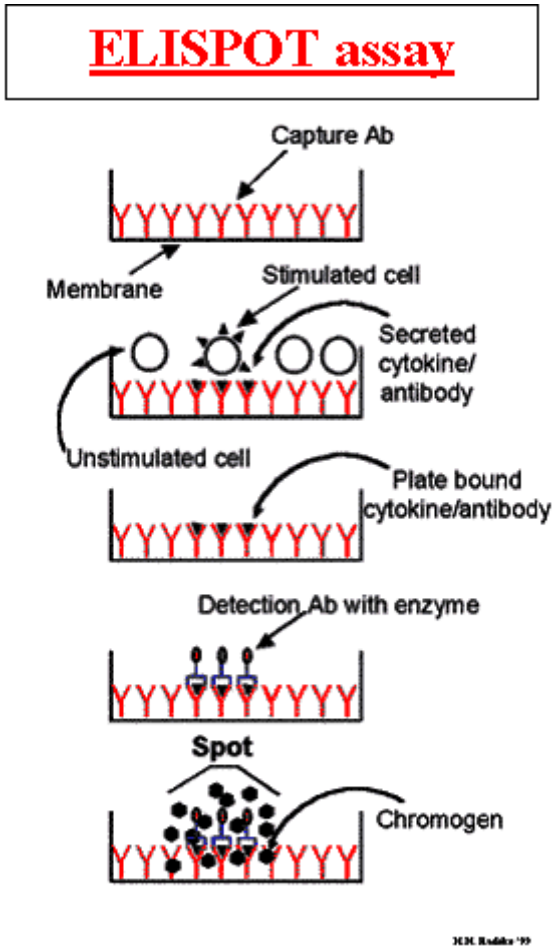



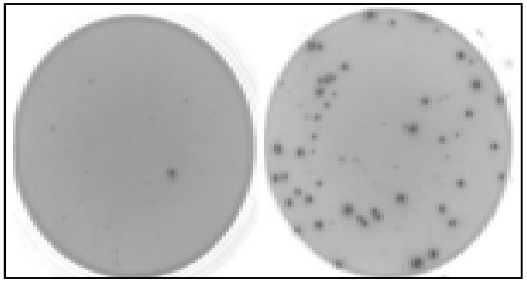




*Gros et al., J Clin Inv 2014*

# How to measure presence of CTLs?



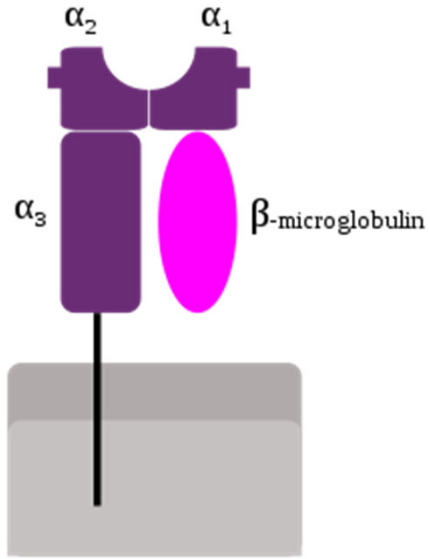



no peptide peptide

no tumor cells tumor cells

# How to quantify antigen specific CTLs?

- several different MHC1 haplotypes exist in the human population
  - any CTL will be reactive only against one particular MHC
  - fortunately, HLA-A*02 is globally common (20-50%)

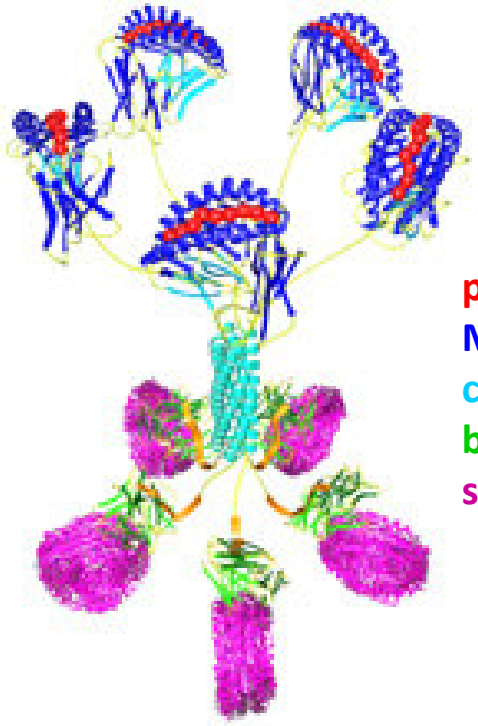



- rules have been identified which help to predict peptides bound to MHC1
  - typically many peptides still have to be tested to identify good binders
  - such peptides are “loaded” onto MHC1 molecules to measure binding/presentation
  - affinity maturation by amino acid substitutions

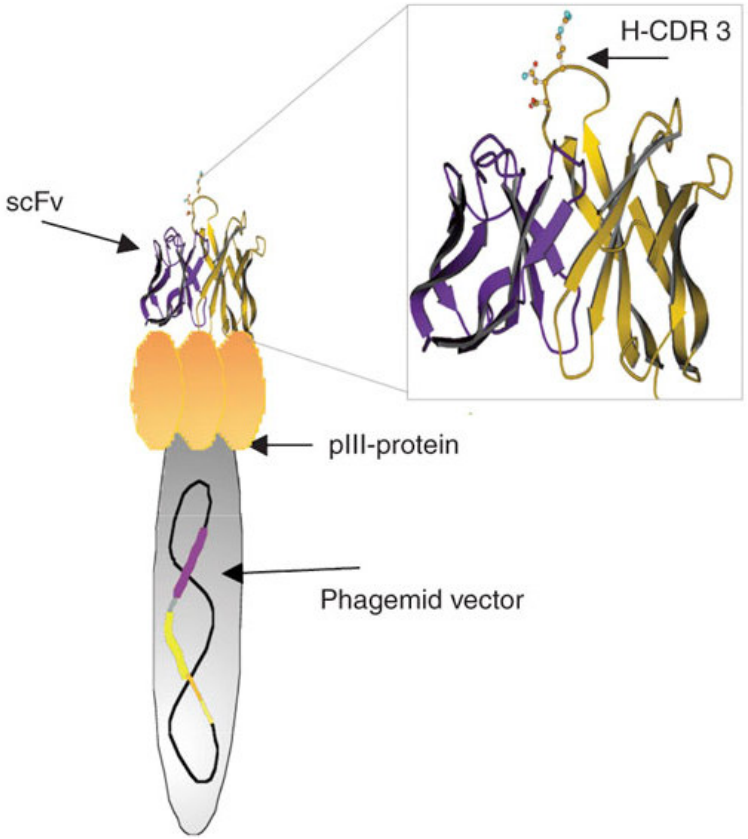
# How to quantify antigen specific CTLs?

- loaded MHC1 molecules can be used to isolate CTLs from patients with tetramers/pentamers and magnetic bead technology

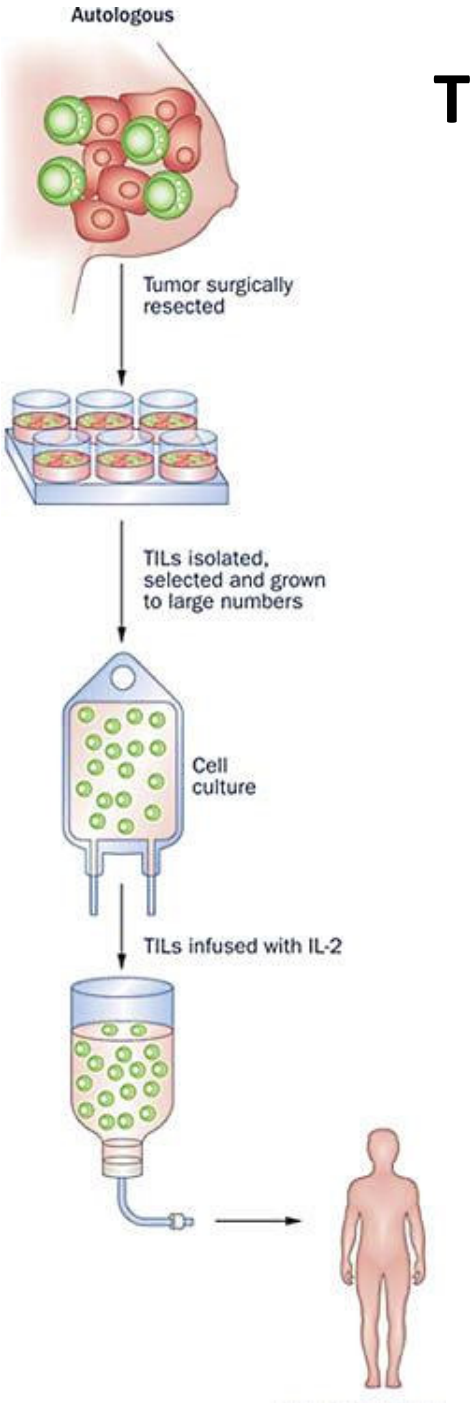
- loaded MHC1 can be also used to perform screens in phage displays expressing TCR fragments



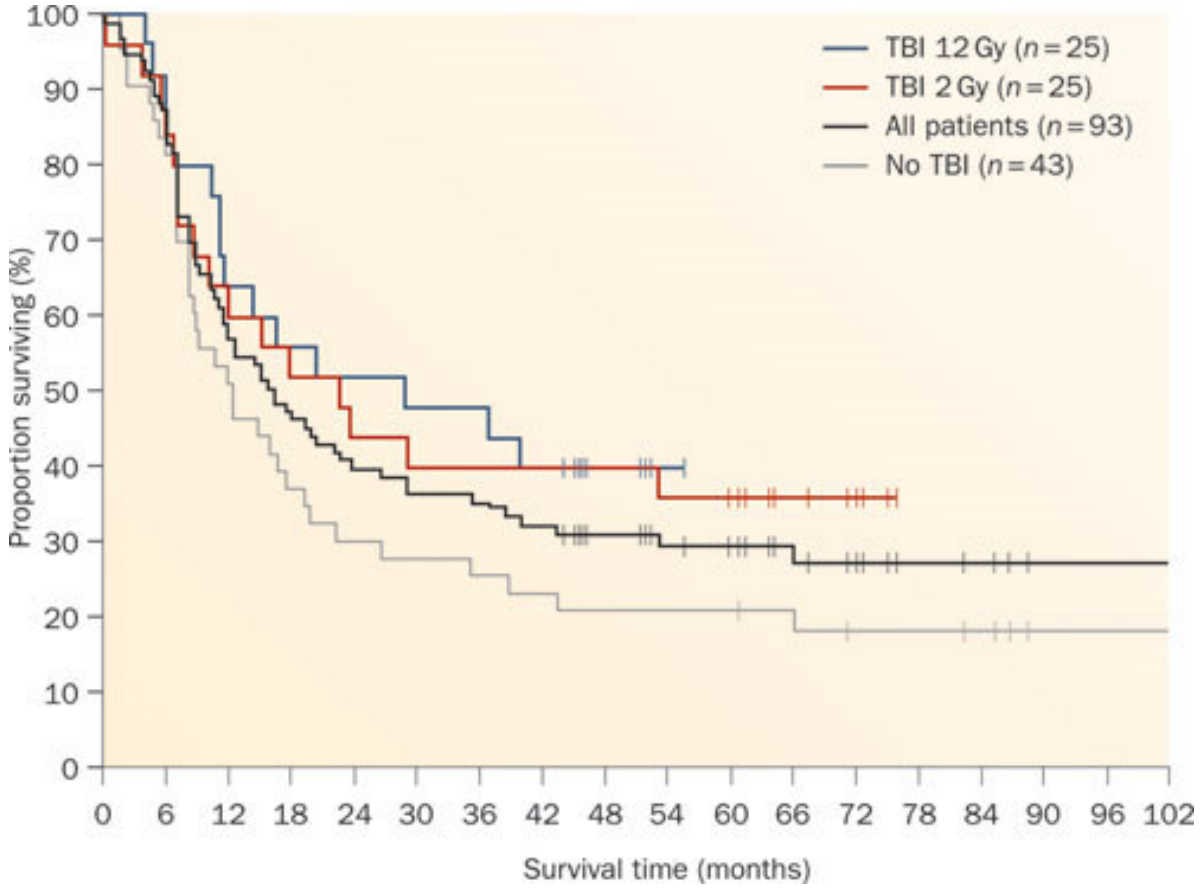
**peptide**
**MHC1**
**coiled-coil**
**biotin**
**streptavidin**



# TIL treatment: clinical outcomes

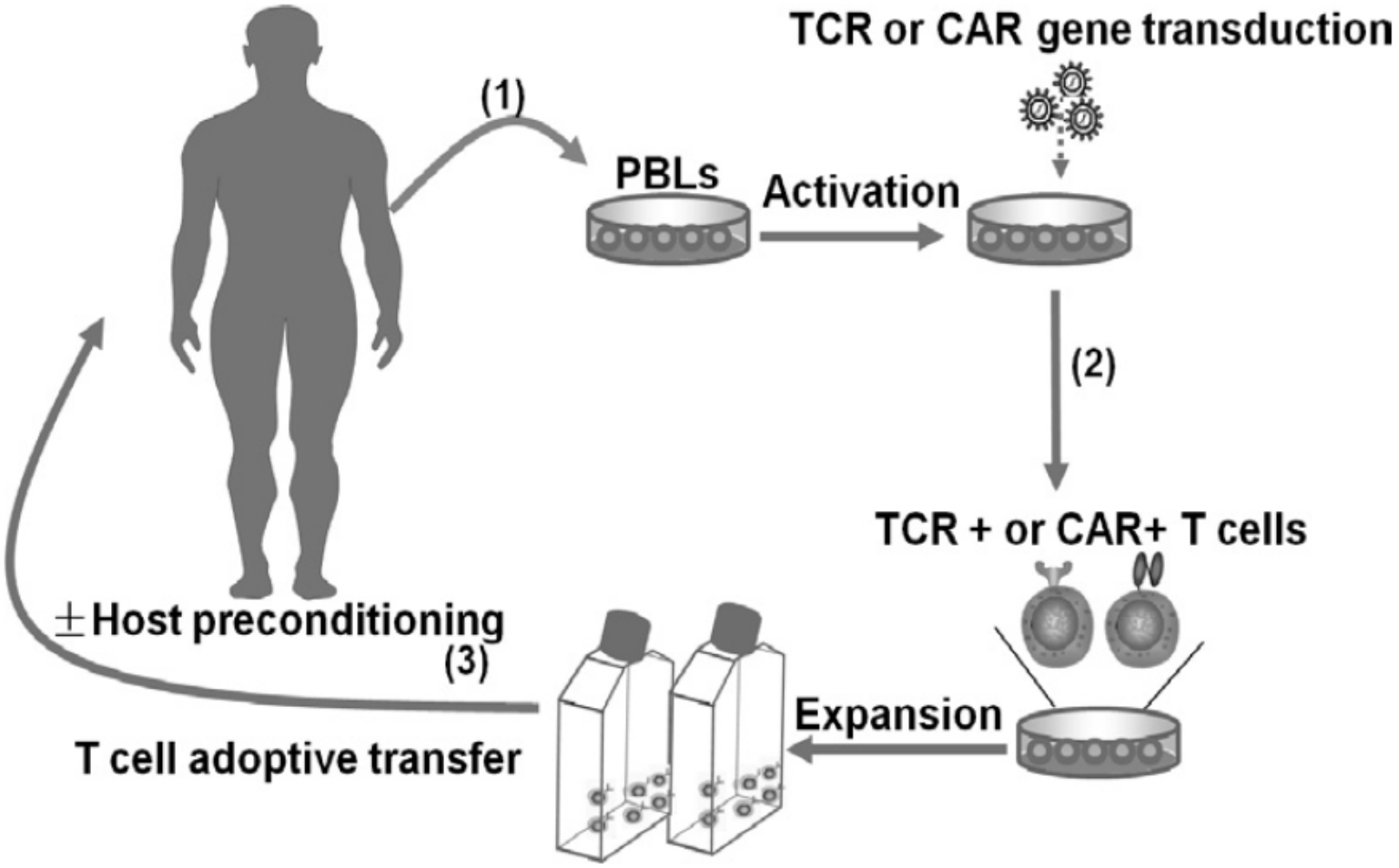



metastatic melanoma treated with autologous TIL

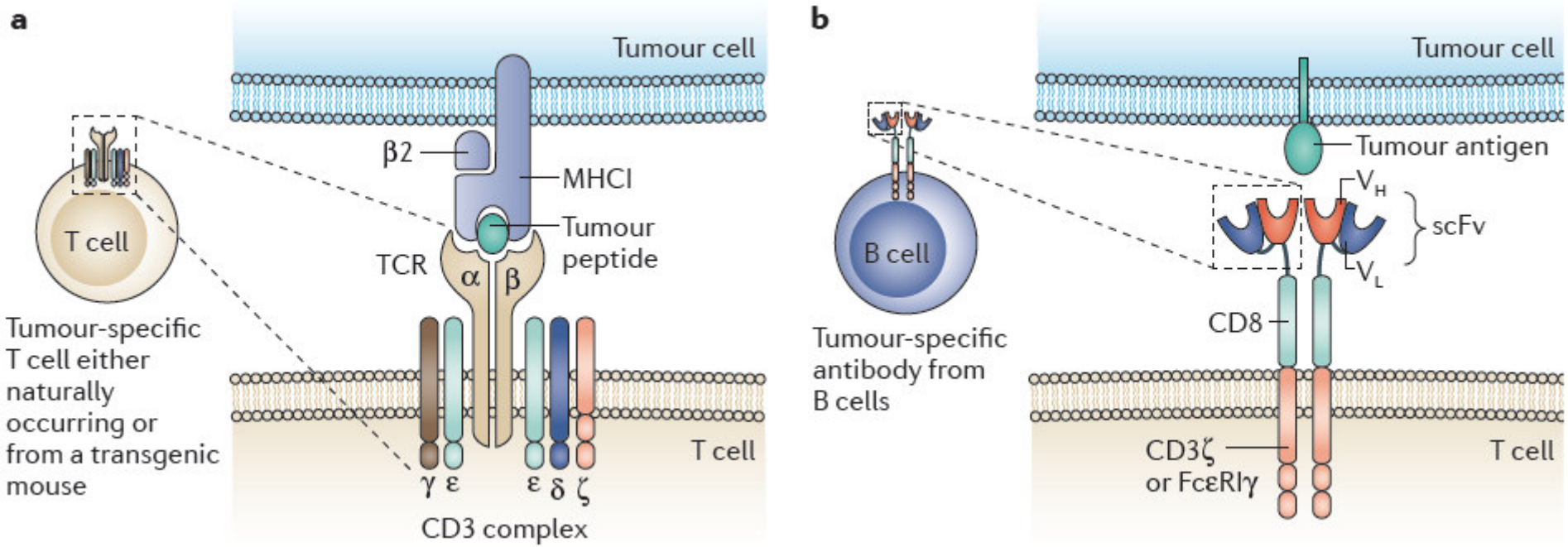



*Rosenberg, Nat Rev Clin Onc 2011*

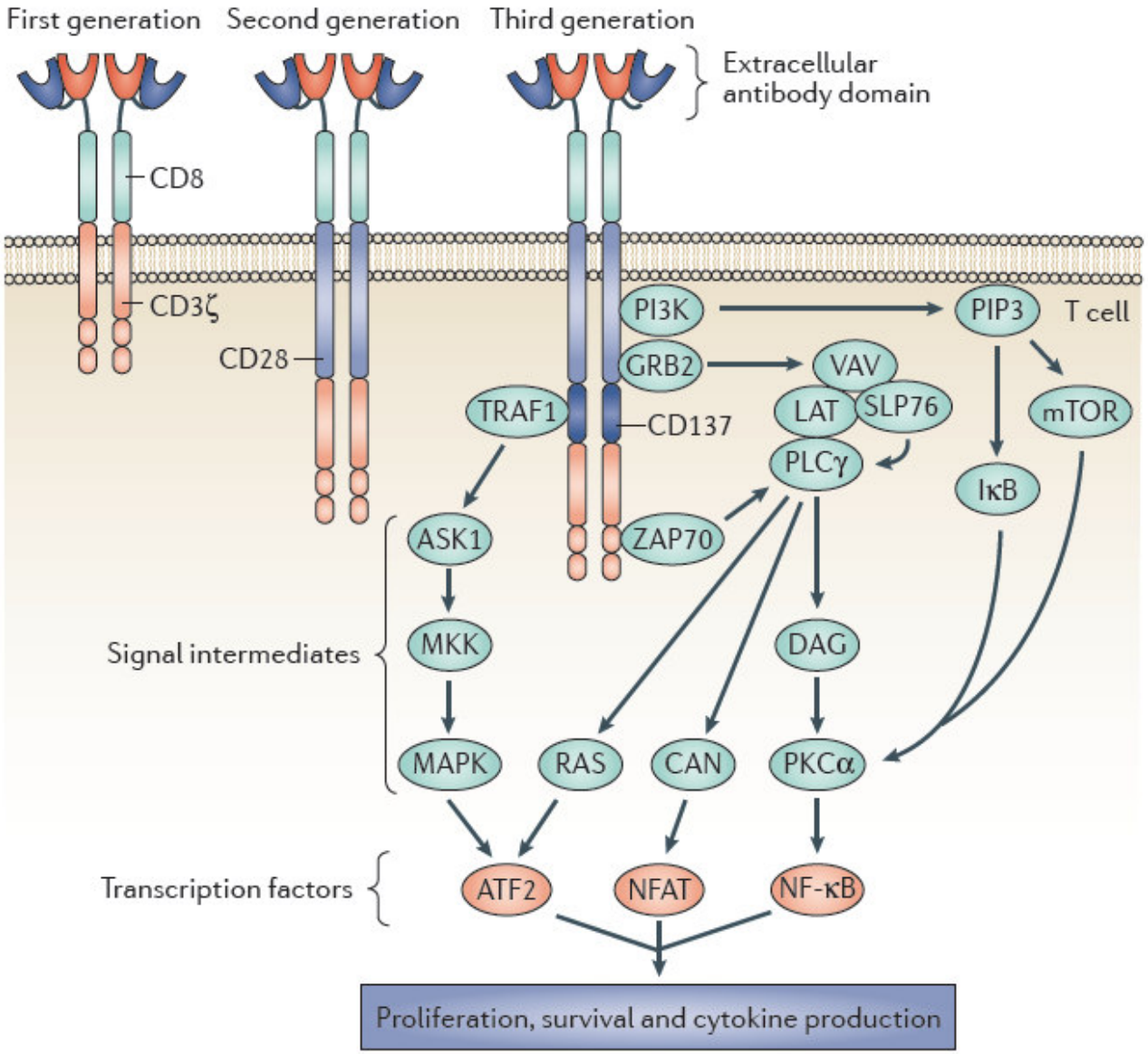
# Adoptive therapy with genetically modified T cells



# CAR T cells: chimeric antigen receptors allow to target additional antigens



# CAR T cells: additional signaling domains allow to create immune “special forces”



# Adoptive transfer of genetically engineered CTLs

## Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes

Richard A. Morgan, Mark E. Dudley, John R. Wunderlich, Marybeth S. Hughes, James C. Yang, Richard M. Sherry, Richard E. Royal, Suzanne L. Topalian, Udai S. Kammula, Nicholas P. Restifo, Zhili Zheng, Azam Nahvi, Christiaan R. de Vries, Linda J. Rogers-Freezer, Sharon A. Mavroukakis, Steven A. Rosenberg*

TCRs recognizing: MART-1, gp100, NY-ESO-1, p53
disease free (2/15 patients)

## Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells

Brian G. Till,[1,2] Michael C. Jensen[3], Jinjuan Wang,[1] Eric Y. Chen,[1] Brent L. Wood,[4] Harvey A. Greisman,[4] Xiaojun Qian,[1] Scott E. James,[1] Andrew Raubitschek,[5] Stephen J. Forman,[6] Ajay K. Gopal,[1,2] John M. Pagel,[1,2] Catherine G. Lindgren,[2] Philip D. Greenberg,[1,2] Stanley R. Riddell,[1,2] and Oliver W. Press[1,2]

disease free (2/7 patients); stable disease (4/7)

## Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19

James N. Kochenderfer,[1] Wyndham H. Wilson,[2] John E. Janik,[2] Mark E. Dudley,[1] Maryalice Stetler-Stevenson,[3] Steven A. Feldman,[1] Irina Maric,[4] Mark Raffeld,[3] Debbie-Ann N. Nathan,[1] Brock J. Lanier,[1] Richard A. Morgan,[1] and Steven A. Rosenberg[1]


CTL transduced with anti-CD19 scFv fused to CD28 and TCRζ
complete regression (1/1 patient)

# Safety of clinical trials using gene modified T cells

| Cell type | Antigen | Cancer type | Toxicities | Comments |
|---|---|---|---|---|
| TCR-T cells | MART1 | Melanoma | No toxicity | No toxicities because of the low avidity TCR |
| | MART1 and gp100 | Melanoma | Inflammation and destruction of normal melanocytes in the skin, eye, and ear | Various on-target toxicities due to the high-avidity TCRs |
| | CEA | Colorectal cancer | Colitis | On target toxicity due to the TCR-T cells recognizing CEA expressed within normal colonic mucosa |
| | NY-ESO-1 | Synovial sarcoma and melanoma | No toxicity | No on-target toxicities were seen because of the lack of NY-ESO-1 expression of normal tissues |
| CAR-T cells | CAIX | Renal cell carcinoma | Liver toxicity | On-target toxicity because of unanticipated expression of CAIX in biliary epithelium |
| | L1-CAM (CD171) | Neuroblastoma | Grade 3 lymphopenia, neutropenia, low hemoglobin, bacteremia and pneumonitis | No apparent evidence of toxicities to tissues known to express CD171 (e.g. adrenal medulla, central nervous system and sympathetic ganglia) |
| | Her2 | Colorectal cancer | Lung toxicity | Patient passed away may due to the cytokine storm and respiratory failure triggered by the recognition of low levels of Her2 on lung epithelial cells |
| | FRα | Ovarian cancer | No toxicity | FRα is relative safe target because the expression in normal tissues is restricted to the apical surface of some polarized epithelial cells of normal tissues |
| | CD19 | Lymphoma | Depletion of normal B cells | CD19 expression in normal tissues is limited to "non-vital" tissues, and thus represents attractive tumor target |
| | CD20 | Lymphoma | No toxicity | Like CD19, CD20 is also an attractive tumor target |

Abbreviations: CEA, carcinoembryonic antigen; CA IX, carbonic anhydrase IX; L1-CAM, L1-cell adhesion molecule; FRα, Folate receptor-alpha.

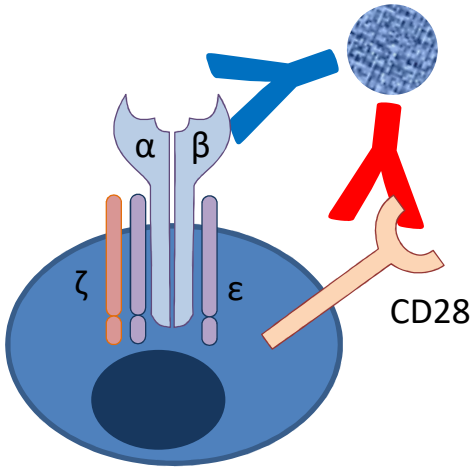
| | |
|---|---|
| off-target toxicity: | does basically not occur |
| on-target, off-tumor toxicity: | weak antigen expression on normal cells of other tissue, can be reduced by lower affinity CARs |
| too high on-target efficacy: | high killing rate will induce **cytokine-storm**, can be avoided by slower dose-escalation scheme |

# Scaling up adoptive T cell therapy

Is it only a **boutique therapy** that is impossible to **commercialize**?

**automating production:**

- routine expansion of T cells by CD3/CD28 bead technology (sufficient quantities of T cells within ten days)
- use of RNA electroporation (safety and quality control)




**regulation issues:**

- rules set up by the US FDA and the European Medicines Agency are largely intended for drugs prepared and tested far in advance of medical need
- regulations and standards must evolve to reflect products that are personalized and sometimes administered in settings of urgent medical need

- for classical drugs, a single production run serves thousands of patients and allows extensive quality controls (**scale up**)
- personalized therapies are produced for a single patient, so mainly “know how” has to be ‘**scaled out**’ to multiple medical centers with technically advanced operating theatres, laboratories and medical staff

# Genetic engineering strategies