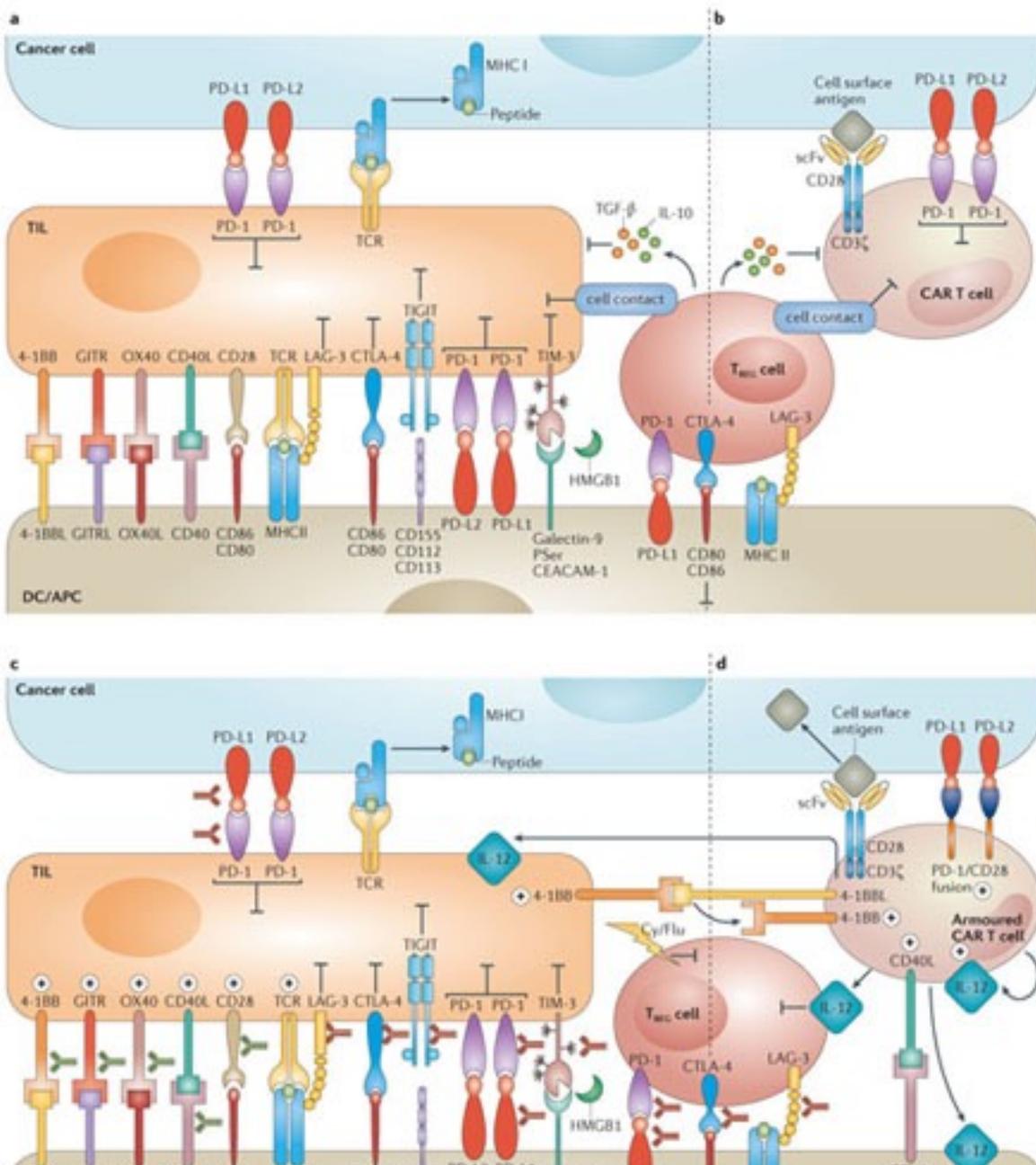


# Review of cancer immunotherapy

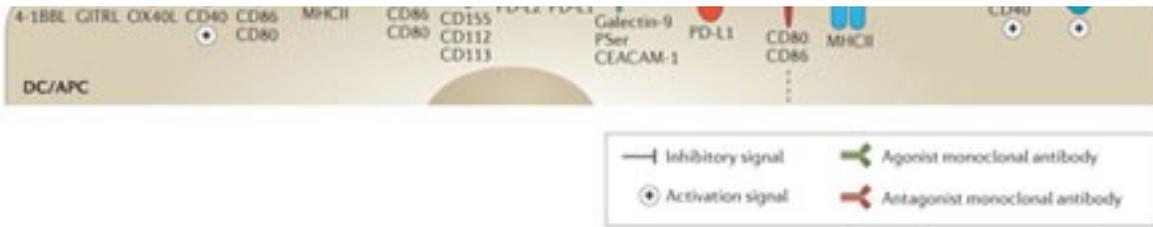
## Key points:

- \* Cancer immunotherapies have the potential to generate robust anti-tumor responses; through antibodies or adoptive cellular therapy
- \* Since 2010, clinical trials using different immunotherapeutic approaches to treat patients
- \* In contrast with therapies that act on the tumor itself, immunotherapy-dependent anti-tumor
- \* The optimal efficacy of immunotherapy will likely be achieved with designs that include c

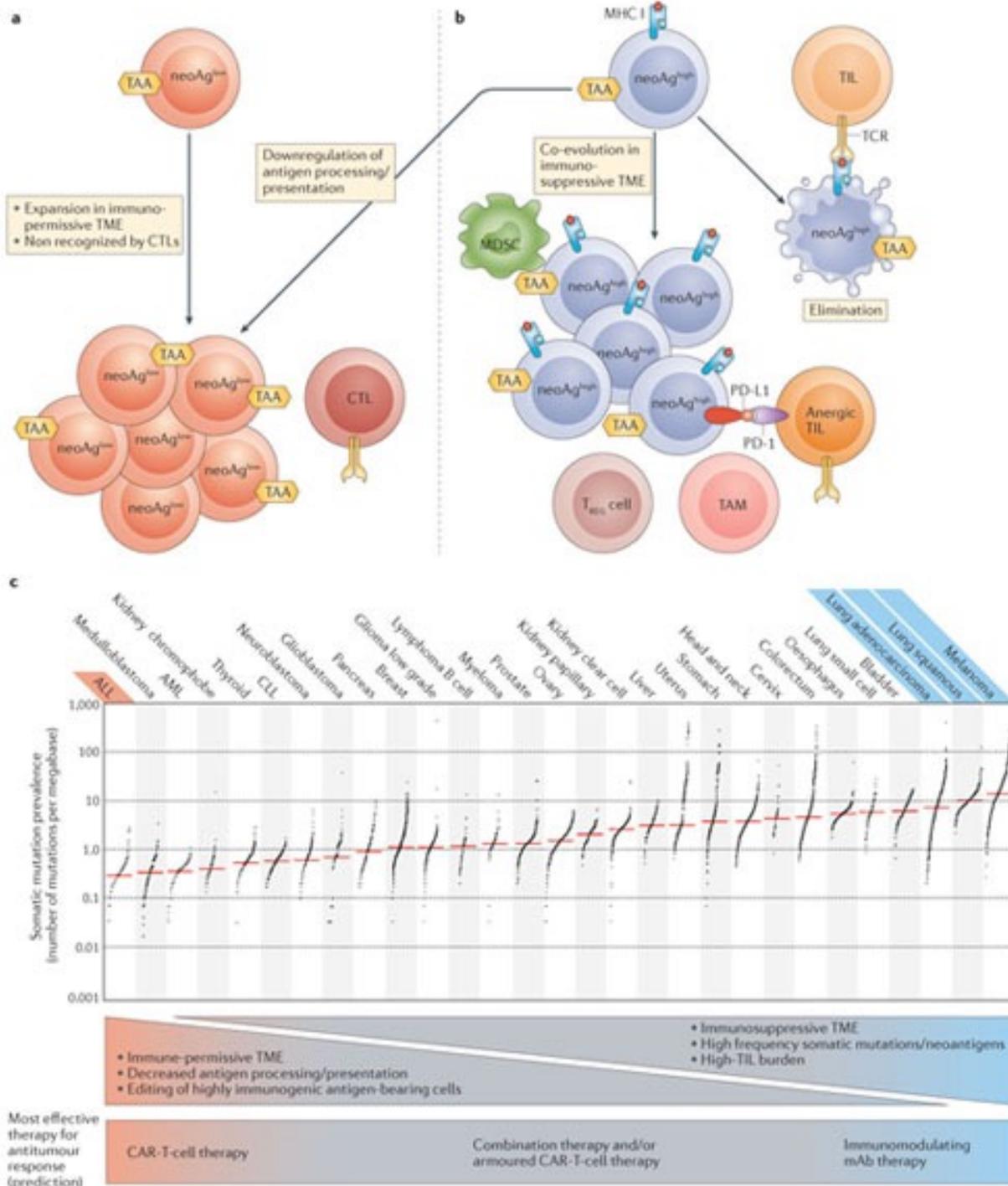


is can be achieved through several methods, such as modulatory

s with several tumor types have yielded unprecedented results  
umor responses can be sustained after the treatment has finished  
combinations of different immunotherapeutic approaches, or im



munotherapy combined with



in other cancer



## **Immune modulation**

Using mAbs to target cancer cells is complicated by the fact that cancer cells are self and vary greatly. **more reliably used to activate or inhibit the action of the patient's immune system.** These mAbs target particularly T-cells, that can then respond to cancer antigens if they are presented to the T-cells. For any type of vaccination, they must have primed T-cells that are actively being held in check by the immune system [Khalil et al., 2016]

Patients with C-reactive protein (a liver protein whose levels rise when inflammation is present) or immune modulation. Patients with high levels of myeloid-derived suppressor cells or high levels of soluble TIGIT (a marker of maturation) of T cells), are less likely to respond to immune modulation [Khalil et al., 2016]

## **PD-1 (programed cell-death protein 1)**

Programed cell death (aka apoptosis) is a form of intentional and highly regulated cell death that occurs in Apoptotic cells fragment and 'bleb' into small particles that are then cleaned up by macrophages. PD-1 is a receptor that maintains self-tolerance (prevents the body from attack by its own immune system) through 'checkpoint signaling'. Blocking PD-1 with mAbs prevents tumor-targeting T-cells from death and is used against melanoma & non-small-cell lung cancer. [Khalil et al., 2016]

**T-cell co-stimulation** uses mAbs to stimulate T-cell co-receptors, increasing activation of T-cells

**Creation of immune memory** against tumors could prevent recurrence and escape of tumors. Tumor cells in patients who received immunotherapy. [Khalil et al., 2016]

## **CTLA-4 (cytotoxic T-lymphocyte antigen-4)**

T-cells are primed when their T-cell receptor (TCR) binds to a peptide fragment of their antigen presented by a protein on the surface of an antigen presenting cell (APC) such as a dendritic cell. But primed T-cells also need the CD80 or the CD86 protein on the surface of the APC. Without this second signal, primed T-cells can lead to autoimmune responses. [Khalil et al., 2016]

In activated T-cells, CTLA-4 moves to the cell surface where it binds to the APCs CD80 or CD86 and 'dampens' T-cell activation and thus restricts the ability of T-cells to kill tumor cells; CTLA-4 controls

Regulatory T-cells always express CTLA-4 on their cell membranes and use it to suppress the activation of T-cells by binding to the CTLA-4 on the primed T-cells and on regulatory T-cells and preventing CTLA-4 from binding to antigen presenting cells. [Khalil et al., 2016]

Anti-CTLA-4 mAbs (ipilimumab) allowed patients with advanced melanoma to mount a better immune response and continued to survive even after mAb treatment stopped. [Khalil et al., 2016]

greatly with the type of cancer and the individual. However, **mAbs can be** mAbs essentially act as adjuvant since they activate the immune system, s. Since some cancer patients have strong responses to these mAbs without the cancer; active suppression of a potentially functional immune response.

) and good lymphocyte levels are most likely to benefit from immune (T-cell) soluble CD25 (soluble interleukin-2 receptor; IL-2 stimulates differentiation (aka

at removes targeted self cells without provoking an immune response to self. es.

une system) by killing T-cells that target self. This process is called 'immune d thus allows the immune response to the tumor. Anti-PD-1 mAbs are being

. [Khalil et al., 2016]

This type of effective memory response has occurred in some melanoma

(a target protein) presented in an MHC (major histocompatibility complex) -cells do not become active until their CD28 surface protein binds to either cells become quiet (anergic) or commit suicide (apoptosis); this prevents

more strongly than CD28 does. By competing with TCR activation, CTLA-4 strains T-cell responses. [Khalil et al., 2016]

tivity of other primed T-cells. Anti-CTLA-4 mAbs allow T-cells to be active by om disrupting the (stimulatory) binding of T-cell CD28 to CD80 & CD86 on

immune response to their tumors. The 20% of treated patients who survived

Immune-related adverse effects (irAE) can occur after treatment with anti-CTLA-4 mAb when the goal is maintaining the immune response. And some patients given anti-CTLA-4 mAb have some tumor responses [Khalil et al., 2016]

Anti-CTLA-4 mAbs may be most effective in combination with other therapy where the mAb blocks the immune response. Anti-CTLA-4 mAb, ipilimumab, in combination with chemotherapy and cryoablation all show better responses with anti-CTLA-4 mAb. [Khalil et al., 2016]

### **PD-1**

PD-1 is also an inhibitory receptor expressed on the surface of T-cells. APCs and cancer cells express B7-1 and B7-2 on their surface. Binding of B7-1 or B7-2 to PD-1 on T-cells prevents activation of primed T-cells; effectively suppressing the T-cell response. In a phase 1 trial, pembrolizumab (anti-PD-1 mAb) caused responses in 40% of patients with advanced melanoma, including those who did not respond to ipilimumab. Anti-PD-1 mAbs is even more effective and is now the treatment of choice. Two anti-PD-1 mAbs, pembrolizumab and nivolumab, are now being used to treat a number of other cancers. There are also anti-PD-1L mAbs now being used to treat cancer. [Khalil et al., 2016]

Anti-PD-1 mAbs generally produce fewer irAE side effects than anti-CTLA-4 mAbs, though pulmonary toxicity is still a concern.

**Lymphocyte-activation gene 3 (LAG-3)** is expressed on the cell surface of activated T-cells (CD4+), regulatory T-cells, and exhausted T-cells. It sends an inhibitory signal to T-cells but stimulates the ability of regulatory T-cells to suppress immune responses. 'exhausted' and are dysfunctional; this is seen in cases of chronic infection. Soluble LAG-3 is seen in the blood of patients with chronic infection. LAG-3 can function. Under development and showing promise: soluble LAG-3-Ig fusion protein.

**T-cell membrane protein 3 (TIM-3)** (aka hepatitis A virus cellular receptor 2) is also seen on exhausted T-cells. Blocking TIM-3 increases proliferation and function of T-cells and increases anti-tumor activity in murine models of melanoma & sarcoma, more when combined with blockade of PD-1. [Khalil et al., 2016]

**T-cell immunoreceptor with Ig and ITIM domains (TIGIT)** is expressed on T-cells and also associated with anti-tumor activity. [Khalil et al., 2016]

### **T-cell co-stimulation**

This strategy uses agonist (stimulating) mAbs to co-stimulatory molecules on T-cells to activate T-cells. The BTLA-1 (CD226) and HVEM (CD270) family are promising targets. [Khalil et al., 2016]

**T-cell antigen 4-1BB homolog (4-1BB)** (aka CD137 or TNFR superfamily member 9) is expressed on T-cells. 4-1BBL on the surface of dendritic cells (a potent type of APC) causing T-cell proliferation and immune response. Anti-4-1BB mAb increases preclinical antitumor responses, especially in combination with blockade of PD-1. Anti-4-1BB mAb urelumab is effective in melanoma patients, but caused severe liver toxicity. Anti-4-1BB mAbs should increase natural killer cell destruction of tumors. [Khalil et al., 2016]

the 'disinhibited' immune system attacks self. Most irAE can be managed while tumor growth before a successful immune response against the tumor. [Khalil et al., 2016]

can potentiate the efficacy of the second treatment. Radiation, oncolytic virus

express PD-1 ligands (PD-L1 and PD-L2). Binding of T-cell PD-1 to its ligands on tumor cells leads to immune response. Tumors expressing PD-L1 lead to poorer prognosis. Anti-PD-1 mAbs (pembrolizumab and nivolumab) respond to anti-CTLA-4 mAbs. Treatment with both anti-CTLA-4 and anti-PD-1 mAbs (pembrolizumab (aka keytruda) and nivolumab, are now used against melanoma and lung cancer. [Khalil et al., 2016]

Immune-related adverse events must be watched for and treated. [Khalil et al., 2016]

Regulatory T-cells, B cells and dendritic cells. LAG-3 binds to MHC II on APCs and inhibits immune responses. T-cells that express both LAG-3 and PD-1 are called LAG-3<sup>hi</sup>PD-1<sup>hi</sup> T-cells. High expression in some breast cancer patients and indicates a better prognosis; soluble LAG-3 and LAG-3-blocking mAb. [Khalil et al., 2016]

Exhausted T-cells. TIM-3 blunts T-cell responses and induces apoptosis (suicide) and reduces cytokine production. Blockade of TIM-3 is effective against mouse models of melanoma and lung cancer.

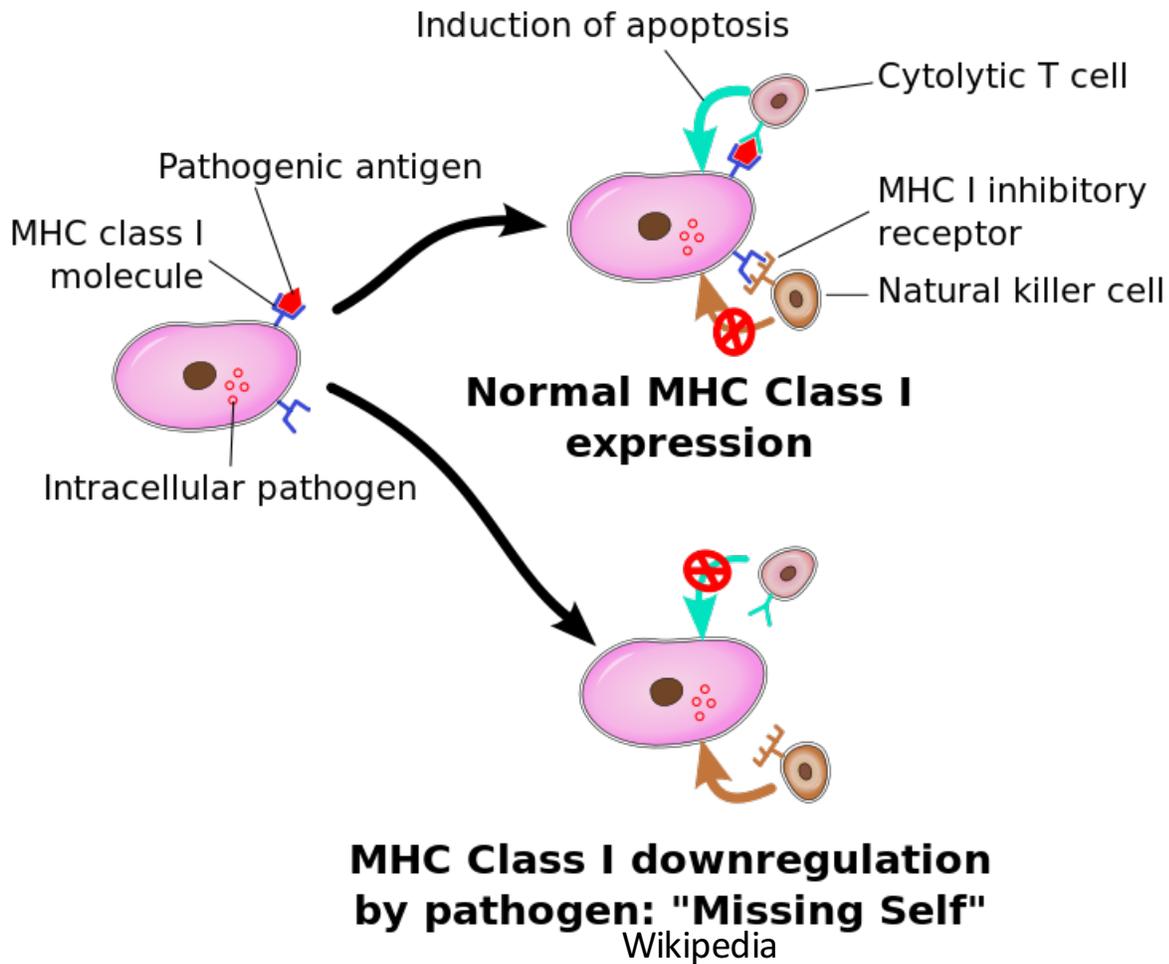
Associated with exhausted T-cells. Blocking TIGIT and PD-L1 and TIM-3 increases

activity of T-cells and enhance their activity. Members of the TNFR (tumor necrosis

receptor family are expressed on the surface of T-cells, natural killer cells & monocytes. 4-1BB binds to HVEM and increases expression of proteins that protect T-cells from apoptosis.

Combination of blocking CTLA-4, activating CD40, use of cellular vaccines, or with radiation and chemotherapy can improve tumor damage. Combination of these mAbs with mAbs targeting tumor cells

Natural killer cells are cells of the innate immune system which kill foreign cells and cancer cells specific antigens, but kill cells that don't express the 'self' antigen MHC I and are therefore not response or tolerance (dampening of the adaptive immune response).



**Glucocorticoid-induced TNFR-related protein (GITR)** (aka superfamily member 18) is expressed on T-cells and is involved in cell proliferation, cytokine production and resistance to suppression. User of anti-GITR agonist mAbs in melanoma, fibrosarcoma, etc. Early clinical studies are in progress.

**CD40** is a member of the TNFR family expressed on APCs: dendritic cells, macrophages, monocytes. T-cells express the ligand CD40L. When T-cell CD40L binds to CD40 on APCs, the APC increases expression and maturation. mAbs can either target CD40 on tumor cells or on immune cells; the latter is probably more effective. anti-CD40 agonist mAbs has ben very effective. [Khalil et al., 2016]

**OX40 (TNFR superfamily 4)** is expressed on T-cells, NK cells and neutrophils (another innate immune cell). OX40 is involved in cell proliferation, survival and secretion of cytokines that stimulate helper T cells and blunts activity of regulatory T cells.

s or cells infected with viruses. NK cells act quickly and are not specific for  
of the body. NK cells also have roles in shaping the adaptive immune

d on activated T-cells but all regulatory T-cells. Activation of GITR increases T-  
mAbs causes tumor rejection & immune memory in mouse models of

ytes, B cells and on cancer cells: melanoma, lymphoma, leukemia. T-cells  
sion of MHC II and pro-inflammatory cytokines. B- cell CD40 stimulates  
ably more significant. For pancreatic cancer the combination of chemo and

mune cell). OX40L is expressed on APCs. Binding of T-cell OX40 causes  
y of regulatory T-cells and increases their death. Preclinical binding of OX40

causes antitumor responses by killer and helper T-cells and immunological memory. Phase I clinical trials are ongoing.

### **Combining checkpoint blockade & co-stimulation**

So, taking off the brake and stomping on the accelerator: co-stimulatory agonist mAbs with blockade of inhibitory checkpoints (e.g., anti-CTLA-4, anti-PD-1, anti-BTLA-1, anti-VISTA, anti-HVEM, anti-TIGIT, anti-CD96, anti-CD138, anti-CD137, anti-CD137L, anti-CD137R1, anti-CD137R2, anti-CD137R3, anti-CD137R4, anti-CD137R5, anti-CD137R6, anti-CD137R7, anti-CD137R8, anti-CD137R9, anti-CD137R10, anti-CD137R11, anti-CD137R12, anti-CD137R13, anti-CD137R14, anti-CD137R15, anti-CD137R16, anti-CD137R17, anti-CD137R18, anti-CD137R19, anti-CD137R20, anti-CD137R21, anti-CD137R22, anti-CD137R23, anti-CD137R24, anti-CD137R25, anti-CD137R26, anti-CD137R27, anti-CD137R28, anti-CD137R29, anti-CD137R30, anti-CD137R31, anti-CD137R32, anti-CD137R33, anti-CD137R34, anti-CD137R35, anti-CD137R36, anti-CD137R37, anti-CD137R38, anti-CD137R39, anti-CD137R40, anti-CD137R41, anti-CD137R42, anti-CD137R43, anti-CD137R44, anti-CD137R45, anti-CD137R46, anti-CD137R47, anti-CD137R48, anti-CD137R49, anti-CD137R50, anti-CD137R51, anti-CD137R52, anti-CD137R53, anti-CD137R54, anti-CD137R55, anti-CD137R56, anti-CD137R57, anti-CD137R58, anti-CD137R59, anti-CD137R60, anti-CD137R61, anti-CD137R62, anti-CD137R63, anti-CD137R64, anti-CD137R65, anti-CD137R66, anti-CD137R67, anti-CD137R68, anti-CD137R69, anti-CD137R70, anti-CD137R71, anti-CD137R72, anti-CD137R73, anti-CD137R74, anti-CD137R75, anti-CD137R76, anti-CD137R77, anti-CD137R78, anti-CD137R79, anti-CD137R80, anti-CD137R81, anti-CD137R82, anti-CD137R83, anti-CD137R84, anti-CD137R85, anti-CD137R86, anti-CD137R87, anti-CD137R88, anti-CD137R89, anti-CD137R90, anti-CD137R91, anti-CD137R92, anti-CD137R93, anti-CD137R94, anti-CD137R95, anti-CD137R96, anti-CD137R97, anti-CD137R98, anti-CD137R99, anti-CD137R100). [Khalil et al., 2016]

**CAR-T-cell therapy** (chimeric antigen receptor) uses ex-vivo (in lab out of body) manipulation of T-cells.

- Expansion of tumor-infiltrating lymphocytes;
- Gene transfer of synthetic sTCR or chimeric antigen receptor (CAR) into T-cells.

CAR uses a single-chain variable fragment (receptor crafted from an antibody gene and the transmembrane domain) that sees peptides that don't need to be bound to MHC (aka HLA). This simplifies T-cell activation and co-stimulation (without APCs??). The next generation of CARs will include more co-stimulatory domains.

Armored CAR T-cells add a second trans gene that gives protection from cell death or increases co-stimulation (e.g., CD40L). [Khalil et al., 2016]

**CD19-targeted CAR-T-cells** are used for B cell lymphomas since CD19 is expressed on B cells but not on T-cells. Chemo to knock down regulatory T-cells prior to the immunotherapy. Some ex-vivo techniques improve persistence of the CAR-T-cells is critical and is higher in pediatric patients. Chemotherapeutic conditioning is better than others. CART therapy may make allogeneic stem cell transplantation unnecessary. B cell lymphoma has a large number of other targets for blood cancers in addition to - or instead of - CD19.

**CART side-effects** include: cytokine-release syndrome; macrophage activation syndrome; neurotoxicity. Side effects can be predicated by serum levels of C-reactive protein. [Khalil et al., 2016]

**CART for solid tumors** is in exploratory stages. Challenges include: immunosuppressive tumor microenvironment; lack of T-cells. [Are parts of the ASPS fusion protein unique?] Clinicals: mesothelioma; ovarian; CNS, etc. See Jackson, H. J., Rafiq, S. & Brentjens, R. J. Driving CART cells forward. Nat. Rev. Clin. Oncol. 14, 103-118 (2018).

**Escape from CART** can occur by tumor antigen shift, loss of CAR T-cells, loss of CART-cell function. Find a way to get CAR T-cells into the tumor site. Add immunomodulatory therapy to be sure CAR T-cells are effective.

### **Three examples of armored CAR T-cells**

- Add second trans gene: chimera of PD-1 extracellular domain with CD28 intracellular signaling domain. Used for melanoma.
- Trans genes for continual expression of co-stimulatory molecules on CAR T-cells: CD40L, CD137, CD137L, CD137R1, CD137R2, CD137R3, CD137R4, CD137R5, CD137R6, CD137R7, CD137R8, CD137R9, CD137R10, CD137R11, CD137R12, CD137R13, CD137R14, CD137R15, CD137R16, CD137R17, CD137R18, CD137R19, CD137R20, CD137R21, CD137R22, CD137R23, CD137R24, CD137R25, CD137R26, CD137R27, CD137R28, CD137R29, CD137R30, CD137R31, CD137R32, CD137R33, CD137R34, CD137R35, CD137R36, CD137R37, CD137R38, CD137R39, CD137R40, CD137R41, CD137R42, CD137R43, CD137R44, CD137R45, CD137R46, CD137R47, CD137R48, CD137R49, CD137R50, CD137R51, CD137R52, CD137R53, CD137R54, CD137R55, CD137R56, CD137R57, CD137R58, CD137R59, CD137R60, CD137R61, CD137R62, CD137R63, CD137R64, CD137R65, CD137R66, CD137R67, CD137R68, CD137R69, CD137R70, CD137R71, CD137R72, CD137R73, CD137R74, CD137R75, CD137R76, CD137R77, CD137R78, CD137R79, CD137R80, CD137R81, CD137R82, CD137R83, CD137R84, CD137R85, CD137R86, CD137R87, CD137R88, CD137R89, CD137R90, CD137R91, CD137R92, CD137R93, CD137R94, CD137R95, CD137R96, CD137R97, CD137R98, CD137R99, CD137R100).
- Trans genes for secreted IL-2, IL-15, or IL-12. CAR T-cells used as micro-delivery system for immunomodulatory therapy.

clinical trial in melanoma is promising. [Khalil et al., 2016]

blockade of PD-1 or PD-L1 is seen as promising and in trials (vs. advanced

of T-cells by several means:

transmembrane, signaling domain of the T-cell receptor and a co-stimulatory  
cell activation: when the transgenic T-cells see antigen they are primed,  
co-stimulatory domains to provide greater activation. [Khalil et al., 2016]

ability to kill or changes the tumor microenvironment: IL-12, 4-1BBL or

that not other immune cells. Clinical success has been seen and seems to require  
do not appear to exhaust T-cells prior to treatment with them. Survival or  
conditioning prior to CAR-T seems critical and some agents and regimens are  
better for B-ALL than CLL. [Khalil et al., 2016] Researchers are looking at a

immunological toxicity. These issues are treated with antibodies or steroids and can

microenvironment; heterogeneity of antigens; collateral damage to normal

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doi:10.1038/nrclinonc.2016.36 (2016).

conditioning. So? Target multiple tumor antigens. Use 'conditioning chemotherapy.  
CAR T-cells can be functional, or use armored CART.

signaling domain. Better persistence and clearance in syngenic mouse

4-1BBL. The latter allows trans-co-stimulation of neighboring T-cells.

or non-toxic local levels of cytokines. Enhances persistence and resistance to

Does ASPS have a high TIL burden?

**References:**

Khalil, D., Smith, E.L., Brentjens, R.J., Wolchok, J.D. (2016) The future of cancer treatment: immunotherapy. *Critical Oncology*, 13.5 (May): 273-

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