Basic Principles of Tumor Immunotherapy

Michael A. Curran, Ph.D.

Disclosures

- AstraZeneca, Threshold, Infinity - Consultant

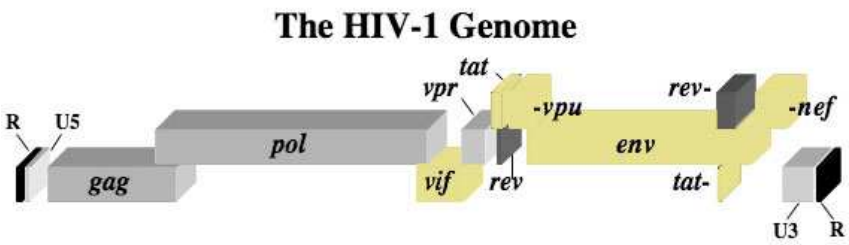
Why does the immune system fail to eliminate cancer?

Antigenic Cancer Cells Grow Progressively in Immune Hosts without Evidence for T Cell Exhaustion or Systemic Anergy

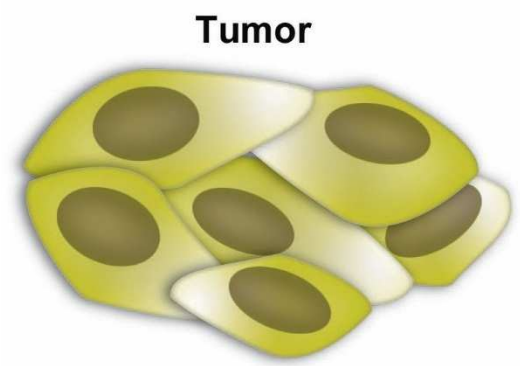
By Maresa Wick,* Purnima Dubey,* Hartmut Koeppen,* Christopher T. Siegel,‡ Patrick E. Fields,§ Lieping Chen,‖ Jeffrey A. Bluestone,§ and Hans Schreiber*

J. Exp. Med. © The Rockefeller University Press
Volume 186, Number 2, July 21, 1997 229–238

Like pathogens, tumors deploy multigenic immune evasion programs

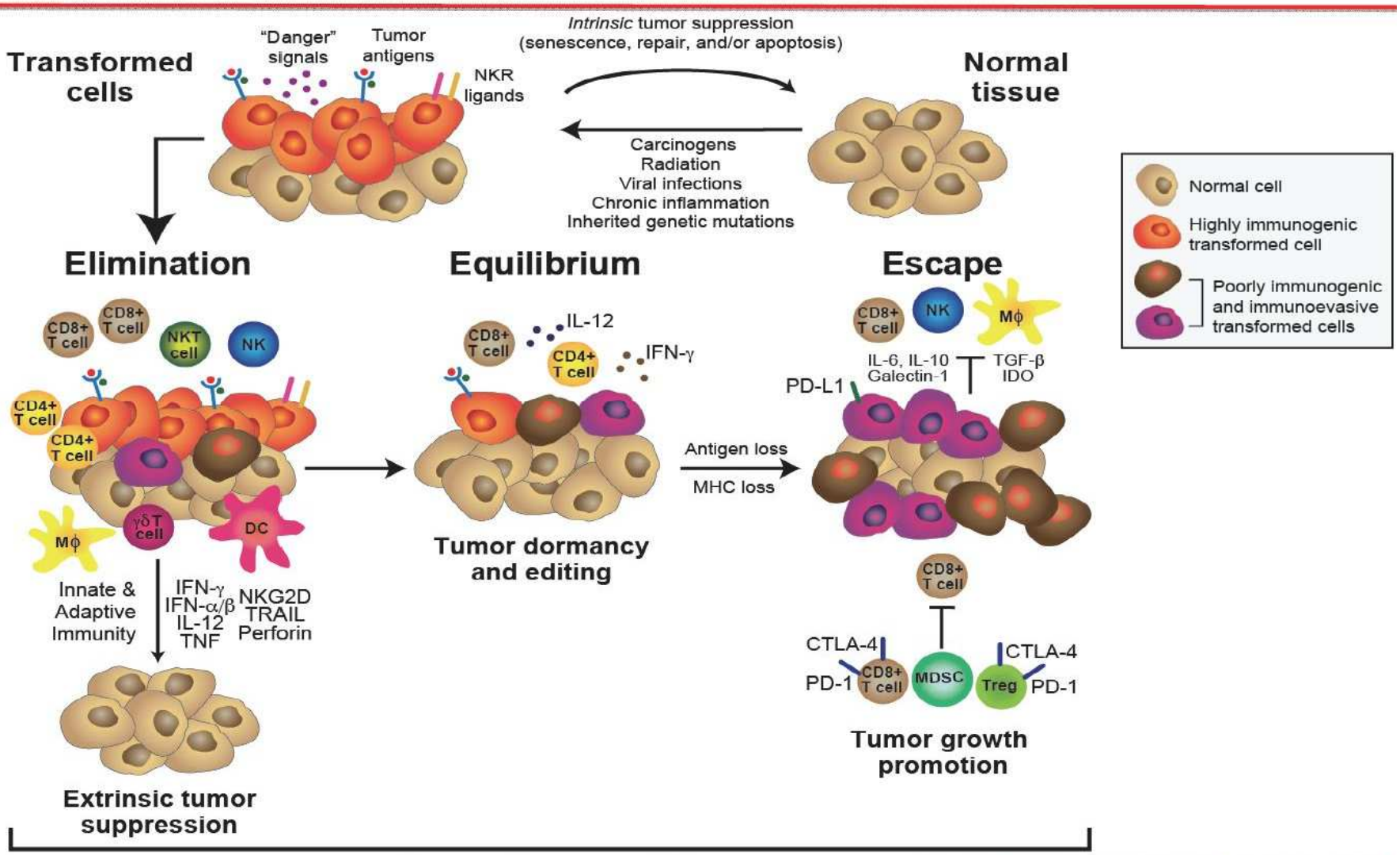


With < 9.8 kB of genome space HIV, like many other viruses devotes a large percentage of its genome to immune evasion.



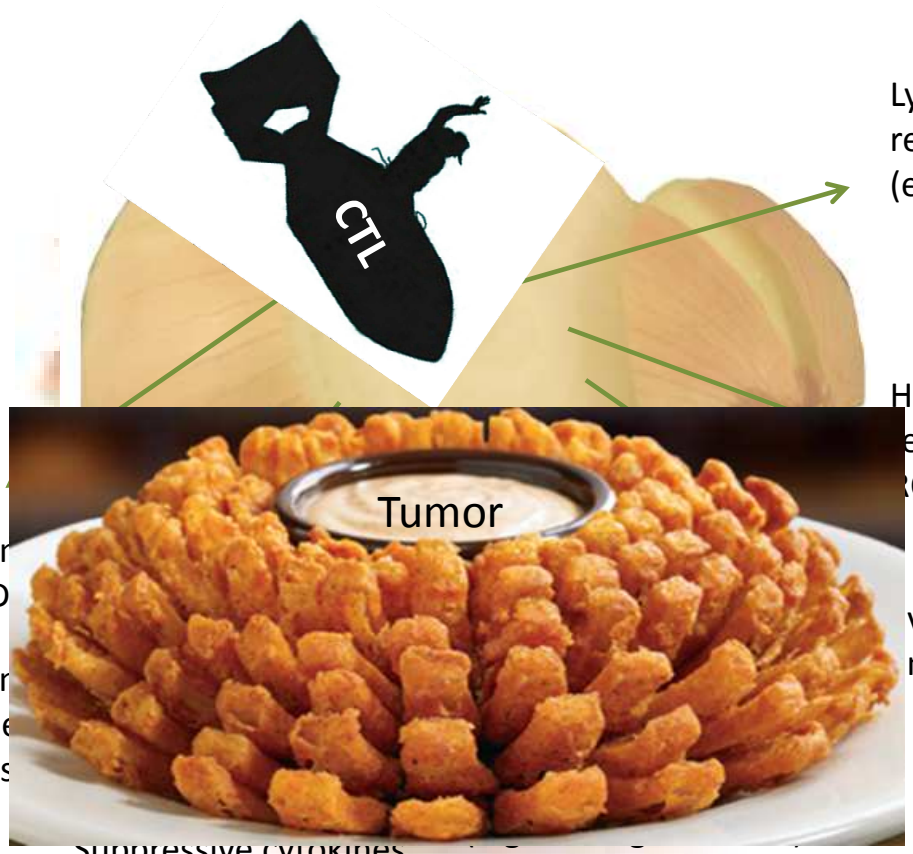
Can access the entire $3x10^9$ base genome for evolutionary as well as adaptive immune evasion.

The 3 Es of Cancer Immunoediting



Schreiber, Old and Smyth, Science 2011

Multi-layered immuno-suppression



Lysis and apoptosis resistant tumor cells (e.g. SPI-6, IAPs)

Hostile metabolism e.g. acidic, hypoxic, ROS, peroxynitrite)

T cell checkpoir ligands (e.g. PD

vasculature no ICAM)

Nutrier enzyme Arginas

Suppressive cytokines (e.g. IL-10, TGF-b)

Tumors insulate themselves with dense layers of immunosuppressive stroma

Overcoming the many layers of inter-connected and often functionally redundant immune suppressive mechanisms represents a daunting challenge for tumor-specific T cells

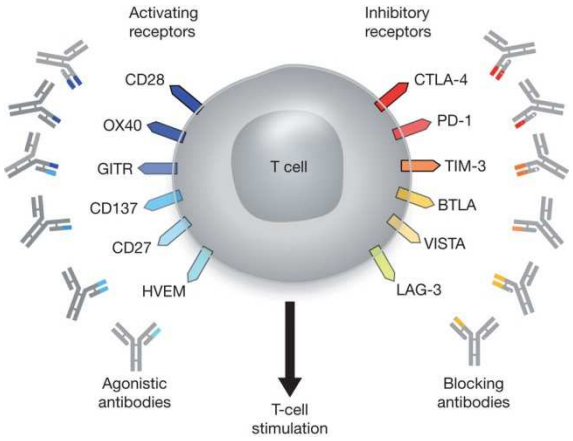
Immunotherapy can peel back the layers of local immune suppression, thereby restoring the capacity of T cells to eradicate the tumor

To exist, tumors must evolve mechanisms to locally disable and/or evade the immune system.

The goal of immunotherapy, then, is to restore the capacity of the immune system to recognize and reject cancer.

Types of Immunotherapy

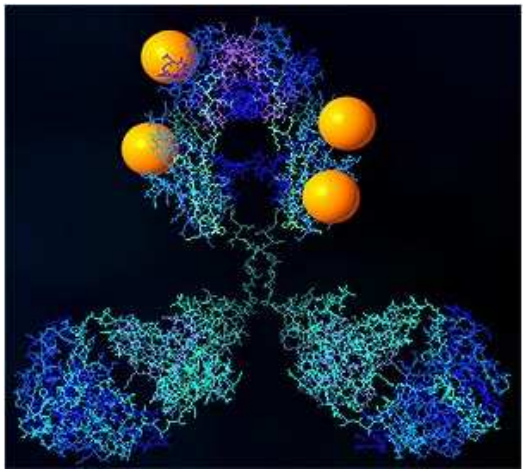
T Cell Checkpoint Modulation



T Cell Adoptive Transfer

Therapeutic Cancer Vaccines

Effector antibodies and ADCs



Order matters…

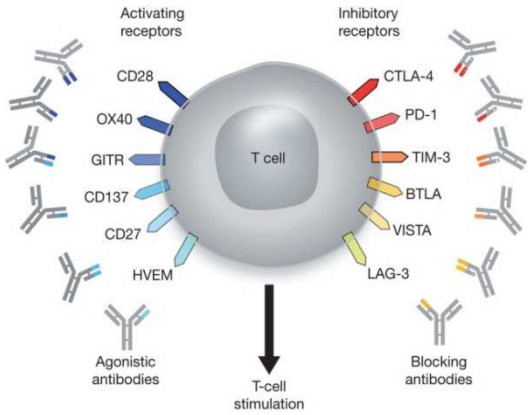
Therapeutic Cancer Vaccines

Prior to the discovery that CTLA-4 blockade could cure murine tumors, the field focused on finding tumor antigens and vaccination.

Unfortunately, vaccine-induced T cells were powerless to overcome local tumor immune suppression prior to the discovery of checkpoint blockade.

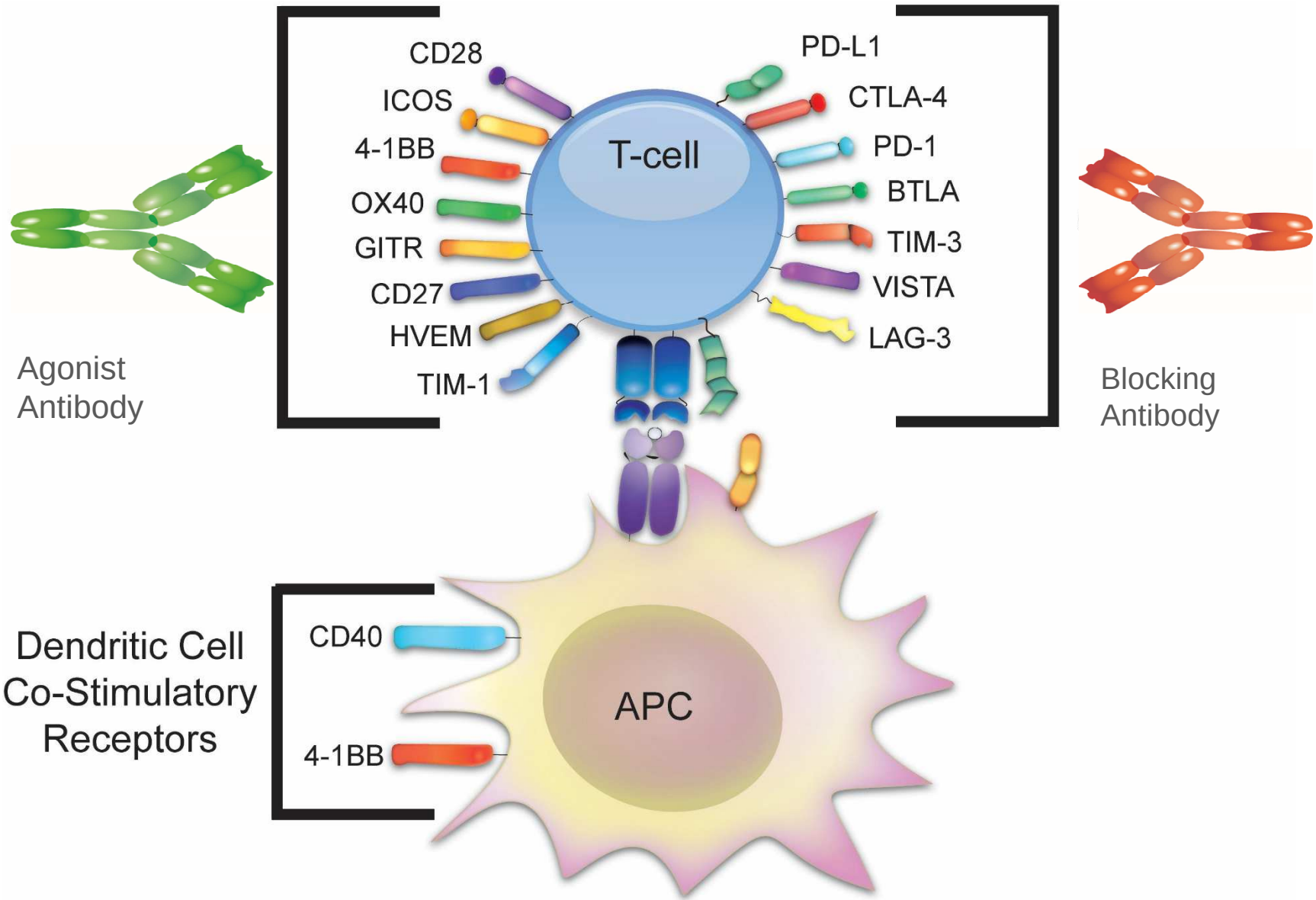
Allows activated T cells to enter, survive in, and kill tumors

T Cell Checkpoint Modulation



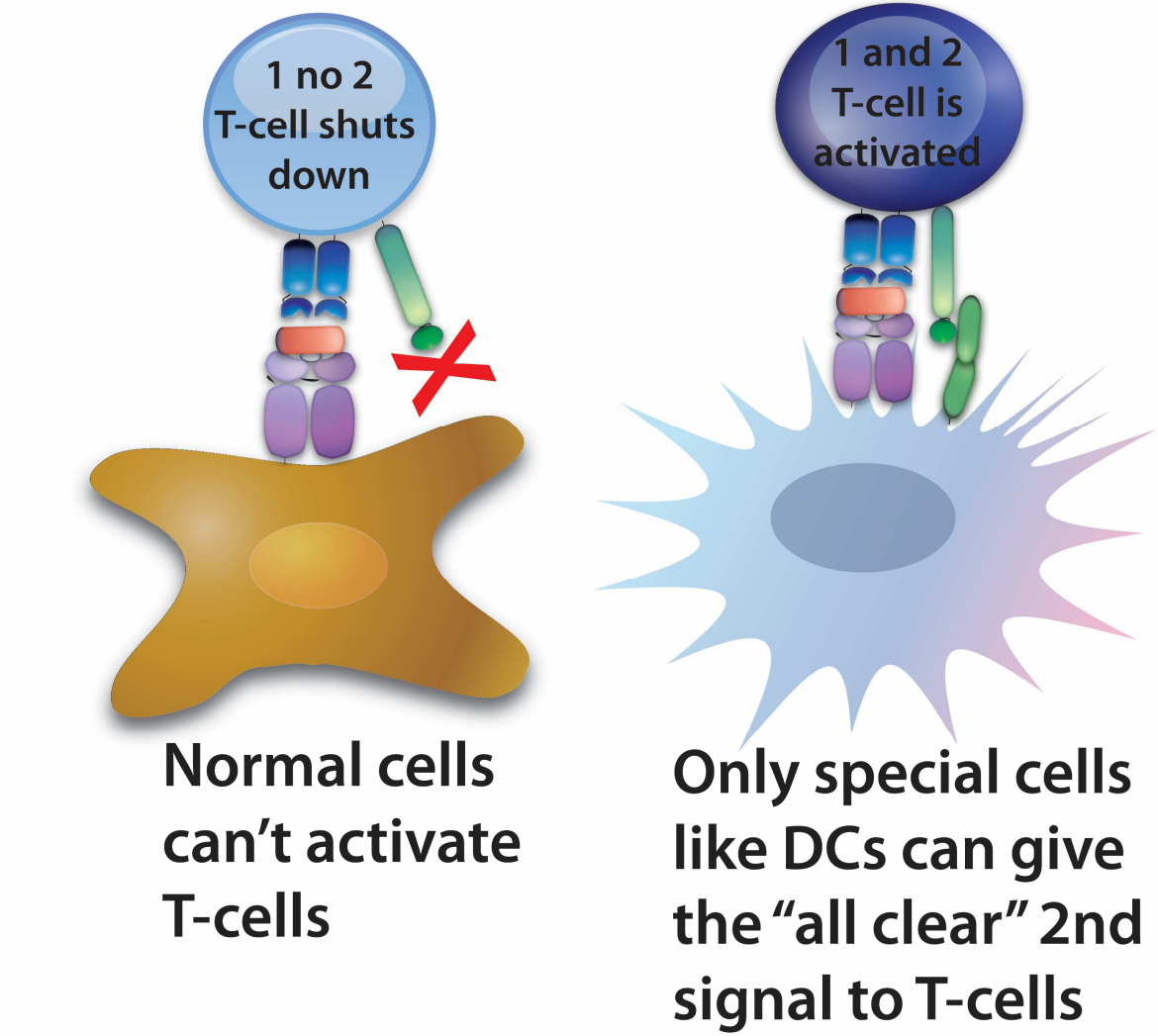
Makes checkpoint blockade more effective and less toxic by keeping focus on tumor-specific T cells

T cell Checkpoint Modulation

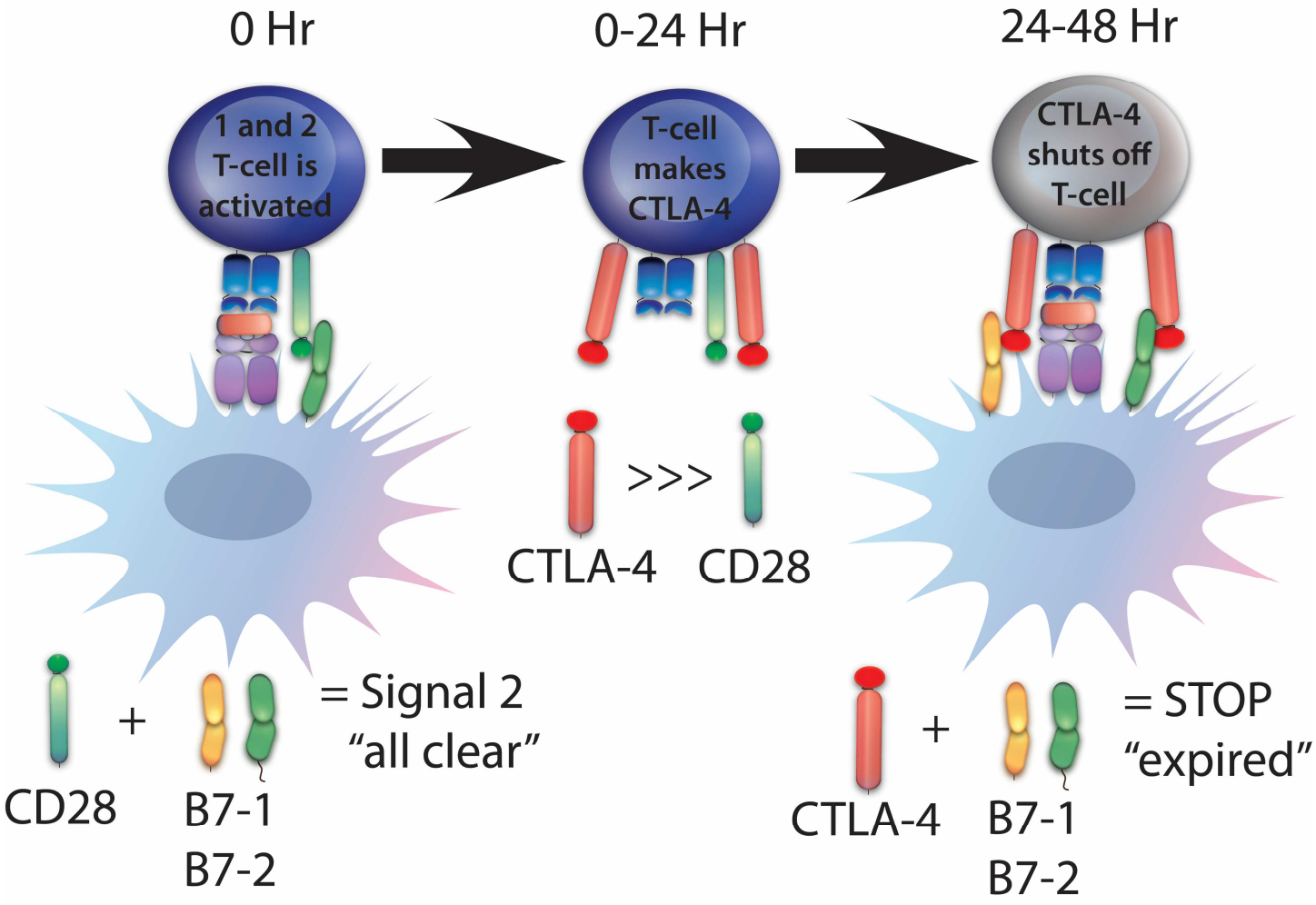


Ai M., **Curran M.** Immune checkpoint combinations from mouse to man. *Cancer Immunology Immunotherapy*, 2015.

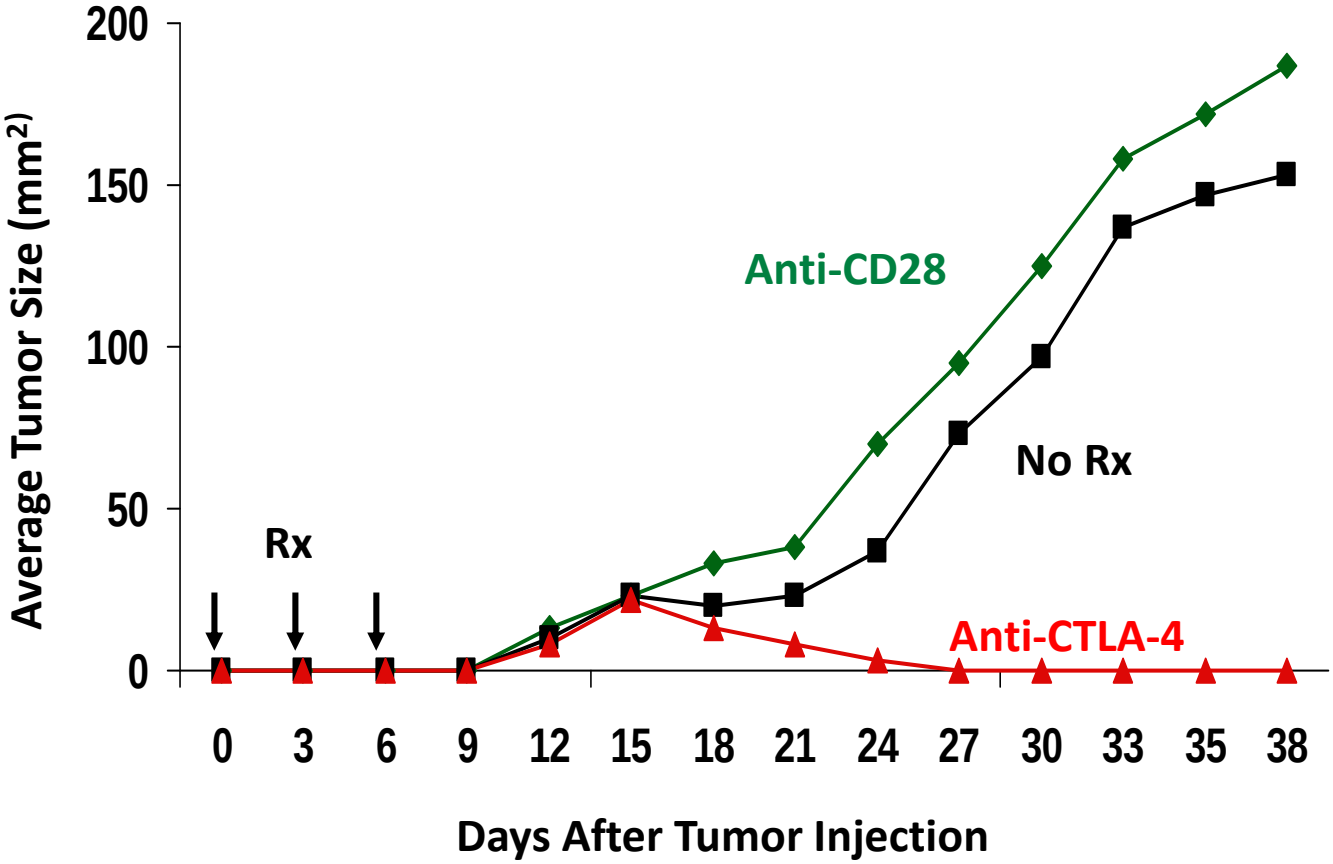
T cells are activated in two steps: T cell receptor ligation and co-stimulation



CTLA-4, a negative regulator of T cell activity, limits the lifespan of activated T-cells



Anti-CTLA-4 Induces Regression of Transplantable Colon Carcinoma

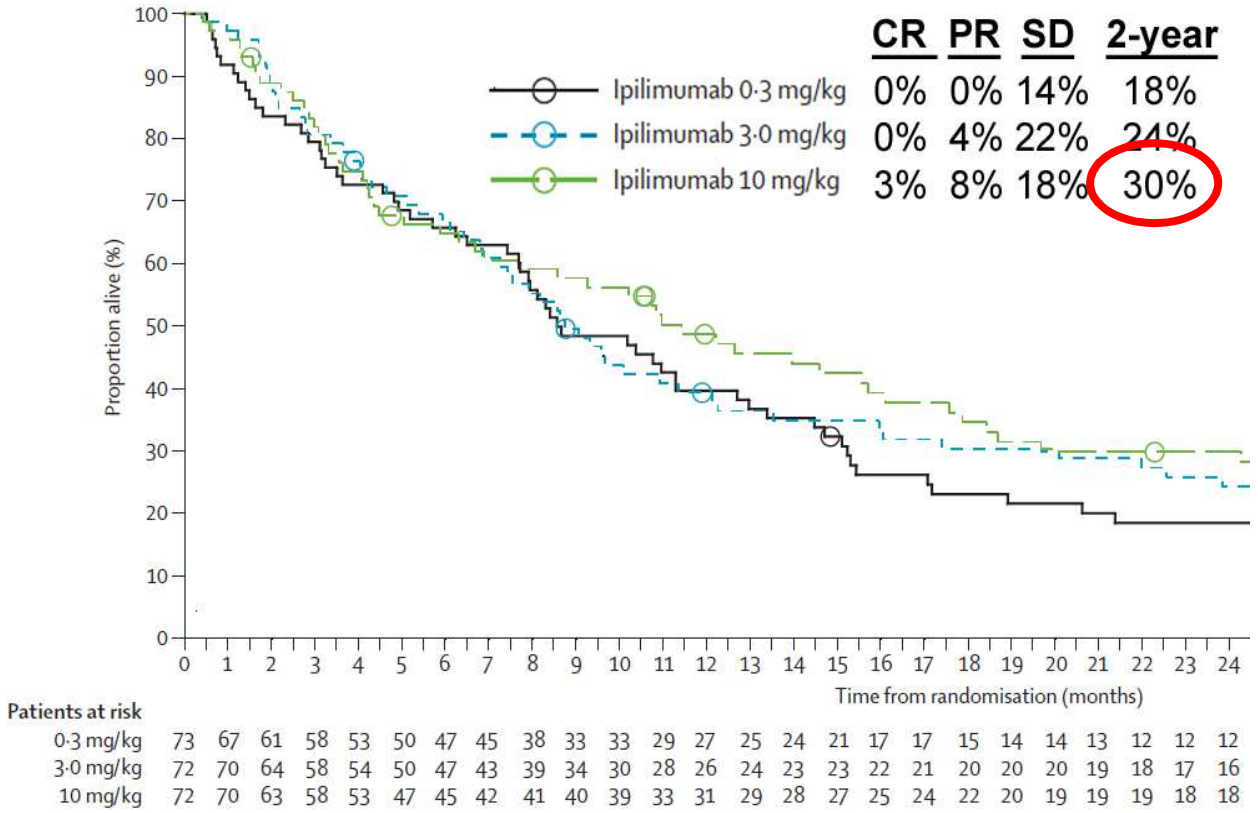


Science. 1996 Mar 22;271(5256):1734-6.

Enhancement of antitumor immunity by CTLA-4 blockade.

Leach DR[1], Krummel MF, Allison JP.

Ipilimumab (anti-human CTLA-4) was approved for the treatment of metastatic melanoma by the FDA in 2010

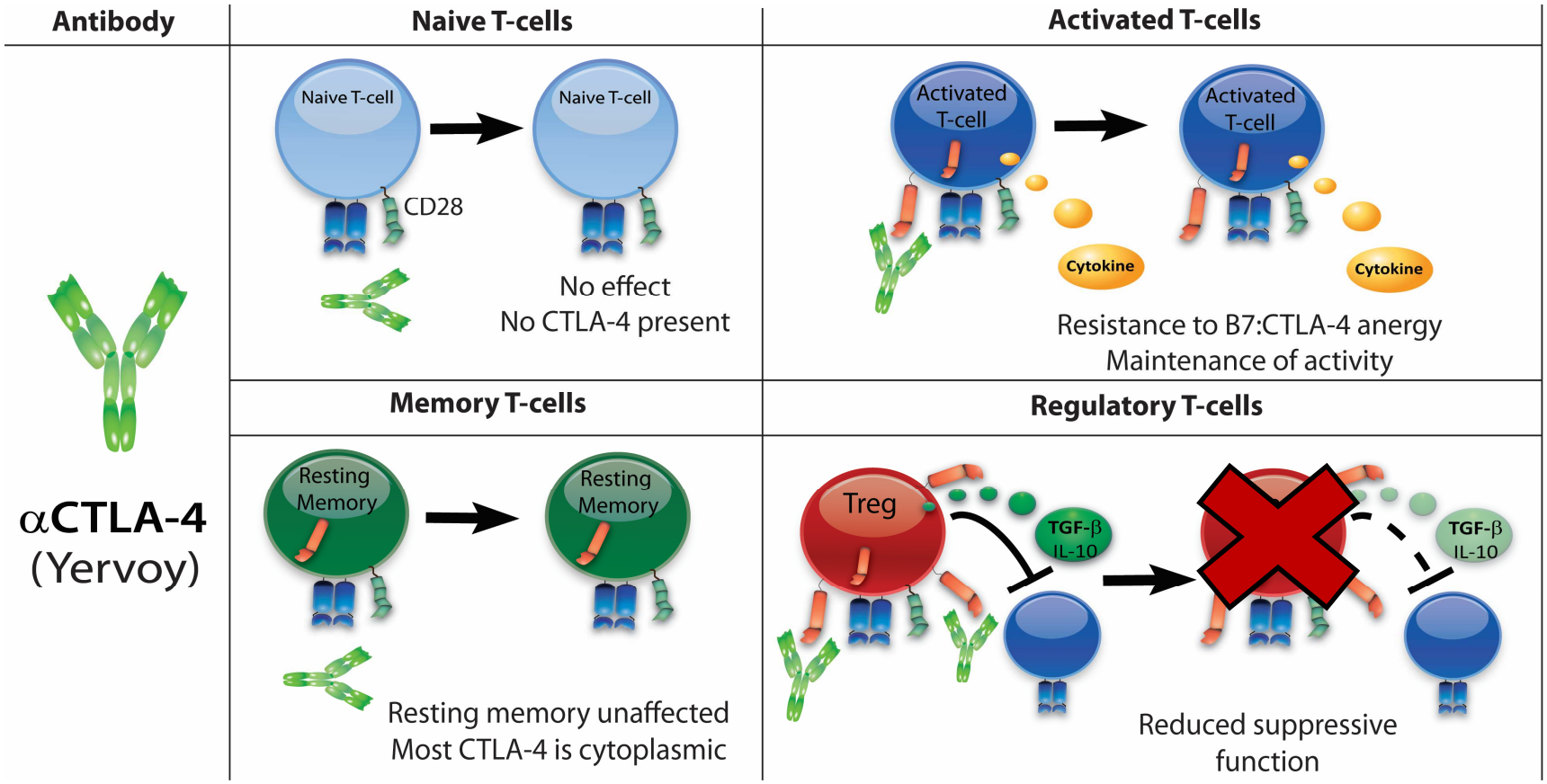


Wolchok et al, *Lancet Oncol*, 2010

Temodar:
CR: 2.5%
PR: 11%
SD: 18%
2yr: 18%

Middleton et.al, *J Clin Oncol*, 2000

Which T-cells are affected by Ipilimumab(αCTLA-4)?



The greater the percentage of active T-cells in a patient targeting the tumor when αCTLA-4 is initiated, the greater the efficacy and selectivity should be.

Immune checkpoint modulating antibodies currently in the clinic

Table 1: T cell immune checkpoint modulating antibodies in the clinic

Target Molecule	Drug	Company	Development Stage
CTLA-4	Ipilimumab	Bristol-Myers Squibb	FDA Approved
	Tremelimumab	Medimmune/Astrazeneca	Phase III Trial
PD-1	Pembrolizumab	Merck	FDA Approved
	Nivolumab	Bristol-Myers Squibb	FDA Approval Pending
	AMP-514/MEDI0680	Medimmune/Astrazeneca	Phase I Trial
PD-L1	MPDL3280A	Genentech/Roche	Phase III Trial
	MEDI4736	Medimmune/Astrazeneca	Phase III Trial
	MSB0010718C	EMD Serono	Phase II Trial
	BMS-936559	Bristol-Myers Squibb	Phase I Trial
4-1BB	Urelumab	Bristol-Myers Squibb	Phase I Trial
	PF-05082566	Pfizer	Phase I Trial
OX-40	MEDI6469	Medimmune/Astrazeneca	Phase I Trial
	MEDI6383 (rOX40L)	Medimmune/Astrazeneca	Phase I Trial
	MOXR0916	Genentech/Roche	Phase I Trial
GITR	TRX518	Tolerx	Phase I Trial
CD27	CDX-1127	Celldex	Phase I Trial
CD40	CP-870,893	Genentech/Roche	Phase I Trial
LAG3	BMS-986016	Bristol-Myers Squibb	Phase I Trial

To exist, tumors must evolve mechanisms to locally disable and/or evade the immune system.

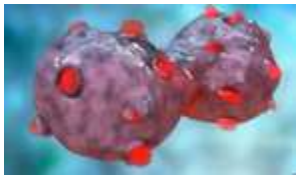
The goal of T cell checkpoint blockade is block the switches on T cells being engaged by the tumor to shut them down and in so doing to restore tumor-specific immunity.

Therapeutic Cancer Vaccines

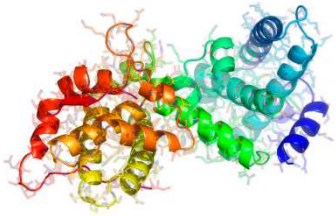
Components of a cancer vaccine

Antigen

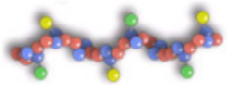
- Whole tumor



- Protein Antigen

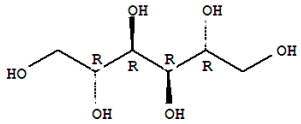


- Antigenic Peptide(s)

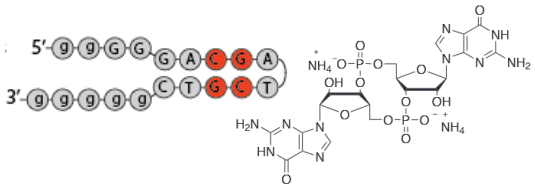


Adjuvant

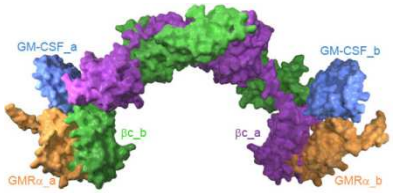
- Emulsifiers



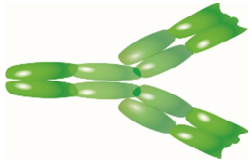
- Innate agonists



- Cytokines



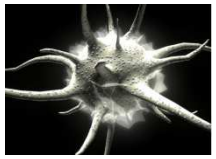
- Antibodies



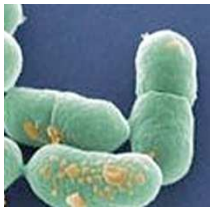
Vector

- Viral Vectors

- Dendritic Cells



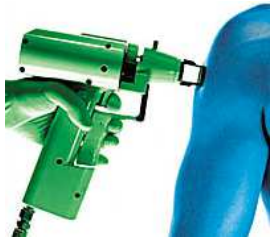
- Attenuated Bacteria



Vehicle

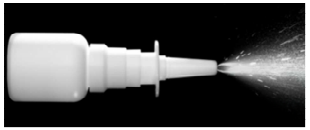
- Injection

- Gene Gun



- Systemic Infusion

- Nasal Spray



The first therapeutic cancer vaccine approved for human use was Provenge for prostate cancer in 2010.

Many others are in Phase III development as shown here and dozens more are currently in Phase I and Phase II.

The momentum in the field is moving toward targeting the mutated epitopes unique to each patient's cancer which are the targets for the most efficacious anti-tumor responses.

Table 1 | Active immunotherapies in phase III development*

Immunotherapy	Targeted antigens	Adjuvants/ immune modulators	Study population	*n*	Outcomes	References
Prostate cancer						
Autologous cell vaccine: sipuleucel-T, Provenge®	PAP	GM-CSF	Metastatic, castration-resistant prostate cancer	512	OS: 25.8 months vs 21.7 months (HR 0.78; P=0.03) PFS: 3.7 months vs 3.6 months (HR 0.95; P=0.63) T-cell response in 73.0% vs 12.1% of patients	50–55
Allogeneic tumour cell vaccine: GVAX	Tumour cell	GM-CSF	Castration-resistant prostate cancer	626	OS: 20.7 months vs 21.7 months with docetaxel plus prednisone (HR 1.03; P=0.78)‖	70, 194
Allogeneic tumour cell vaccine: GVAX	Tumour cell	GM-CSF	Castration-resistant prostate cancer	408	OS: 12.2 months in combination with docetaxel vs 14.1 months docetaxel plus prednisone (HR 1.70; P=0.0076)§	71, 195
Breast cancer						
Peptide vaccine: Theratope	Sialyl-Tn	KLH	Metastatic breast cancer, in remission after first-line chemotherapy	1,028	Median OS: 23.1 months vs 22.3 months (P=0.916) With concomitant endocrine therapy, OS: 39.6 months vs 25.4 months (P=0.005) Median TTP: 3.4 months vs 3.0 months (P=0.353) With concomitant endocrine therapy: 10.6 months vs 6.3 months (P=0.078)	76, 77
Lung cancer						
Peptide vaccine: tecemotide (L-BLP25)	MUC1	Liposomal monophosphoryl lipid A plus cyclophosphamide	Unresectable stage III NSCLC; after chemo-radiotherapy	1,239	Median OS: 25.6 months vs 22.3 months (HR 0.88; P=0.123); OS with concurrent chemotherapy: 30.8 months vs 20.6 months (HR 0.78; P=0.016); OS with sequential chemotherapy: 19.4 months vs 24.6 months (HR 1.12; P=0.38)	79–81, 197
Peptide vaccine: GSK1572932A	MAGE-A3	Liposomal AS15	Completely resected stage IB–II NSCLC	182	Trial terminated owing to failure to meet primary end points of extended DFS. Not possible to identify gene signature predicting benefit	85, 86
Allogeneic tumour cell vaccine: belagenpumatucel-L, Lucanix™	Tumour cell	Anti-TGF-β	Stage IIIB–IV NSCLC	532	Median OS: 20.3 months vs 17 months (HR 0.94; P=0.594) Non-adenocarcinoma: 19.9 months vs 12.3 months (HR 0.55; P=0.036)	93, 198
Melanoma						
Peptide vaccine	gp100	IL2 plus Montanide™ ISA51	Locally-advanced stage III or stage IV melanoma	185	OS: 17.8 months vs 11.1 months (P=0.06) PFS: 2.2 months vs 1.6 months (P=0.08) T-cell responses in 7 of 37 (19%) patients Higher levels of $CD4^+foxp3^+$ cells in patients with clinical response (P=0.01)	35, 198
Peptide vaccine: GSK 2132231A	MAGE-A3	QS-21	Resected melanoma	1,349	Failed to meet primary end point of DFS; ongoing for end point of DFS in patients with predictive gene signature	100
Pancreatic cancer						
Peptide vaccine: GV1001	Telomerase	GM-CSF	Locally-advanced and/or metastatic pancreatic cancer	1,062	OS: 8.4 months (concurrent with chemotherapy) and 6.9 months (sequential chemotherapy) vs 7.9 months with chemotherapy alone (NS)	113, 199, 200
Colorectal cancer						
Autologous tumour cell vaccine: OncoVAX®	Tumour cell	BCG	Resected stage II–III colon cancer; after resection	254	42% reduction in the risk of recurrence and/or death (P=0.032); greatest effect in stage II disease with 60% reduction in risk of recurrence and/or death (P=0.007) and 54% reduction in risk of death	121
Haematological malignancies						
Autologous anti-idiotype vaccine	Idiotype	KLH	Advanced follicular lymphoma, with complete response after chemotherapy	177	PFS: 23.0 months vs 20.6 months (P=0.256) ≥1 blinded vaccination: 44.2 months vs 30.6 months (P=0.047)	130, 201

Finding a broadly-relevant “off the shelf” tumor antigen is difficult and the outcomes often sub-optimal

PMC full text: J Natl Cancer Inst. Apr 18, 2012; 104(8): 599–613.
Published online Mar 6, 2012. doi: 10.1093/jnci/djs033

Table 2

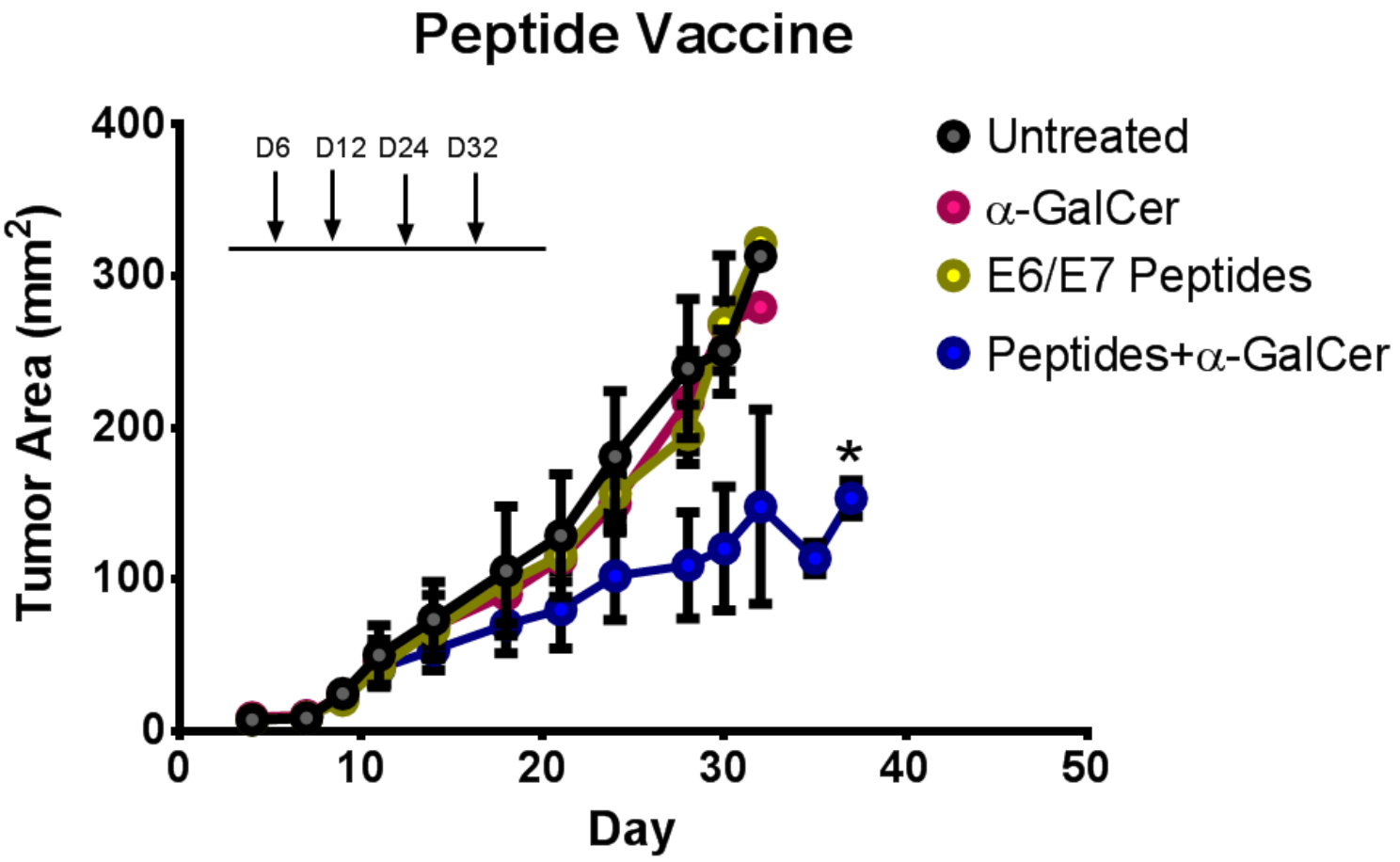
Spectrum of current and potential therapeutic cancer vaccine targets*

Target type	Examples	Selected references
Oncoprotein	point mutated: ras, B-raf, frame shift mutations, undefined unique tumor mutations; HER2/neu, MUC-1 C-terminus, p53	(1,7,8,48,49)
Oncofetal antigen	CEA, MUC-1	
Cancer–testes	MAGE-A3, BAGE, SEREX	
Tissue lineage	PAP, PSA, gp100, tyrosinase	
Stem cell/EMT	Brachyury, SOX-2, OCT-4,	
Viral	HPV, HCV	
Glycopeptides	STn-KLH	(15,16)
Antiangiogenic	VEGF-R	(65,66,67)
B-cell lymphoma	Anti-id	(11–14)

The HPV E6/E7 oncoproteins are ideal targets for therapeutic vaccination as: 1) they are immunologically foreign to the host; 2) they are necessary to maintain the transformed state

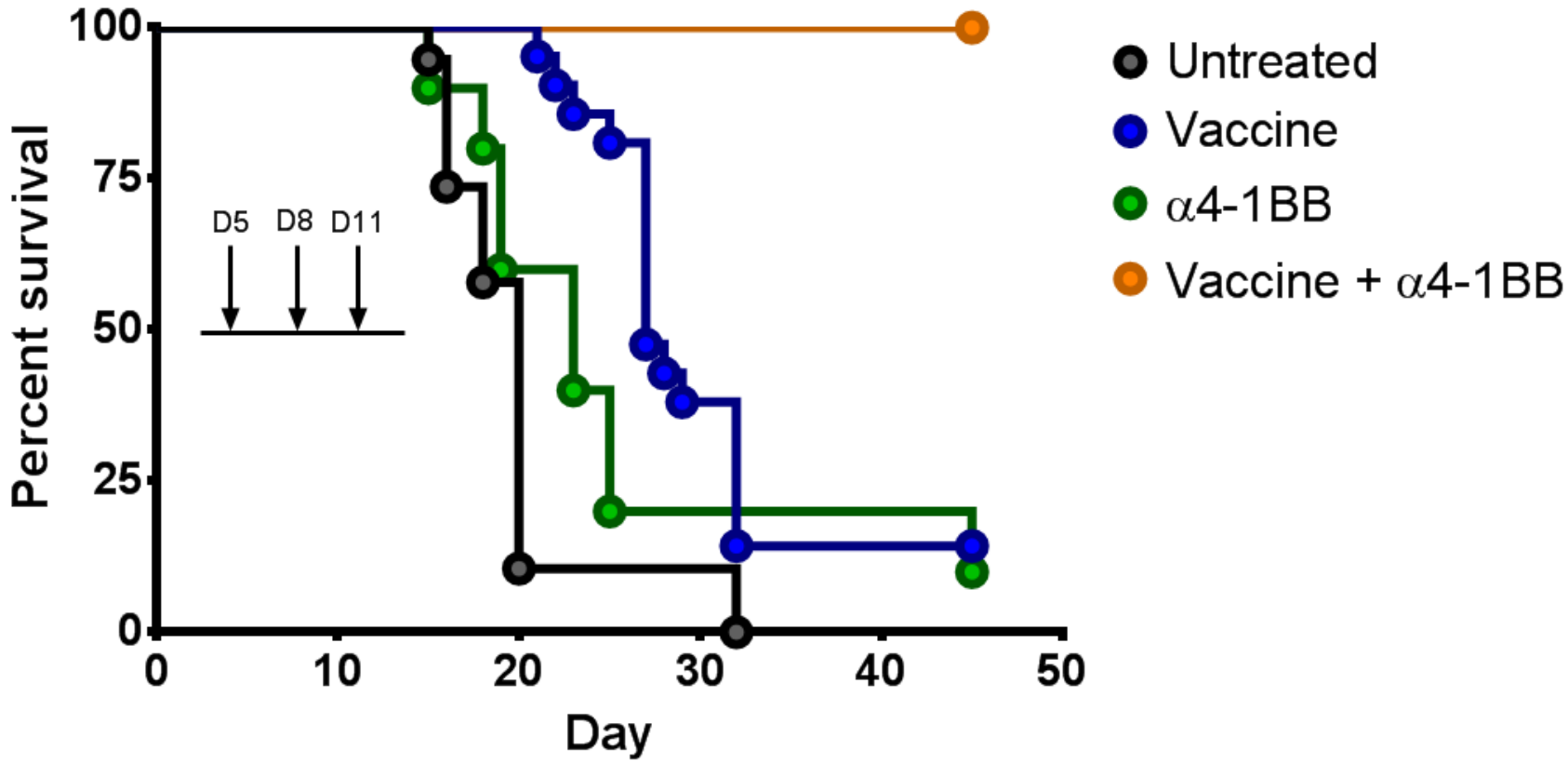
*BAGE = B melanoma antigen; CEA = carcinoembryonic antigen; EMT = epithelial–mesenchymal transition; gp100 = glycoprotein 100; HCV = hepatitis C virus; HPV = human papillomavirus; MAGE-A3 = melanoma-associated antigen-A3; MUC-1 = mucin 1; NY-ESO = New York esophageal carcinoma antigen 1; OCT-4 = octamer-binding transcription factor 4; PAP = prostatic acid phosphatase; PSA = prostate-specific antigen; SOX-2 = (sex determining region Y)-box-2; STn-KLH = sialyl-Tn–keyhole limpet hemocyanin; TERT = telomerase reverse transcriptase; VEGF-R = vascular endothelial growth factor receptor.

An intra-nasal HPV E6/E7 : α-GalCer vaccine slows growth of TC-1 tumors



Shailbala Singh

4-1BB agonist antibody and HPV E6/E7 vaccine synergize in curing TC-1 tumors



Todd Bartkowiak, M.S.

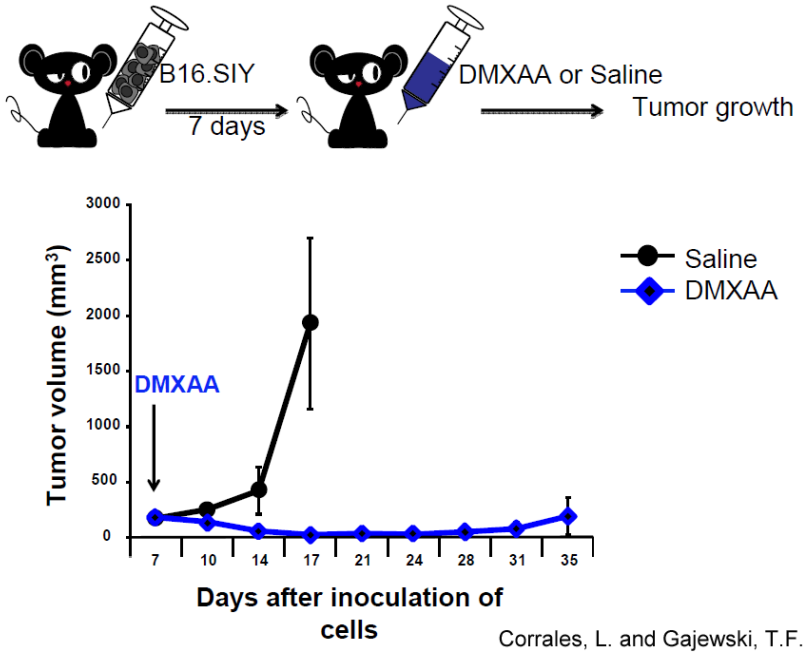
Intratumoral injection of innate immune agonists – the direct vaccination approach

Table 1 | **Nucleic acid-sensing PRRs: localization, sensed pathogens and agonists**

PRR	Localization	Sensed pathogens	Natural agonists	Synthetic agonists
TLR3	Endolysosomal compartment	dsRNA viruses, ssRNA viruses, dsDNA viruses	dsRNA	Polyl:C, polyU
TLR7	Endolysosomal compartment	ssRNA viruses, bacteria, fungi, protozoan parasites	GU-rich ssRNA	Imidazoquinolines (R848, imiquimod, 3M001), guanosine analogues
TLR8	Endolysosomal compartment	ssRNA viruses, bacteria, fungi, protozoan parasites	GU-rich ssRNA	Imidazoquinolines (R848, 3M002), guanosine analogues
TLR9	Endolysosomal compartment	dsDNA viruses, bacteria, protozoan parasites	DNA	CpG ODNs
RIG-I	Cytoplasm	ssRNA viruses, DNA viruses, *Flaviviridae*, reovirus, bacteria	Short RNA with 5′ppp and/or base pairing	Short polyI:C
MDA5	Cytoplasm	*Picornaviridae*, vaccinia virus, *Flaviviridae*, reovirus, bacteria	Long dsRNA	PolyI:C
NOD2	Cytoplasm	RNA viruses	ssRNA	–
DDX3	Cytoplasm	RNA viruses	RNA	–
DDX1–DDX21–DHX36	Cytoplasm	RNA viruses	dsRNA	PolyI:C
DDX60	Cytoplasm	RNA viruses, DNA viruses	ssRNA, dsRNA, dsDNA	–
DHX9	Cytoplasm	DNA viruses, RNA viruses	dsDNA, dsRNA	CpG-B ODNs
DHX36	Cytoplasm	DNA viruses	dsDNA	CpG-A ODNs
DDX41	Cytoplasm	DNA viruses, bacteria	DNA	–
AIM2	Cytoplasm	DNA viruses, bacteria	DNA	–
IFI16	Cytoplasm and nucleus	DNA viruses	dsDNA	–
ZBP1	Cytoplasm	DNA viruses, bacteria	dsDNA	–
LRRFIP1	Cytoplasm	DNA viruses, bacteria	dsDNA, dsRNA	–
STING	Cytoplasm	Bacteria	Cyclic di-GMP	–

5′ppp, 5′ triphosphate end; AIM2, absent in melanoma 2; dsRNA, double-stranded RNA; IFI16, IFNγ-inducible protein 16; LRRFIP1, leucine-rich repeat flightless-interacting protein 1; MDA5, melanoma differentiation-associated protein 5; NOD2, nucleotide-binding oligomerization domain protein 2; ODN, oligodeoxynucleotide; polyI:C, polyinosinic–polycytidylic acid; PRR, pattern-recognition receptor; RIG-I, retinoic acid-inducible gene I; ssRNA, single-stranded RNA; STING, stimulator of IFN genes; TLR, Toll-like receptor; ZBP1, Z-DNA-binding protein 1.

Intratumoral DMXAA (mouse STING agonist) triggers rejection of B16 melanoma



Corrales, L. and Gajewski, T.F.

Current question: Can local injection of one lesion evoke rejection of distant ones. This is known as the **Abscopal Effect**.

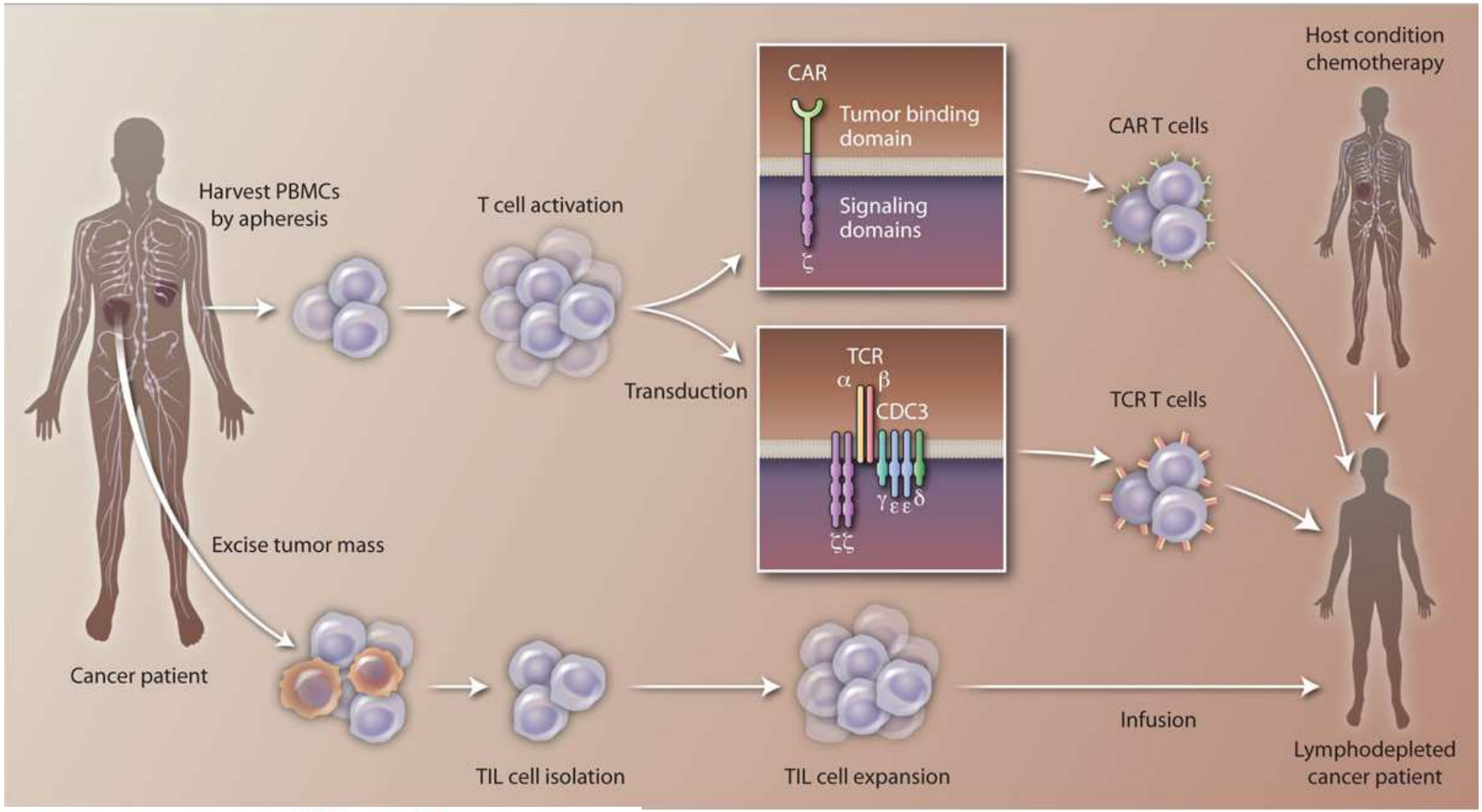
To exist, tumors must evolve mechanisms to locally disable and/or evade the immune system.

The goal of therapeutic cancer vaccination is to increase the immunogenicity of tumor antigens which are poorly presented by the tumor in order to generate a high frequency of tumor-specific T cells.

T cell adoptive transfer

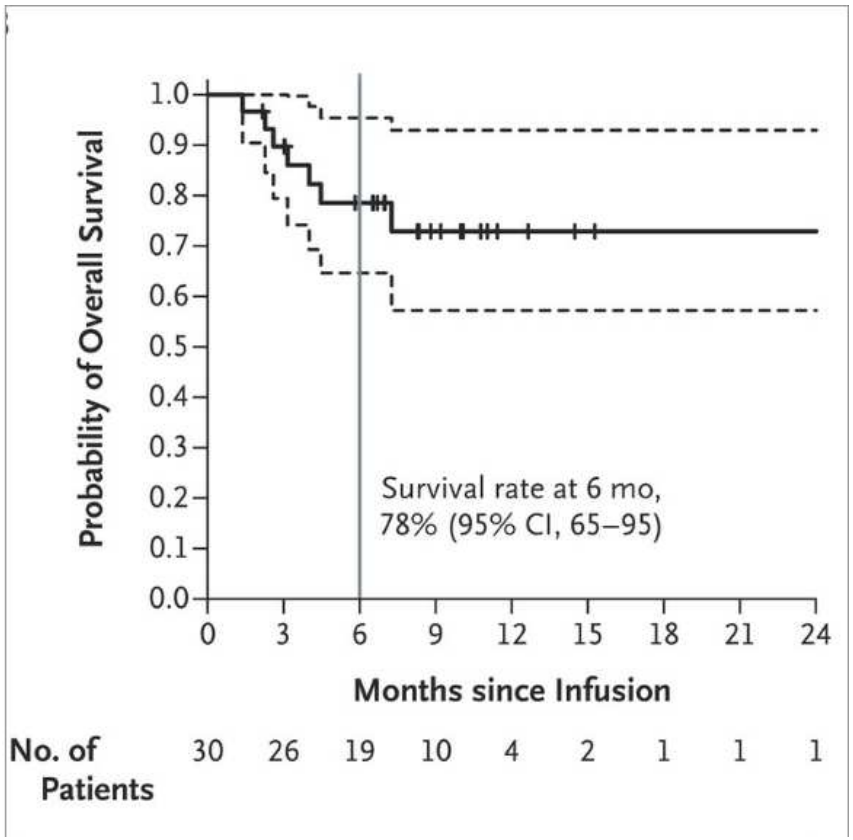
CARs, TIL, engineered PBMC etc…

Adoptive T cell therapy can involve engineered (CAR, TCR) or patient derived (TIL, PBMC) T cells



Citation: C. H. June, S. R. Riddell, T. N. Schumacher, Adoptive cellular therapy: A race to the finish line. *Sci. Transl. Med.* **7**, 280ps7 (2015).

Effective treatment of relapsed B cell ALL with CD19 CAR T cell therapy



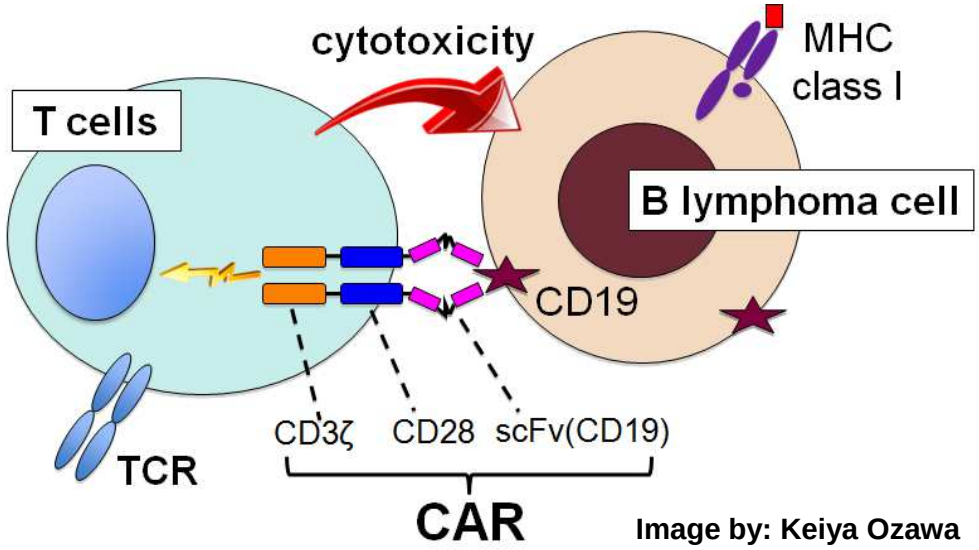


Image by: Keiya Ozawa

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

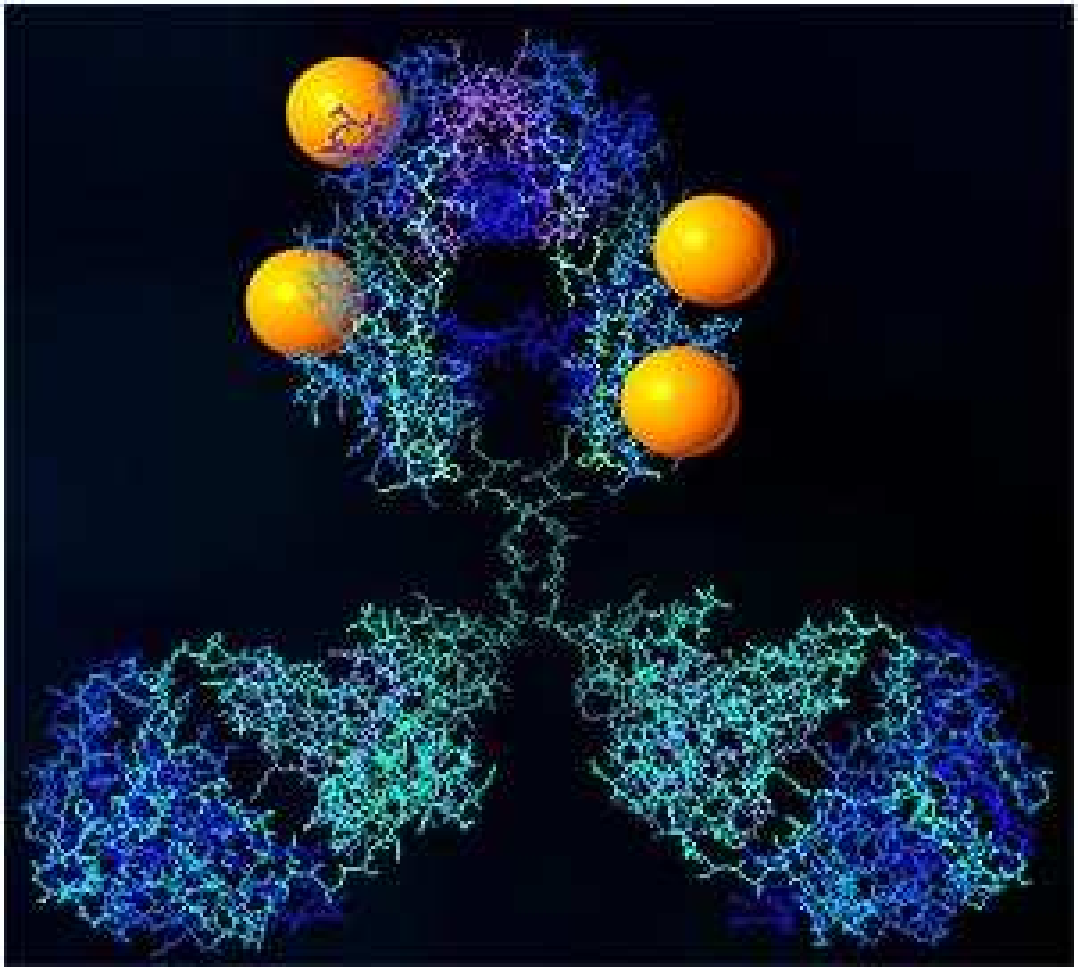
Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia

Shannon L. Maude, M.D., Ph.D., Noelle Frey, M.D., Pamela A. Shaw, Ph.D., Richard Aplenc, M.D., Ph.D., David M. Barrett, M.D., Ph.D., Nancy J. Bunin, M.D., Anne Chew, Ph.D., Vanessa E. Gonzalez, M.B.A., Zhaohui Zheng, M.S., Simon F. Lacey, Ph.D., Yolanda D. Mahnke, Ph.D., Jan J. Melenhorst, Ph.D., Susan R. Rheingold, M.D., Angela Shen, M.D., David T. Teachey, M.D., Bruce L. Levine, Ph.D., Carl H. June, M.D., David L. Porter, M.D., and Stephan A. Grupp, M.D., Ph.D.

To exist, tumors must evolve mechanisms to locally disable and/or evade the immune system.

The goal of T cell adoptive transfer is to win the numbers game and overwhelm the tumor with a higher frequency of tumor-specific T cells than it is capable of suppressing.

Effector Antibodies and ADCs



Key ADC / Antibody principles

- Specificity: the more tumor specific the target antigen is the higher the agent can be dosed without limiting toxicity
- Internalization: the target tumor surface protein must internalize to deliver the toxin – it should do so frequently and to a suitable endosomal compartment.
- Stability: the toxin must remain inert and tethered to the antibody until it is delivered to its target cell.

SGN-70A in the clinic for NHL and RCC

Bioconjug Chem. 2013 Jul 17;24(7):1256-63. doi: 10.1021/bc400217g. Epub 2013 Jun 28.

A potent anti-CD70 antibody-drug conjugate combining a dimeric pyrrolobenzodiazepine drug with site-specific conjugation technology.

Jeffrey SC[1], Burke PJ, Lyon RP, Meyer DW, Sussman D, Anderson M, Hunter JH, Leiske CI, Miyamoto JB, Nicholas ND, Okeley NM, Sanderson RJ, Stone IJ, Zeng W, Gregson SJ, Masterson L, Tiberghien AC, Howard PW, Thurston DE, Law CL, Senter PD.

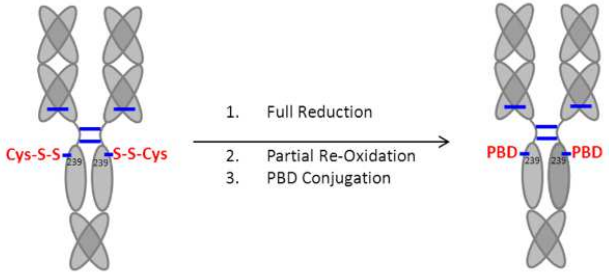
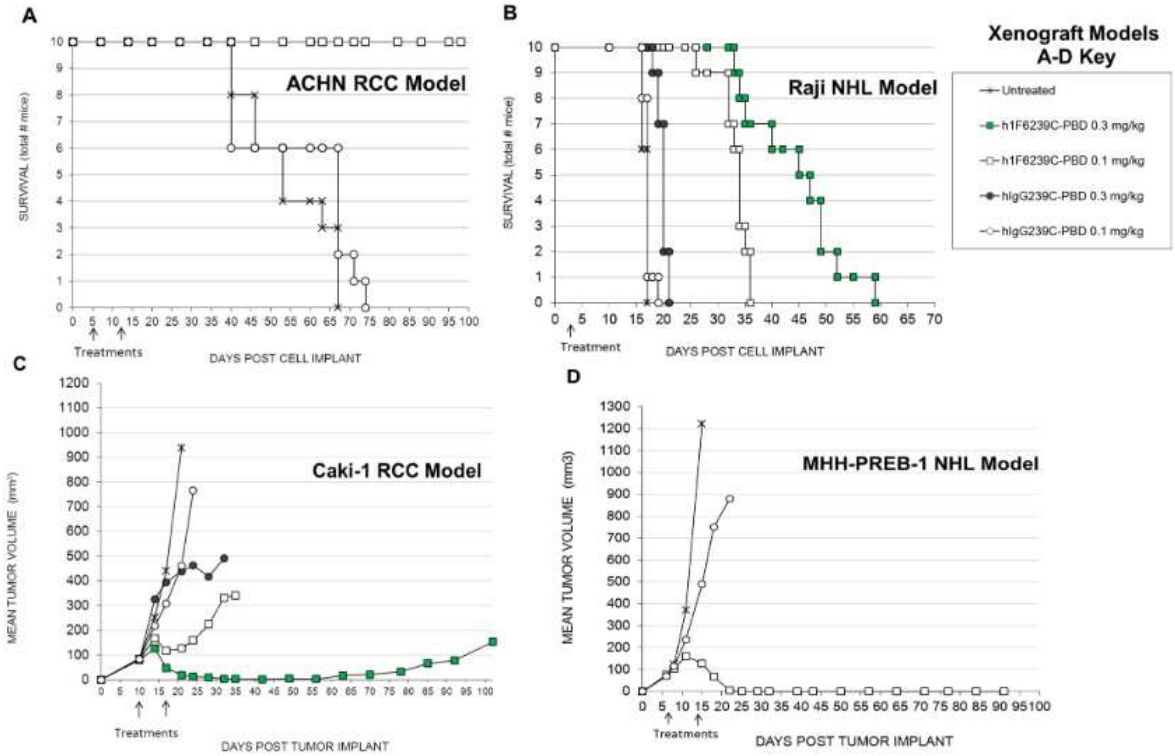


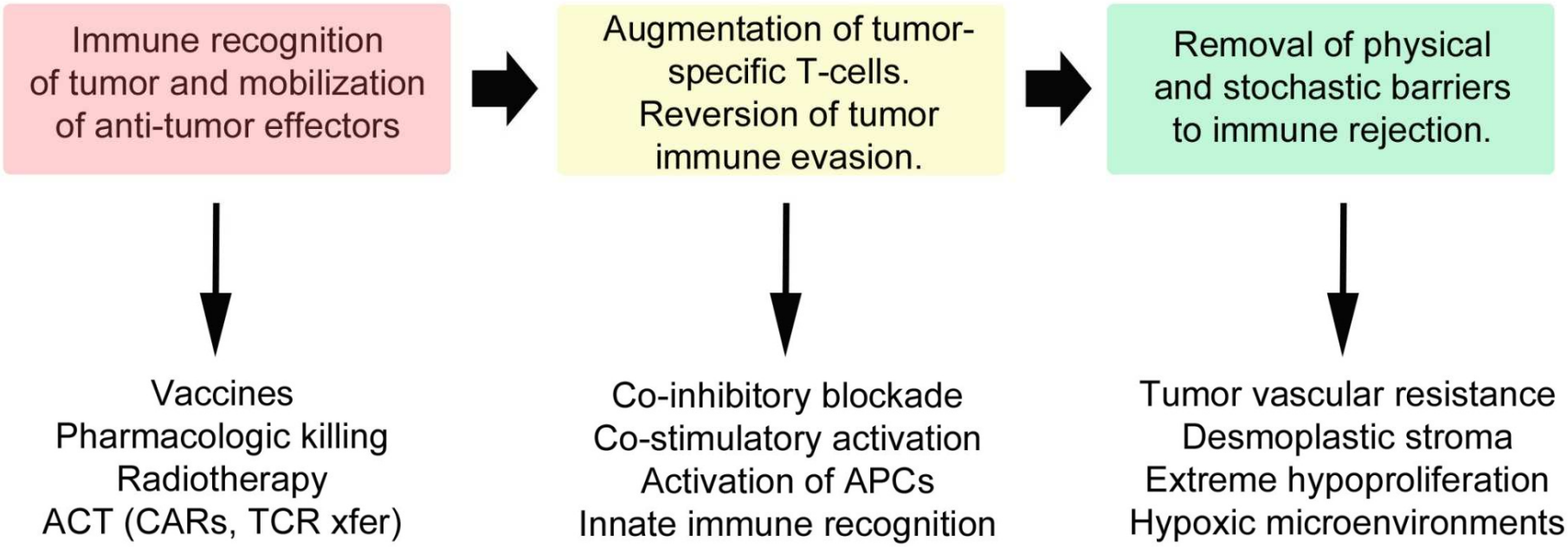
Figure 2. Conjugation process for the 239C antibody format. The engineered antibody, expressed in CHO cells, was isolated as the cysteine disulfide at position 239. The antibody was fully reduced with TCEP and partially reoxidized with dehydroascorbic acid. The resulting free cysteines at position 239 were conjugated to the PBD-linker to give the PBD ADC with nominally 2 drugs/mAb.



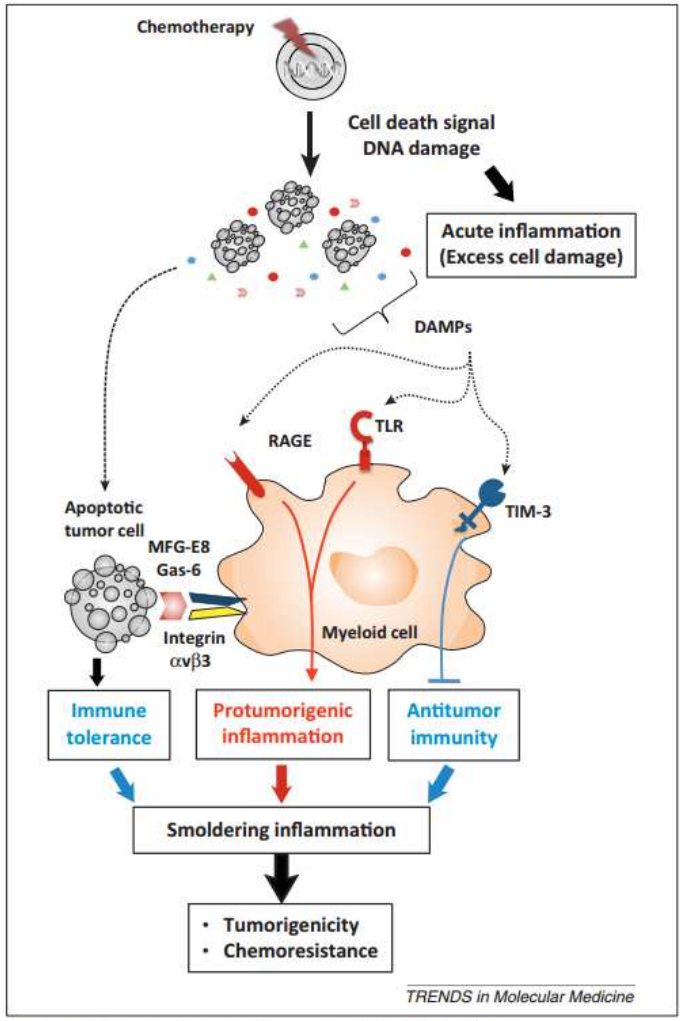
To exist, tumors must evolve mechanisms to locally disable and/or evade the immune system.

The goal of effector antibodies is to utilize the exquisite sensitivity of antibodies to specifically target and kill tumor cells using mechanisms which are difficult to evade or suppress.

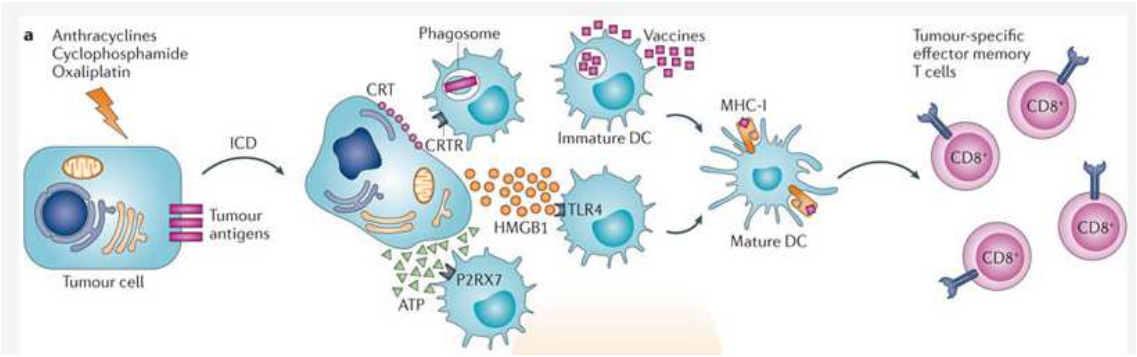
Seeking combinations outside of T cell checkpoint immunotherapy



A different perspective on chemotherapy: immunogenic vs. non-immunogenic cell death



Trends in Molecular Medicine September 2013, Vol. 19, No. 9



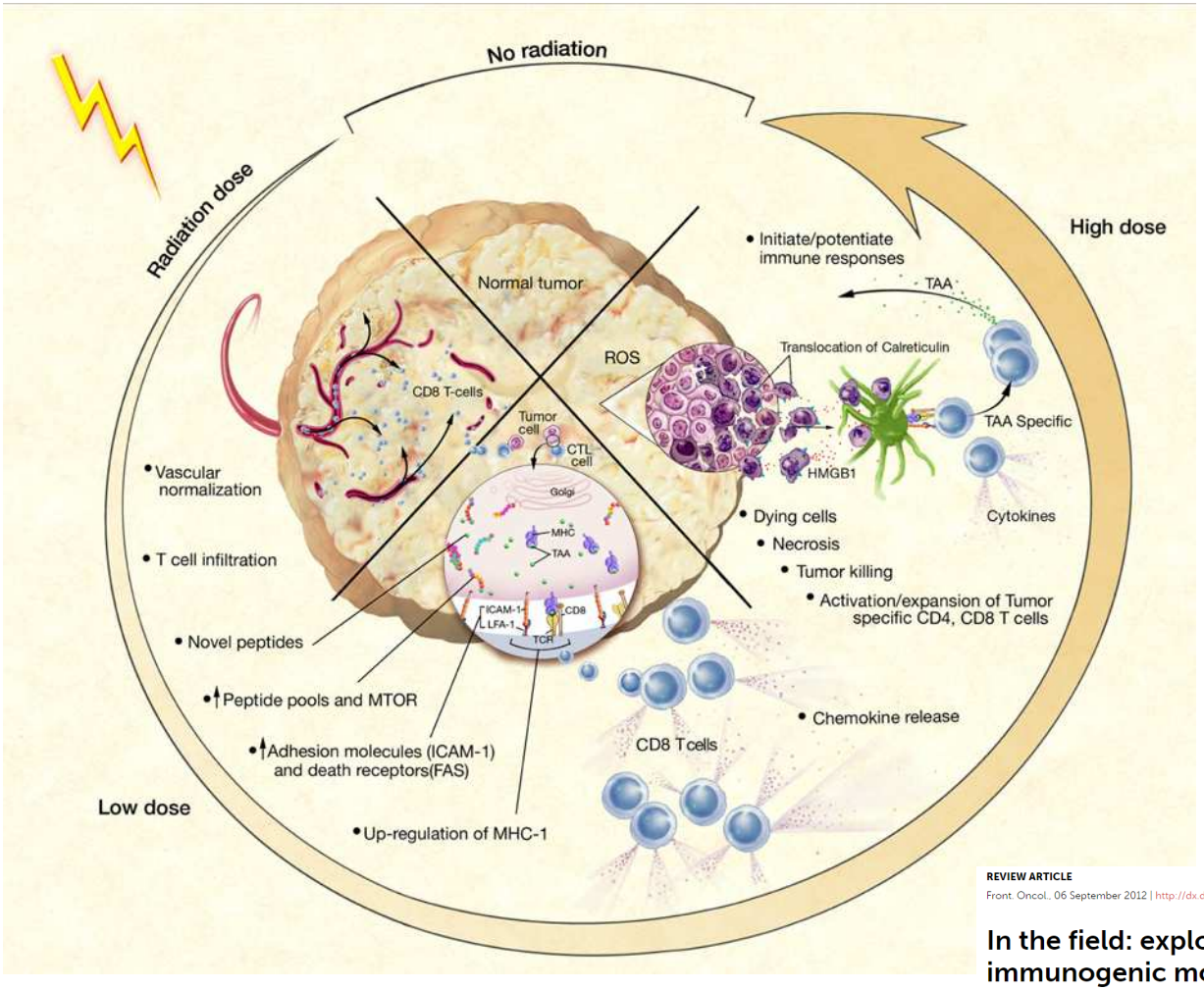
Agent	Indications	Notes
Cyclophos-phamide	Lymphoma, leukaemia, solid tumours	• Immunosuppressive at high doses • Increases class I HLA expression on cancer cells • Selectively inhibits T_{reg} cells and MDSCs, perhaps with a preferential activity on intratumoural populations • Favours the differentiation of IL-17-producing CD4+ cells • Stimulates the expansion of CD8α+ DCs • Restores T cell and NK cell functions • Inhibits IL-4, IL-10 and IL-13 production • Induces immunogenic cell death
Doxorubicin	Several solid and haematopoietic tumours	• Favours the proliferation of tumour-specific CD8+ T cells • Promotes tumour infiltration by IL-17-secreting γδ T cells and activated IFNγ-secreting CD8+ T cells • Induces immunogenic cell death • Stimulates antigen presentation by DCs • Increases the permeability of tumour cells to granzyme B • Induces MCP1 expression on tumour cells, in turn driving the establishment of an immunosuppressive stroma
Gemcitabine	NSCLC, pancreatic cancer, bladder cancer, breast cancer	• Increases class I HLA expression • Enhances tumour antigen cross-presentation • Selectively kills MDSCs
Oxaliplatin	Colorectal cancer	• Increases class I HLA expression • Inhibits PDL2 expression • Induces immunogenic cell death

The secret ally: immunostimulation by anticancer drugs

Lorenzo Galluzzi, Laura Senovilla, Laurence Zitvogel & Guido Kroemer

Nature Reviews Drug Discovery **11**, 215-233 (March 2012)

Radiation therapy : a potent adjuvant for tumor immunity

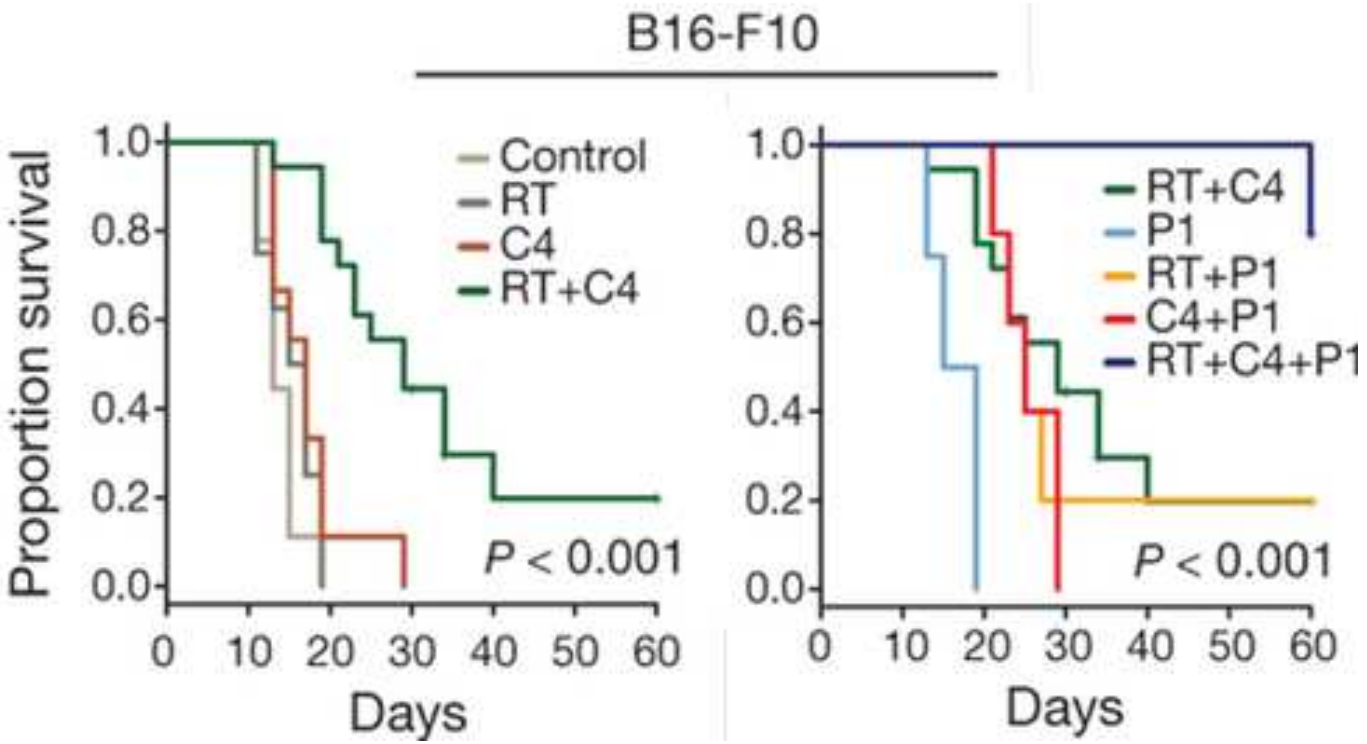


REVIEW ARTICLE
Front. Oncol., 06 September 2012 | http://dx.doi.org/10.3389/fonc.2012.00104

In the field: exploiting the untapped potential of immunogenic modulation by radiation in combination with immunotherapy for the treatment of cancer

Anna R. Kwilas[1], Renee N. Donahue[1], Michael B. Bernstein[2] and James W. Hodge[1*]

Radiotherapy synergizes with blockade of CTLA-4 and PD-1 to cure melanoma lung metastases



Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer

Christina Twyman-Saint Victor, Andrew J. Rech, Amit Maity, Ramesh Rengan, Kristen E. Pauken, Erietta Stelekati, Joseph L. Benci, Bihui Xu, Hannah Dada, Pamela M. Odorizzi, Ramin S. Herati, Kathleen D. Mansfield, Dana Patsch, Ravi K. Amaravadi, Lynn M. Schuchter, Hemant Ishwaran, Rosemarie Mick, Daniel A. Pryma, Xiaowei Xu, Michael D. Feldman, Tara C. Gangadhar, Stephen M. Hahn, E. John Wherry, Robert H. Vonderheide & Andy J. Minn

Nature **520**, 373–377 (16 April 2015) | doi:10.1038/nature14292

More consistent benefit for a larger percentage of patients with a wide range of cancer types