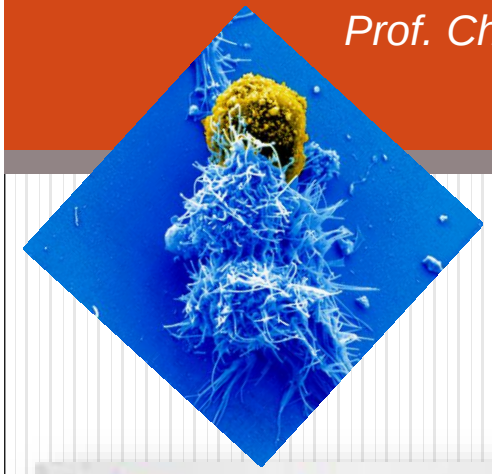
Cancer Immunotherapy Innovations

Prof. Christine DELPRAT, Université Claude Bernard Lyon 1 - FR



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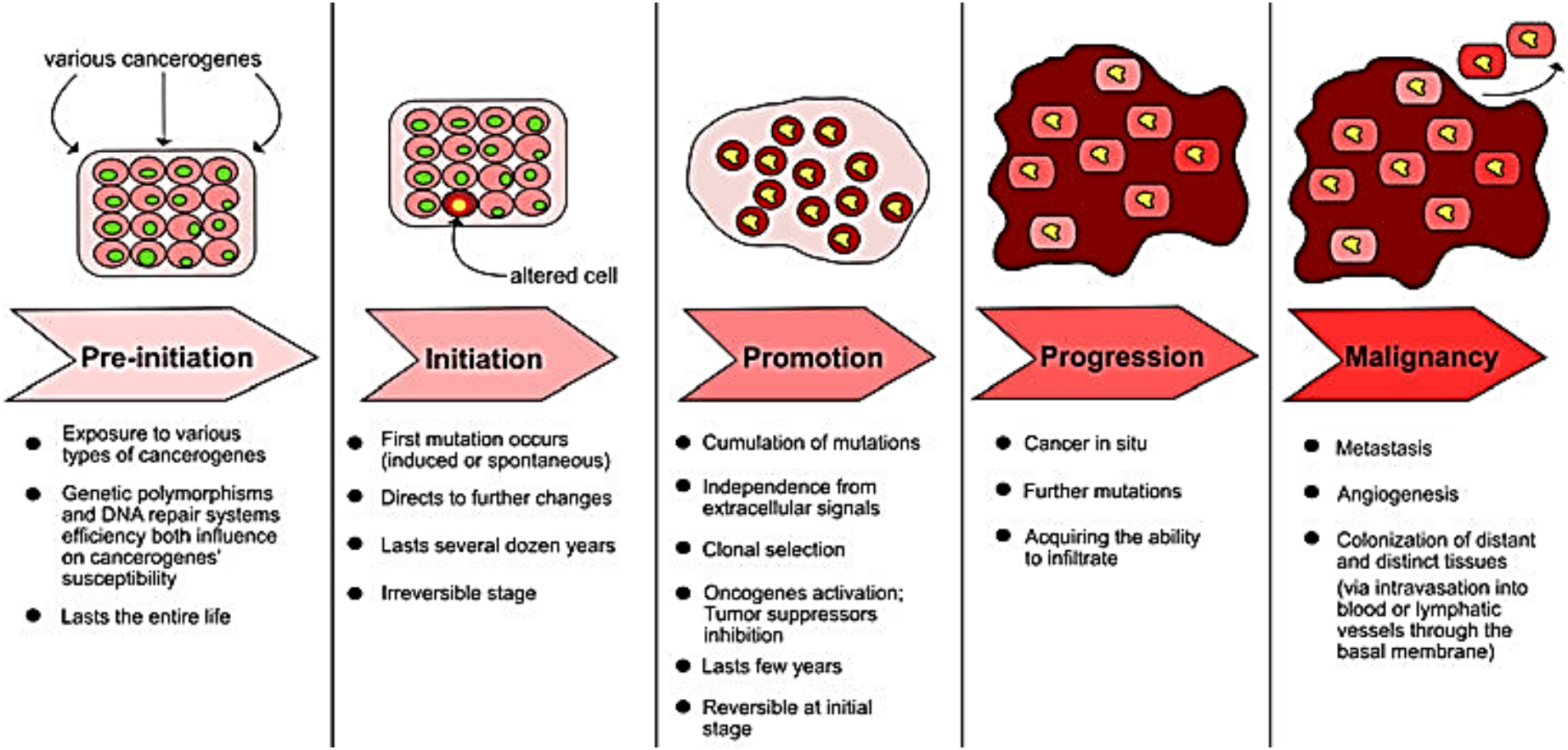
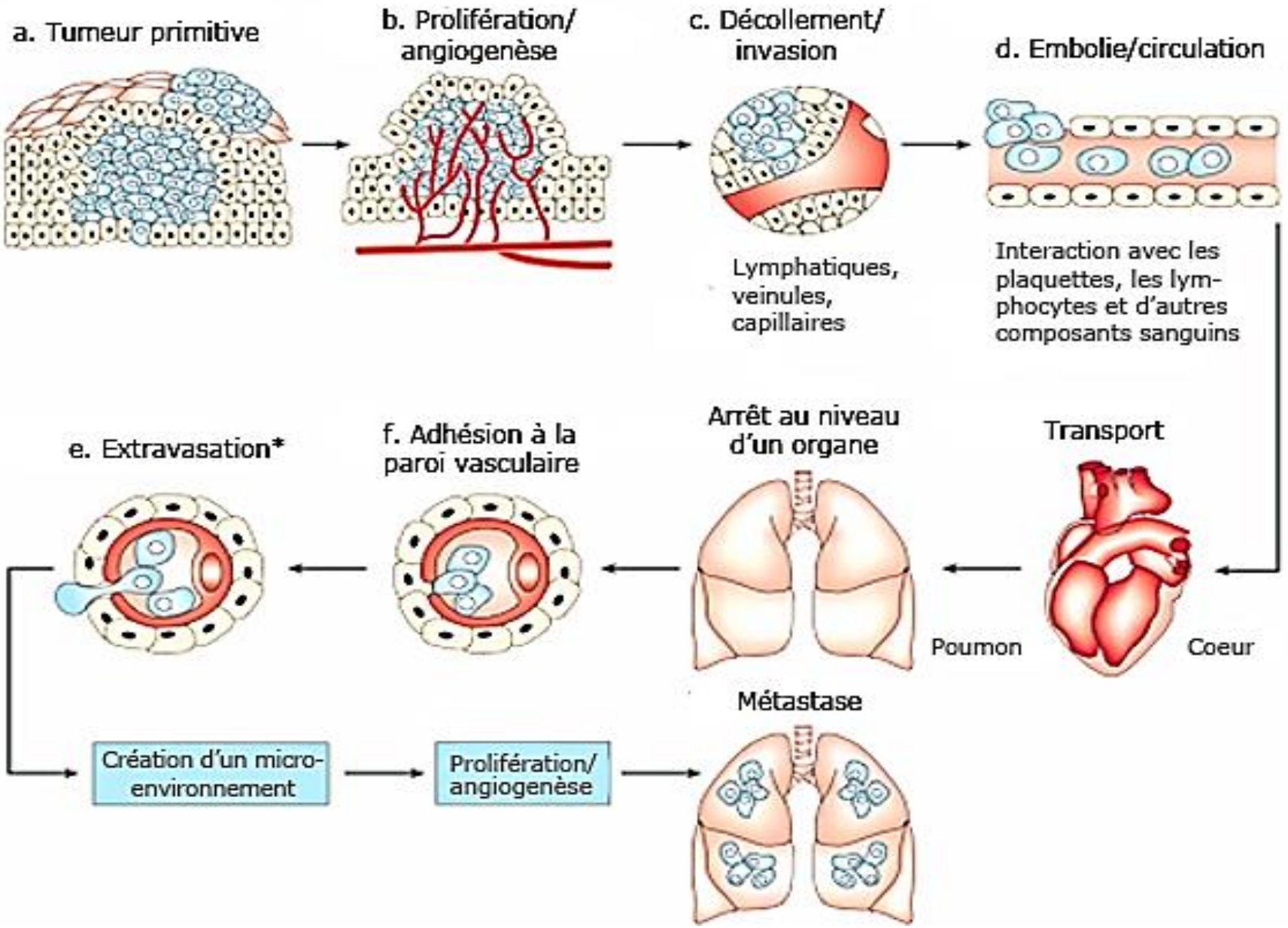
Jan 20, 2020

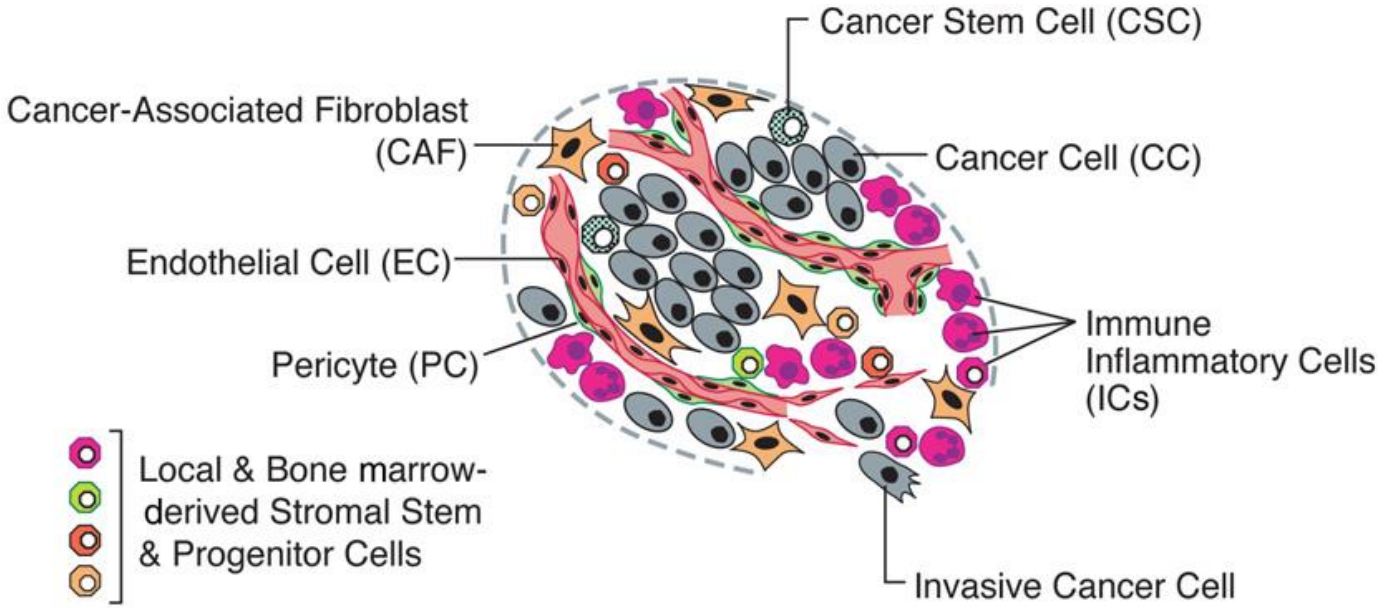
CANCEROGENESIS STEPS

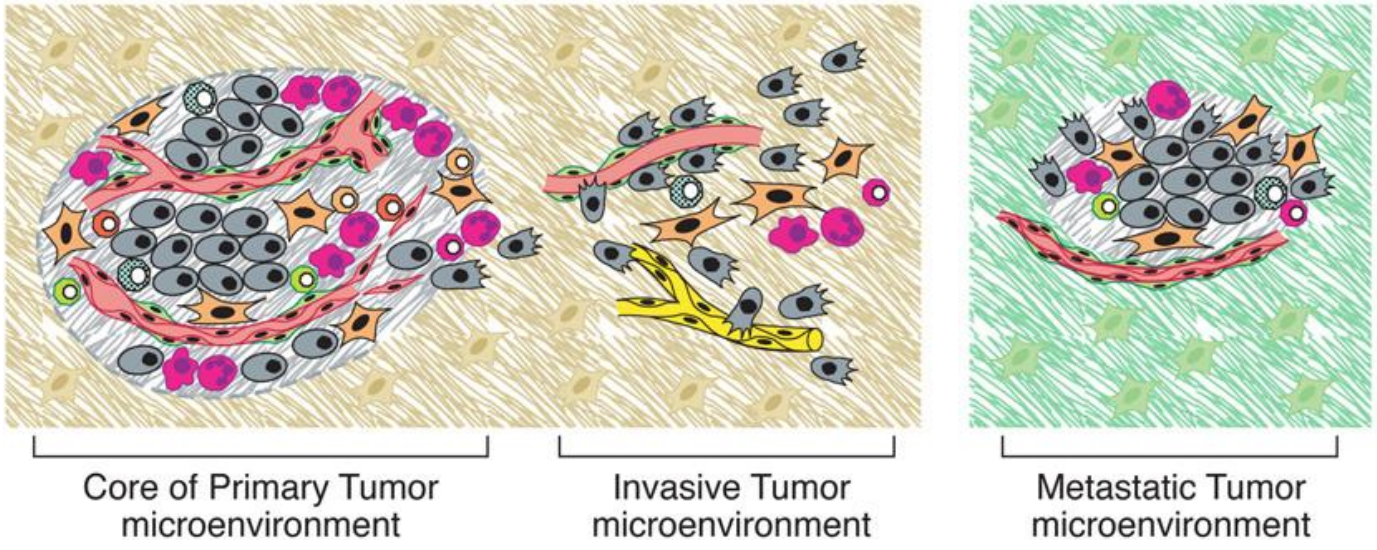


METASTASIS STEPS

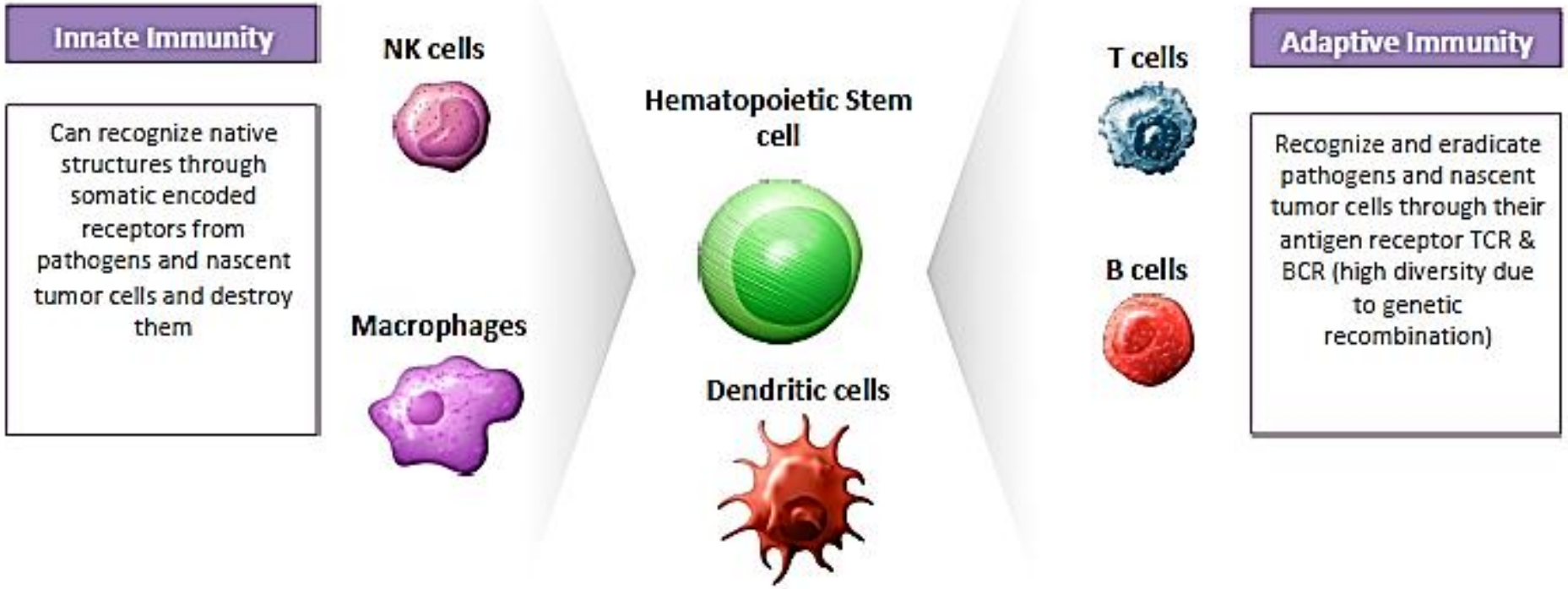


ESTABLISHED CANCER





Immunosurveillance: both innate and adaptive arms of the immune system can fight tumors

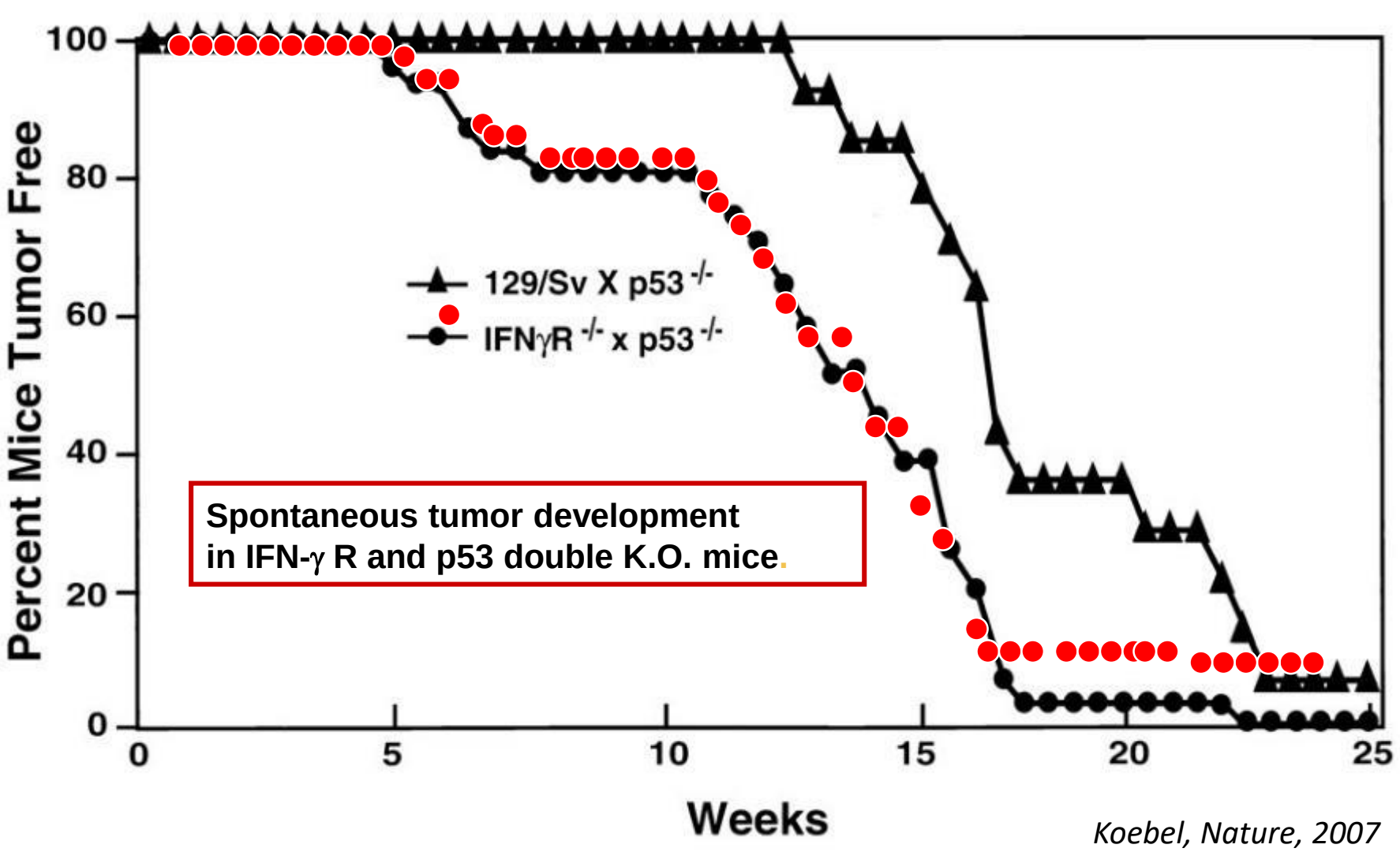


NK = natural killer.

Norvell A. In: Prendergast GC et al. Cancer Immunotherapy. 2nd ed. Elsevier; 2013:11–24.

IMMUNOSURVEILLANCE

Demonstration of an interferon-γ-dependent tumor surveillance system in immunocompetent mice.
Daniel H. Kaplan, Vijay Shankaran, Anand S. Dighe, Elisabeth Stockert, Michel Aguet, Lloyd J. Old, and Robert D. Schreiber.
PNAS 95: 7556, 1998



Koebel, Nature, 2007

IMMUNOSURVEILLANCE

Table 1. Cancer Types Diagnosed and Incidence Comparison in the Transplant (Tx) and Non-transplant (non-Tx) Population

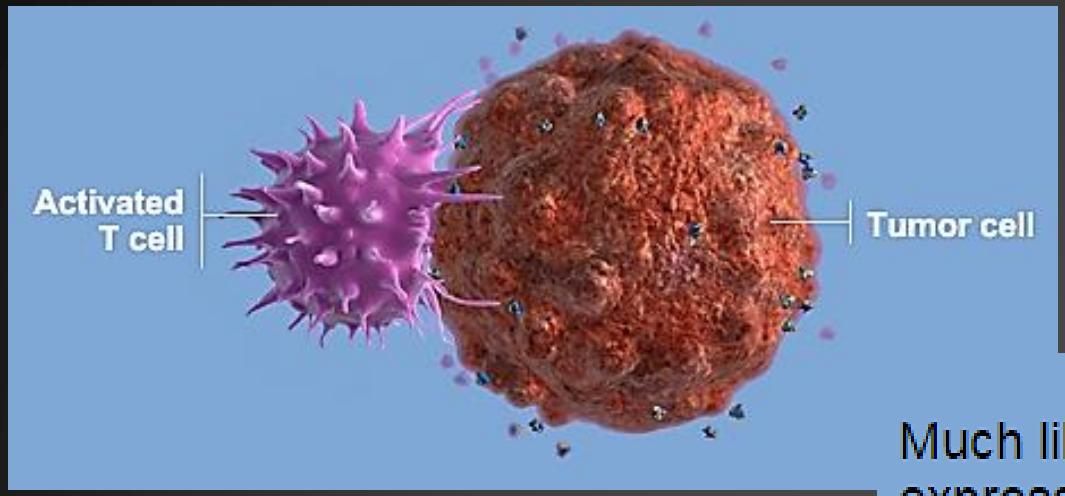
Cancer type	Cases	Incidence in Tx population[a]	Incidence in non-Tx population[b]	RR
Leukemia/lymphoma	33	692.0	26.4	26.2
Head and neck	16	335.5	16.0	21.0
Lung	12	251.6	27.0	9.3
Melanoma	7	146.8	25.6	5.7
Bowel	7	146.8	44.0	3.3
Prostate	5	104.9	61.0	1.7
Colon	4	83.9	N/A	N/A
Breast	2	41.9	26.3	1.6

[a]Incidence per 100,000 person-years.

[b]Age-standardized incidence per 100,000 person-years.

Roitmaier, Lung transplant, 2007

Every patient's immune system has the ability to fight cancer



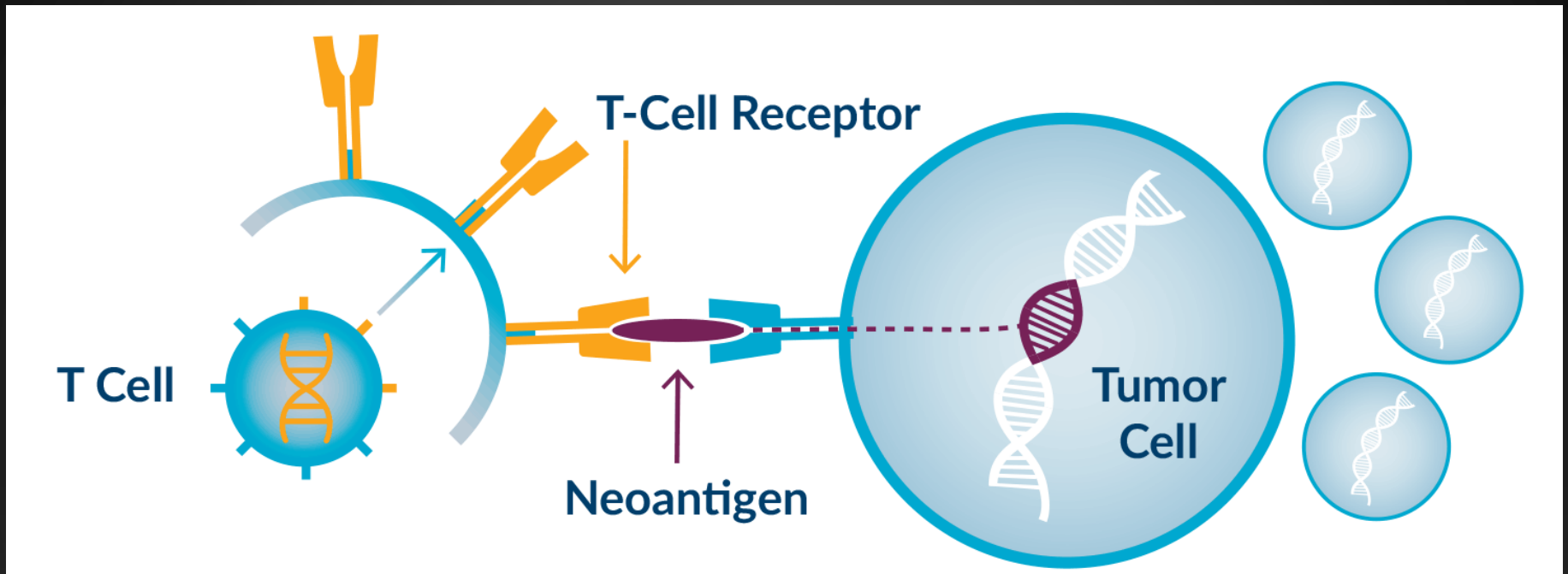
Much like infectious agents, tumor cells express specific antigens that differentiate them from normal cells[1]:

- T cells can recognize and destroy cells displaying tumor antigens[1]
- B cells drive the production of antibodies directed against tumor antigens[1,2]

T cell infiltration within tumors is associated with overall survival (OS) in patients with different cancers.[3-4]

1. Murphy K, Travers P, Walport M, eds. *Janeway's Immunobiology*. 7th ed. Garland Science, Taylor & Frances Group, LLC. New York, NY: 2008.
2. Namm JP, Li Q, Lao X, et al. *J Surg Oncol*. 2012;105:431-435.
3. Zhang L, et al. *N Engl J Med*. 2003;348:203-213.
4. Galon J, et al. *Science*. 2006;313:1960-1964.

Neoantigens: some tumor cells express multiple antigens that are not expressed by normal cells



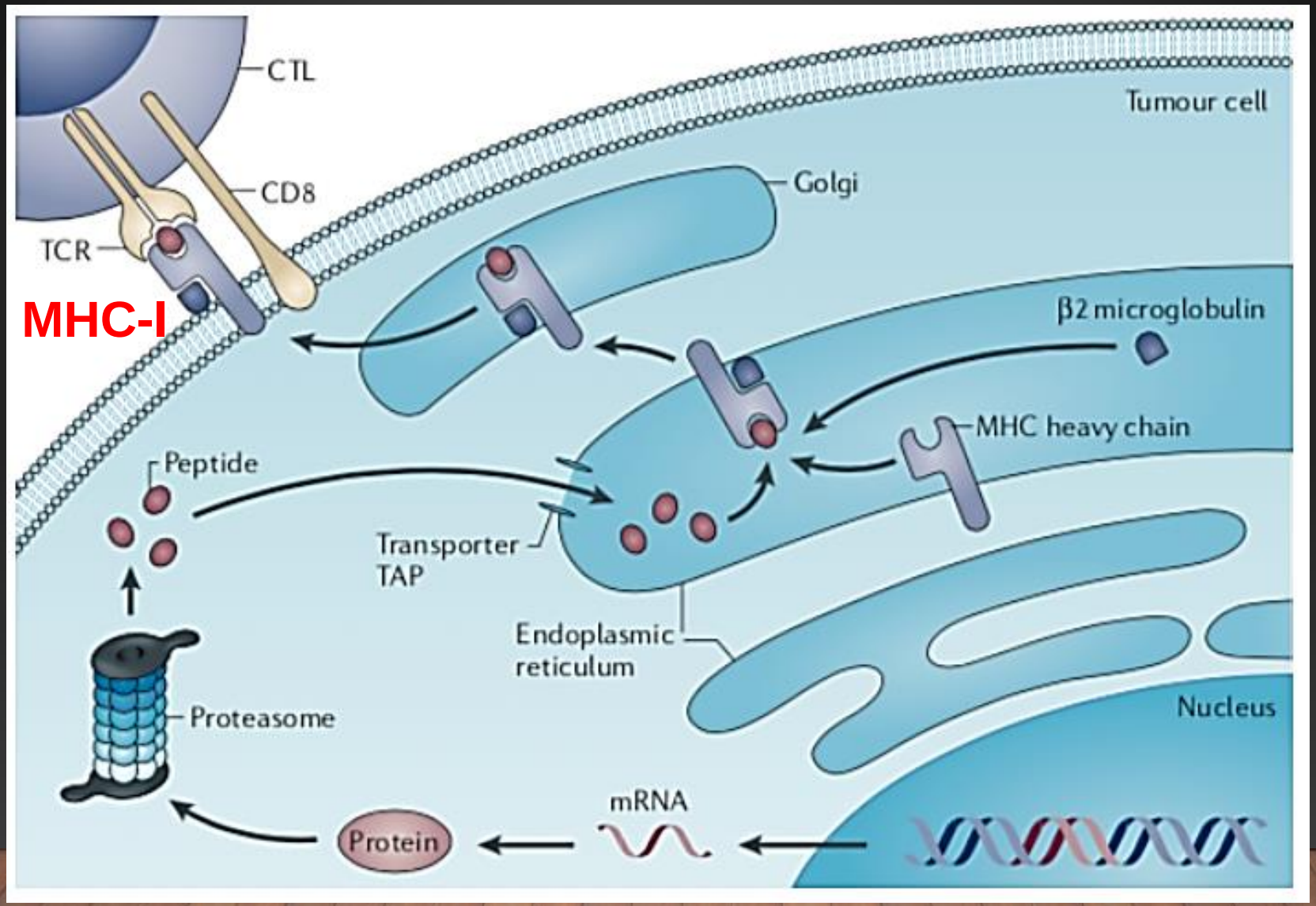
T-Cell receptors

T-cell receptors or TCRs are molecules on the surface of cancer fighting T cells that have the ability to interrogate individual cancer cells and see beneath the cell membrane.

Neoantigens

Antigens are the unique molecules or proteins that help immune cells identify and fight cancer cells. Neoantigens are unique to each patient's tumor cells.

Processing of tumor antigens recognized by CD8+ T cells

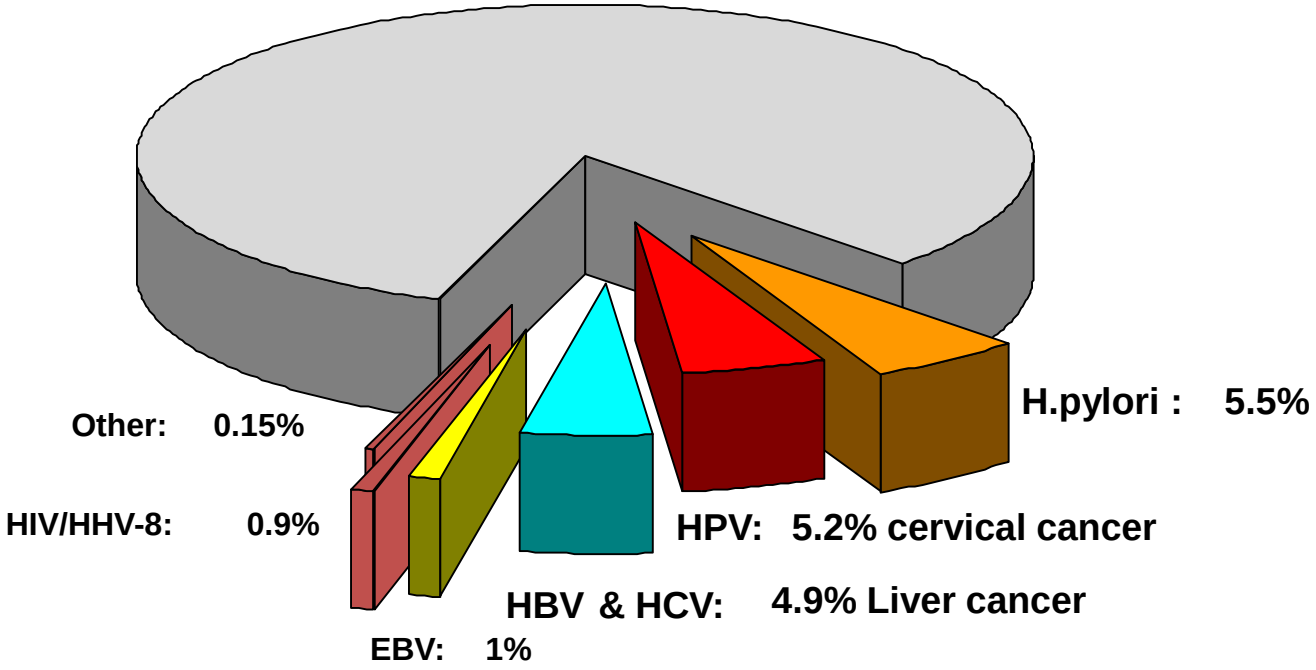


Coulie, Nat Rev Cancer, 2014

Oncopathogens provide antigens

- **1 out 4** cancers attributed to infection worldwide : **>26%**
- **Chronic infection** is a key event in cancer development

Which infections?... Other pathogen candidates for the remaining 74%?



Bouvard et al, Lancet 2009
Transceschi et al, Lancet oncology 2012
IARC monograph 2011
Parkin Br J of medicine, 2010

International Agency for Research on Cancer
World Health Organization

2013 WHO classification of carcinogenic agents

Group 1	*Carcinogenic to humans*	114 agents
Group 2A	*Probably carcinogenic to humans*	69
Group 2B	*Possibly carcinogenic to humans*	283
Group 3	*Not classifiable as to its carcinogenicity to humans*	504
Group 4	*Probably not carcinogenic to humans*	1

Group 1:

- Helicobacter pylori
- HBV, HCV
- HIV1
- HPV -16/18
- HTLV-I, HHV-8, EBV

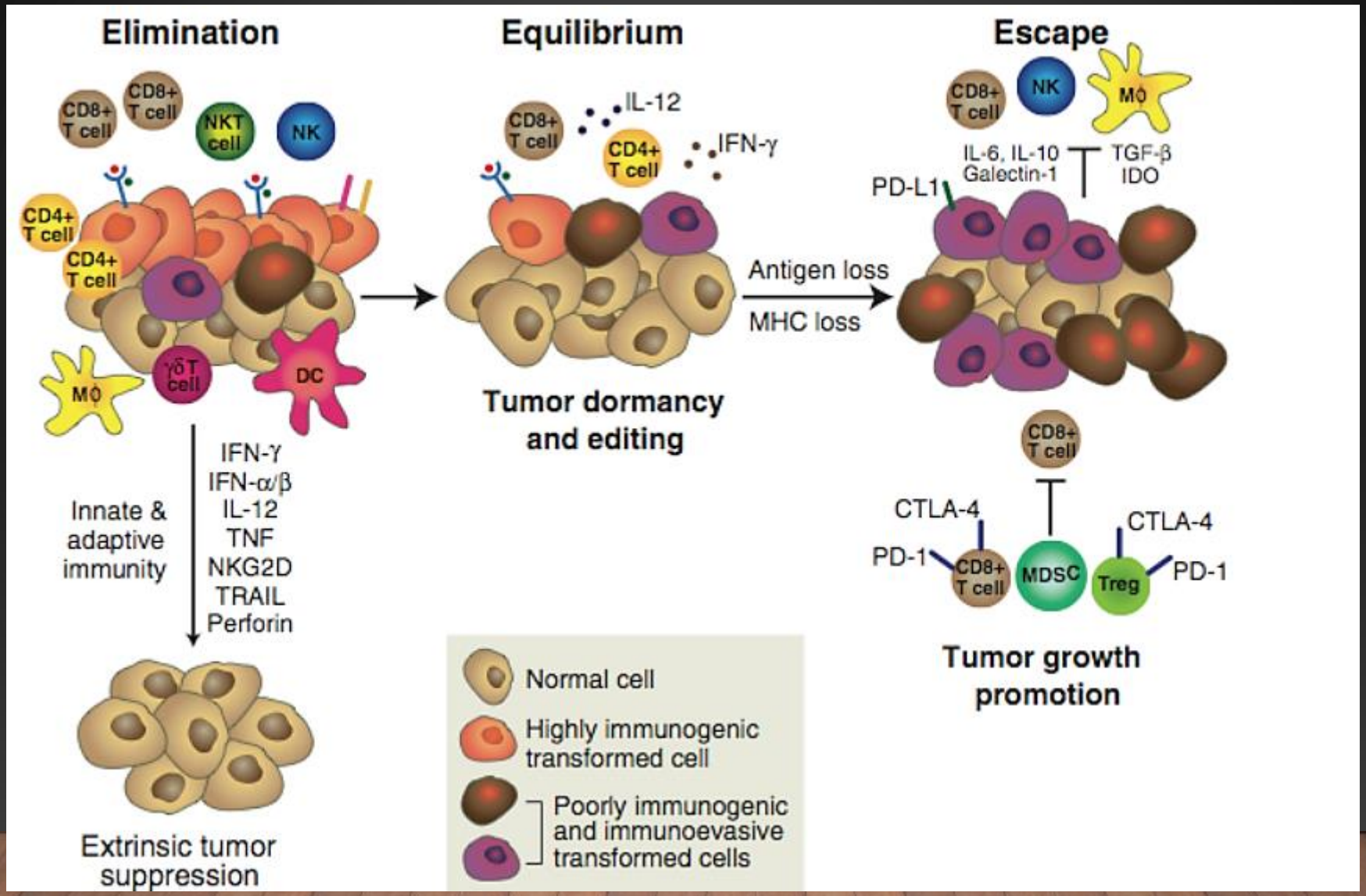
Group 2A: HPV-68, **MCPyV (2013)**

Group 2B: HPV-5/8…, HIV-2, **JCPyV (2013), BKPyV (2013)**

2013:
+3 Polyomavirus

Oncopathogens 2013:
Bacteria: 1, Viruses: **>10**

IMMUNOEDITING: A DYNAMIC HISTORY



Adapted from Schriber, Science, 2011

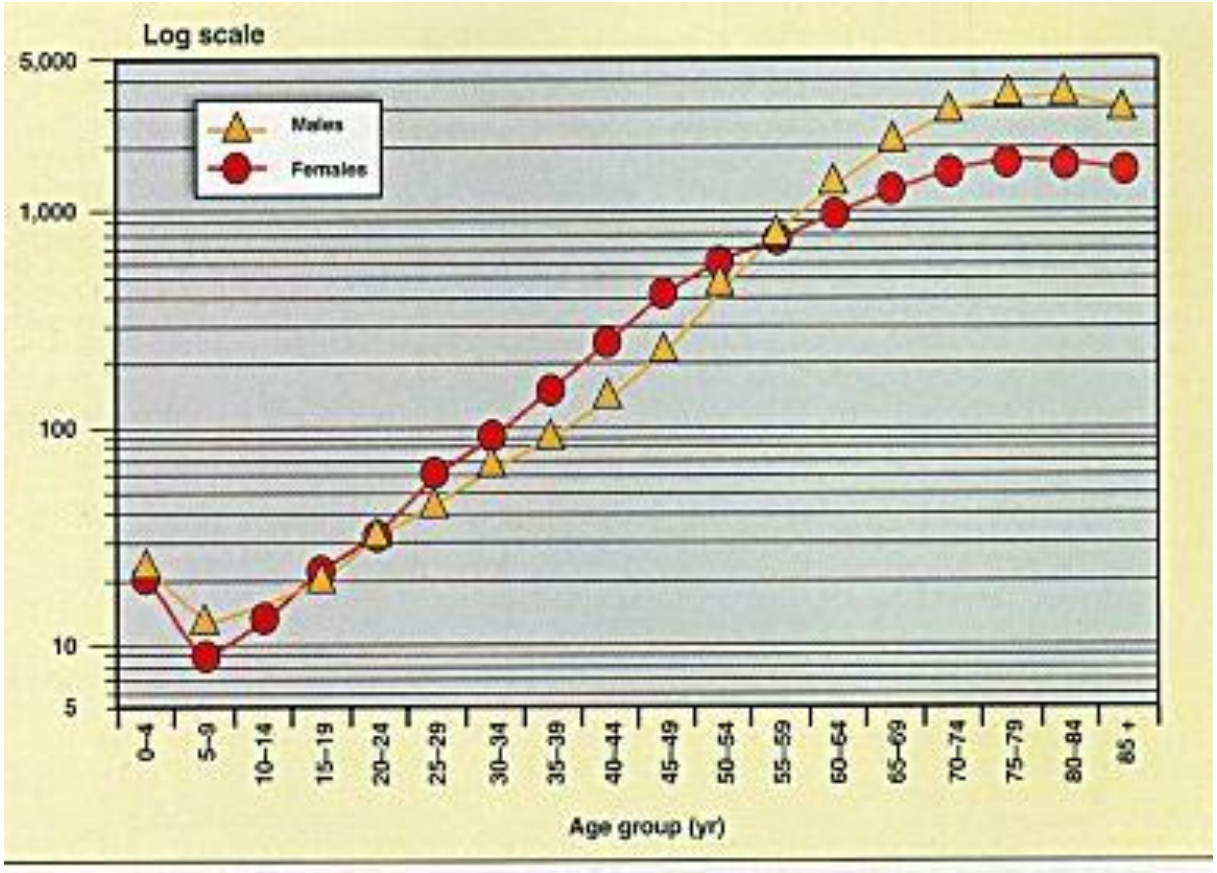
Aging is the single biggest risk factor for developing cancer

✓Aging process is **complex**: each person ages at a different rate
✓ Your actual **age may not reflect your physiologic age**
✓ Lower tolerance of **stress**
✓ Higher susceptibility to **chronic infections**
✓ Lower competence to eliminate cancer cells due to the aging of the **immune system**

Cancer incidence rates by age category (Log scale!)

Specific immune systems:

- **aged people**
- **immunosuppressed patients**



IMMUNOTHERAPY IN SOLID TUMORS

• Cytokines : IL-2, IFNα. Limited clinical activity (melanoma, renal cell carcinoma) and **toxicity issues**

• **Disappointing results** with tumor vaccination: less than 7% of objective clinical responses (Rosenberg Nat Med 2004)

– Use of self antigens and stimulation of T cells with low-affinity TcR unable to mediate effective antitumor response

– Use of monovalent antigen-targeting strategies selecting resistant tumor variants

– Suboptimal delivery systems resulting in weak and short-lived antigen specific T cell response

– Role of immunosuppressive tumor microenvironment and of … checkpoint molecules

→ skepticism about immunotherapy

History of immunotherapy

Immunotherapy has been under evaluation for more than a century, but only recently has it entered a renaissance phase with approval of multiple agents for the treatment of cancer.

ENTHUSIASM IS BACK! from 2010'

ENTHUSIASM PHASE 1978-1985

SKEPTICISM PHASE 1985-1997

RENAISSANCE PHASE 1997-NOW

1890s 1st CA vaccine developed (Coley)

1973 Discovery of the dendritic cell (Steinman)

1976 1st study with BCG in bladder CA

1978 Discovery of tumor specific mABs

1985 1st study with adoptive T cell transfer in CA

1986 IFN-α (cytokine) approved for CA

1990s Discovery of checkpoint inhibitor (Allison)

1997 1st mAB approved for CA

1998 IL-2 (cytokine) approved for CA

2010 1st cellular immunotherapy approved for CA

2011 1st checkpoint inhibitor approved for CA

Adapted with permission from Lesterhuls WJ, et al[2] and Kirkwood JM, et al. *J Clin Oncol*. 2008;26(20):3445-3455.

BCG=Bacillus Calmette-Guérin; CA=cancer

History of immunotherapy

- ✓ 2010: first cellular immunotherapy approved
- ✓ 2011: first checkpoint-inhibitor drug approved
- ✓ 2015: first oncolytic virus treatment approved
- ✓ 2017: first two chimeric antigen receptor (CAR) T-cell treatments approved. Thousands of clinical studies are pitting such immunotherapies against almost every form of cancer.
- ✓ Neoantigen vaccine development is progressing rapidly.
- ✓ NK-based therapies are new concerns in the field.
- ✓ Combining treatments is likely to help an even greater number of people.
- ✓ What cancer will benefit from specific pairing of drugs?
- ✓ Fatal side effects, although rare, are also not fully understood
- ✓ High cost of immunotherapeutic agents will require all stakeholders to agree on appropriate payment.

Two types of immunotherapy for cancer are proven effective

PASSIVE IMMUNOTHERAPY

Passive immunotherapy enhances pre-existing immune response and has a short life. Examples include monoclonal antibodies and cytokines.

ACTIVE IMMUNOTHERAPY

Active immunotherapy engages the immune system and is potentially durable. One example is therapeutic cancer vaccines.

Immunotherapies, such as monoclonal antibodies, checkpoint inhibitors, cytokines, and therapeutic vaccines, have been approved by the FDA to treat certain cancers.[2]

FDA-APPROVED IMMUNOTHERAPIES*[,2-6]

Class	Approvals
Monoclonal antibodies	1997, 1998, 2000, 2001, 2002, 2003, 2004, 2006, 2009
Checkpoint inhibitor	2011
Cytokines	1986, 1992, 1995, 1998
Therapeutic vaccine	2010

*Not inclusive of all immunotherapy classes.

CANCER IMMUNOTHERAPY INNOVATIONS

The study of interactions between the immune system & cancer cells

1. **Advanced checkpoint blockade**
2. Oncolytic viruses
3. CAR T cells & novel CAR cells
4. BiTEs
5. Tumor vaccines & Neo-Ag
6. Reverse vaccinology & mimotopes
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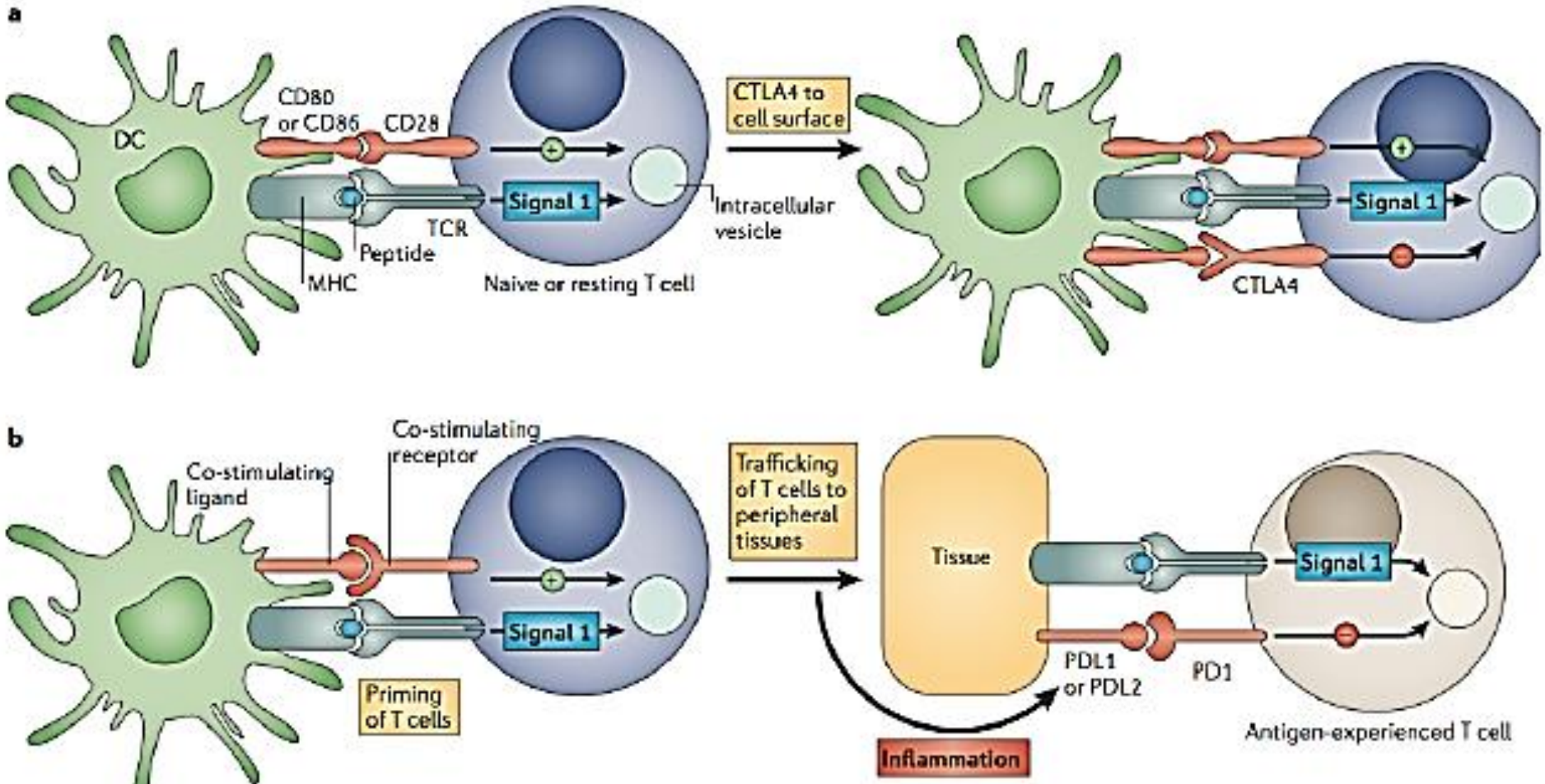
9. Quantitative multivariate model of human immune cell communication

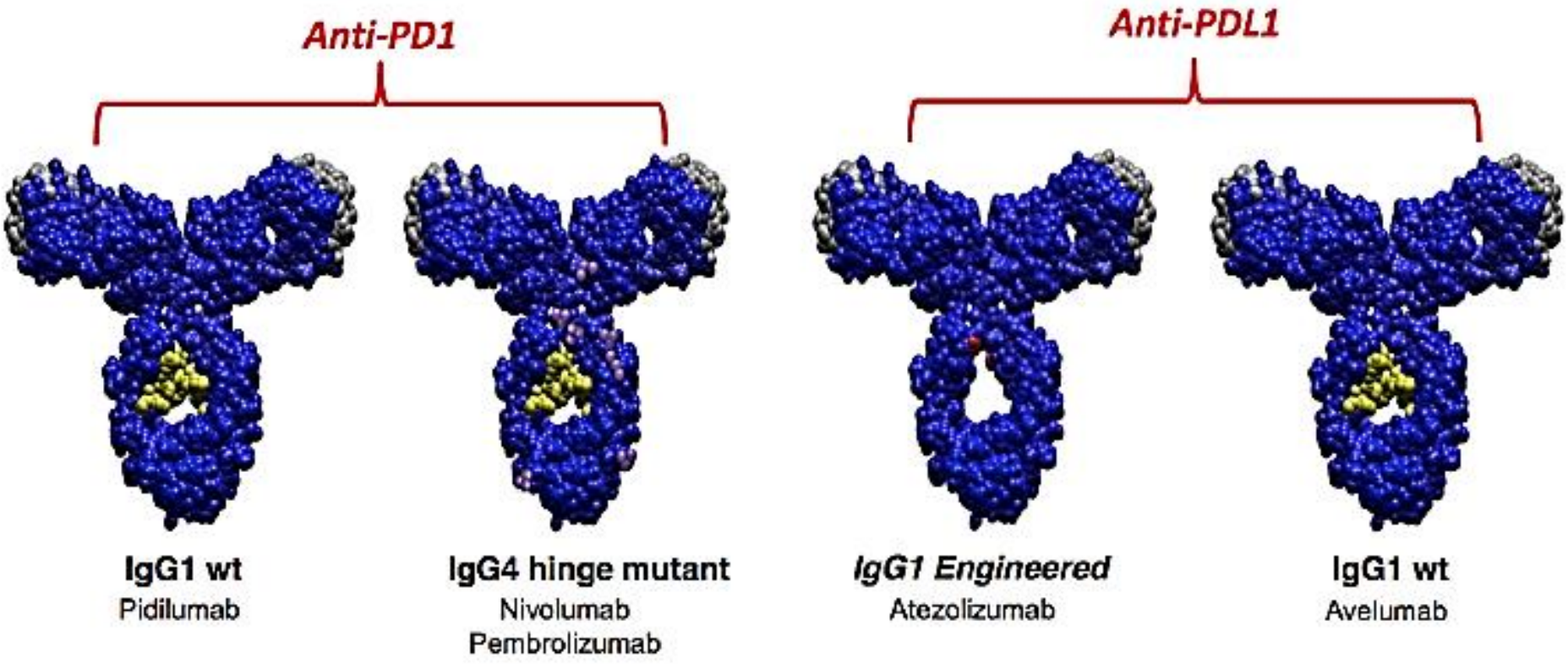
Aims: "Analyzing, understanding and manipulating interactions between tumor cells and the immune system to overcome the cancer progression.“

Anti-CTLA4 and anti-PD1/PDL-1



Pardoll D. Nat Rev Cancer 2012

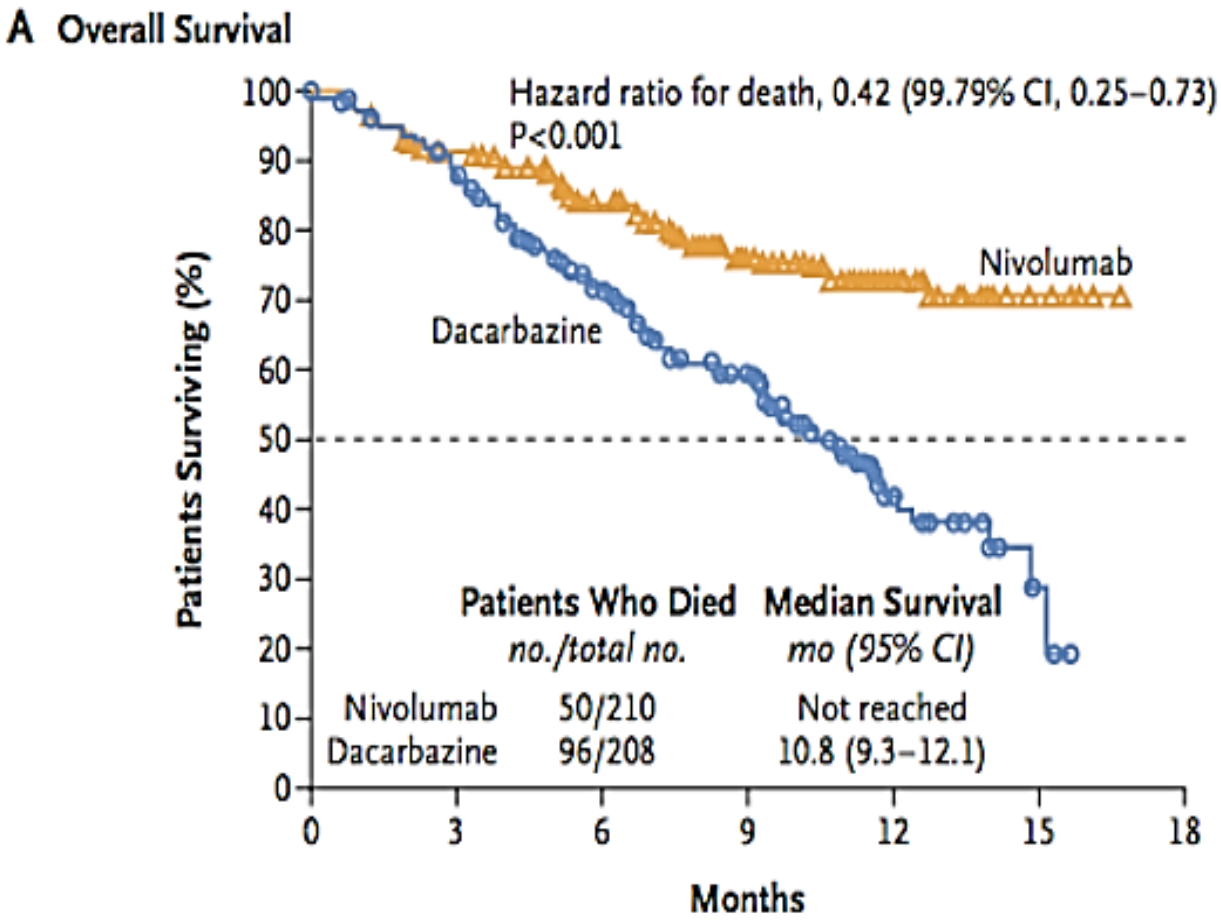
Different antibodies target PD-1 or PD-L1



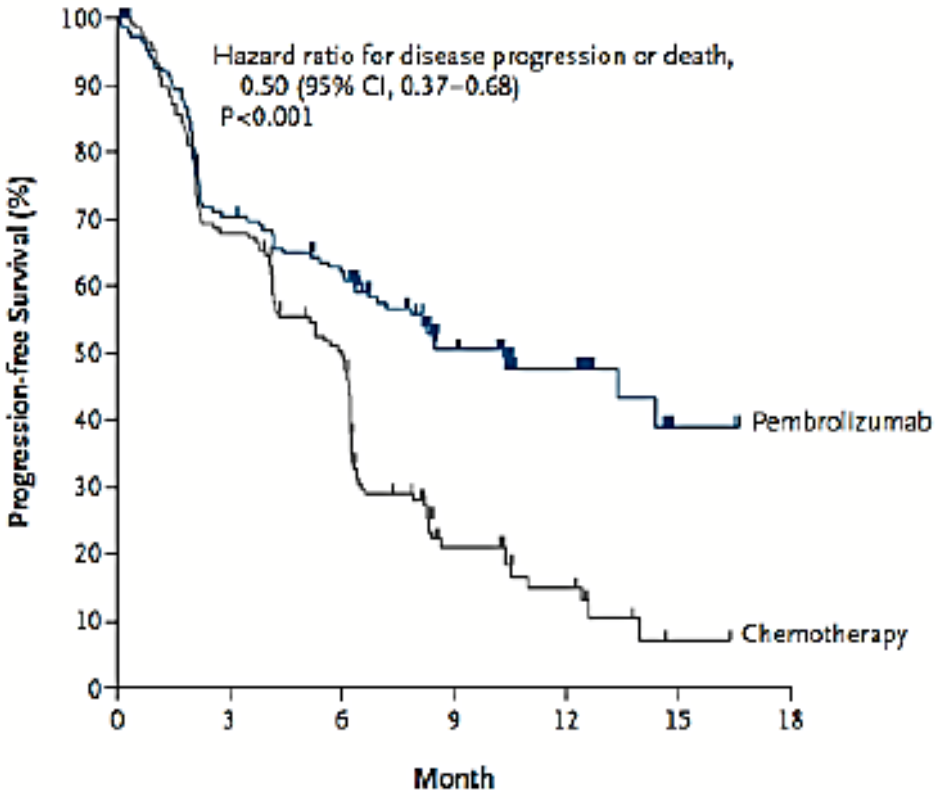
The NEW ENGLAND JOURNAL of MEDICINE

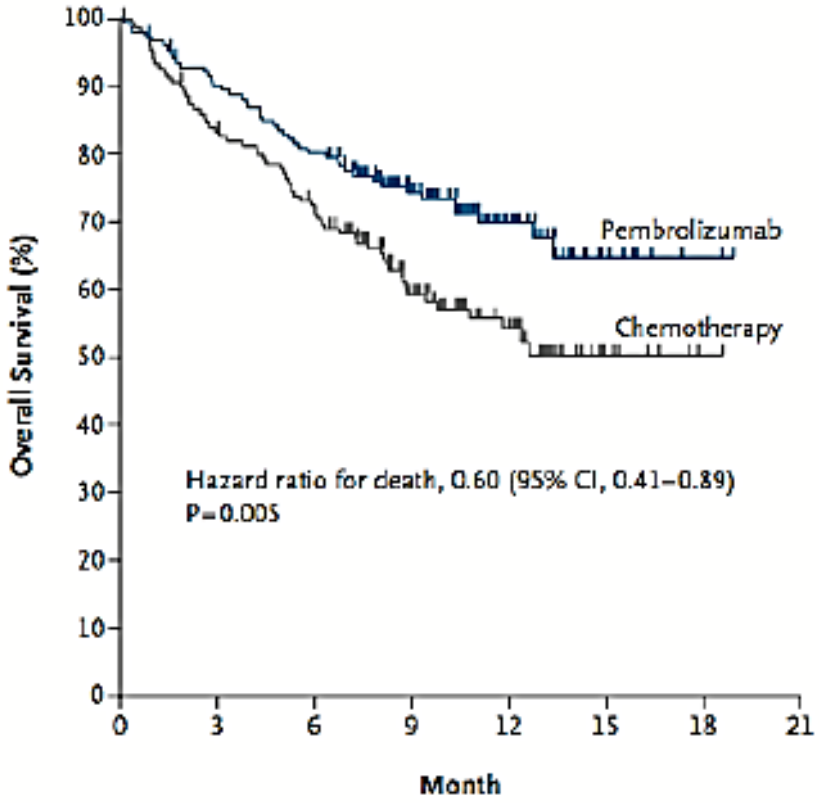
ORIGINAL ARTICLE

Nivolumab in Previously Untreated Melanoma without *BRAF* Mutation



Activity of PD-1 antagonists in first line in selected NSCLC patients





Tumor proportion score **≥ 50%**
Failure of nivolumab with a cut-off at 5%

Reck et al. N Engl J Med 2016

Spectrum of PD-1 / PD-L1 antagonist activity

Active

- Melanoma
- Non Small Cell Lung Carcinoma
- Small Cell Lung Carcinoma
- Renal cell carcinoma
- Head & Neck
- Gastric cancer
- Bladder cancer
- Triple Negative Breast Cancer
- Ovarian cancer
- Mesothelioma
- Merkel cell carcinoma
- Mismatch Repair deficient tumors (colon…)
- Hepatocellular carcinoma
- Glioblastoma
- Hodgkin Lymphoma
- Non Hodgkin Lymphoma

Minimal to no activity

- Prostate cancer (?)
- Non mismatch repair deficient colon cancer
- Pancreatic cancer

Response rate ≈10-40%

An effective immune response is specific, adaptive and sustainable (memory)

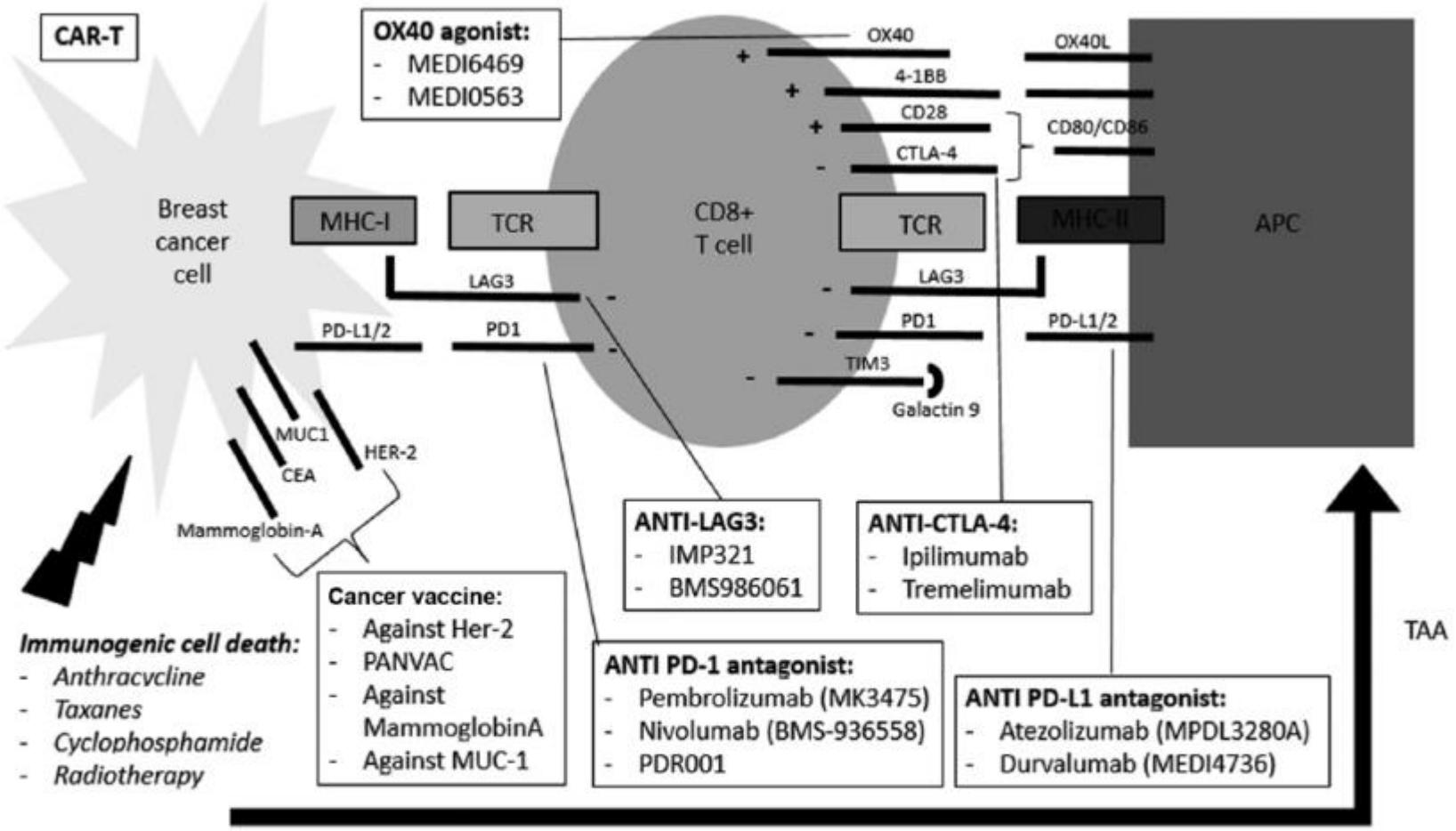
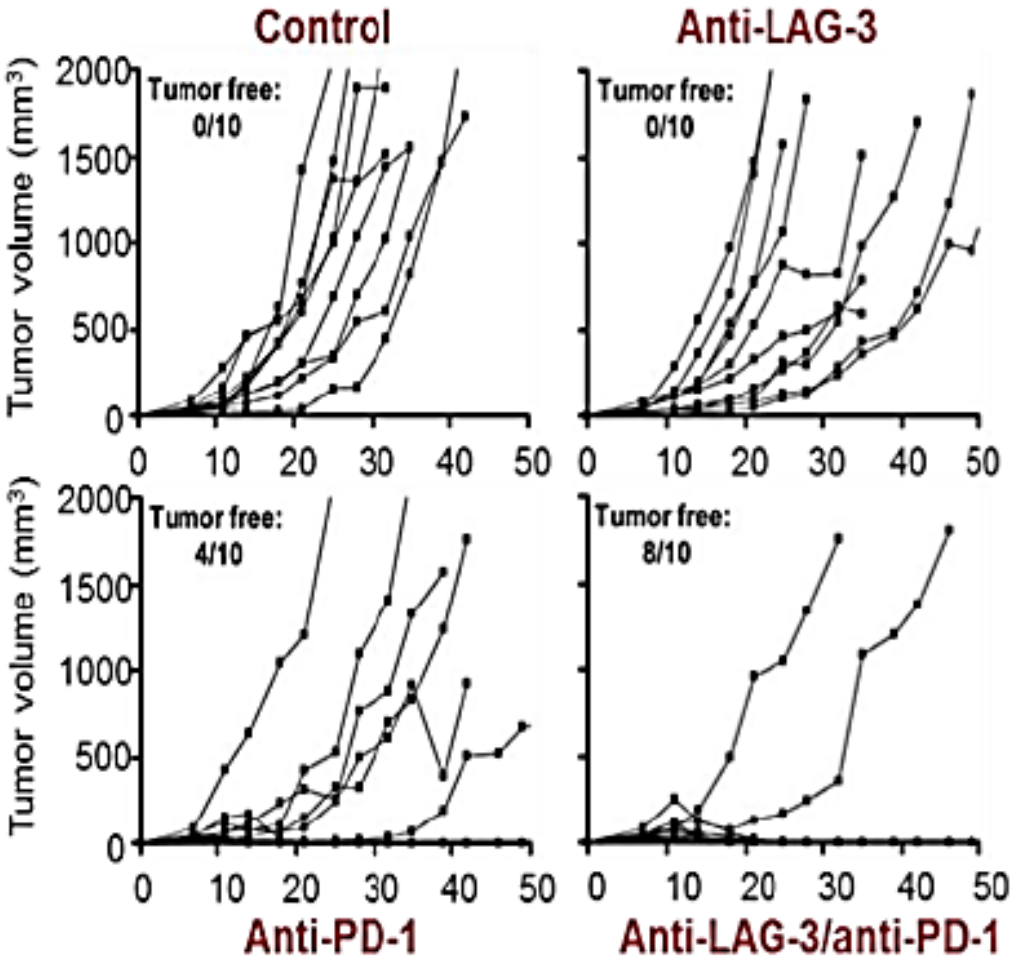
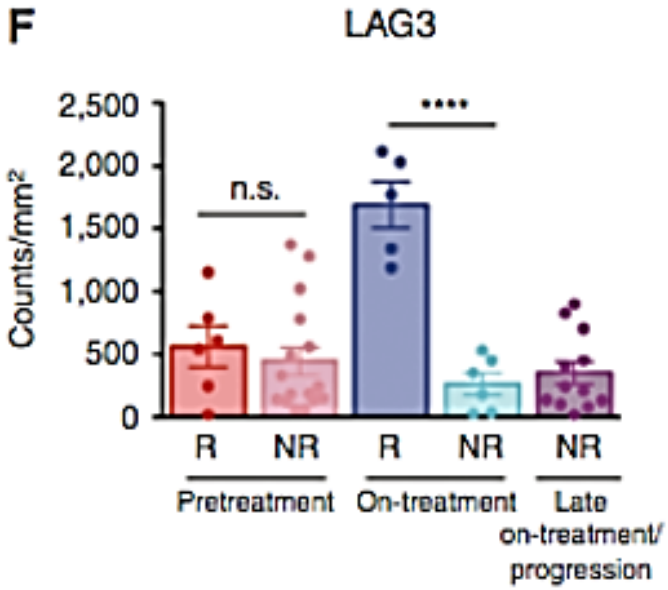


Figure 1. Immunotherapy strategies currently under investigation in BC treatment.
CAR-T, chimeric antigen receptor T cells; CEA, carcinoembryonic antigen; CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; HER-2, human epidermal growth factor receptor 2; LAG-3, lymphocyte activation gene-3; MHC, major histocompatibility complex; MUC1, Mucine 1; PD-1, programmed cell death-1; PD-L1/2, programmed cell death ligand 1/2; TAA, tumor associated antigen; TCR, T-cell receptor; TIM-3, T-cell immunoglobulin mucin-3.

Example combo PD-1 / LAG3



MC38 colon carcinoma; Woo et al *Cancer Res* 2012 72:917



Pei-ling Chen et al. Cancer Discov 2016

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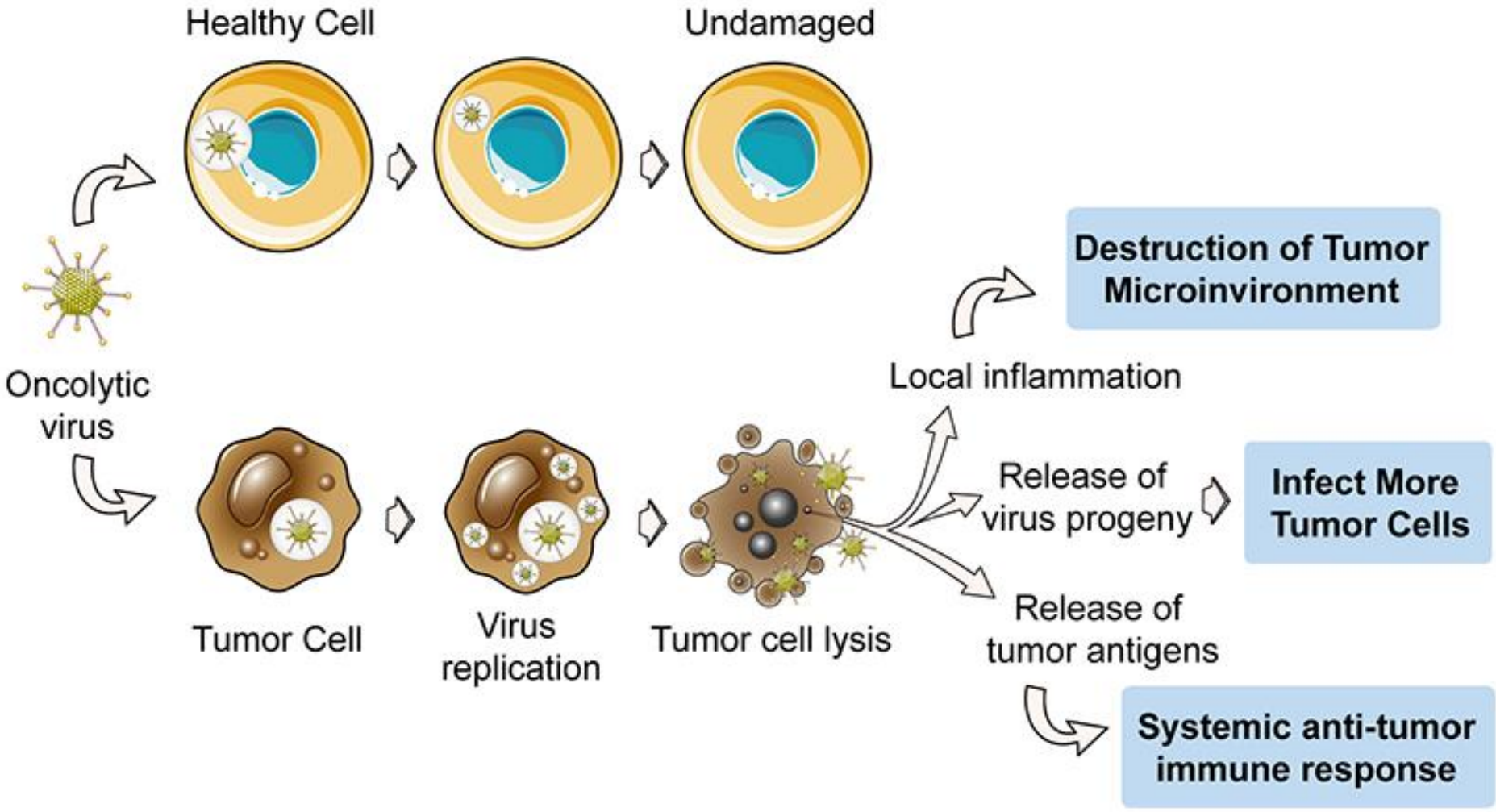
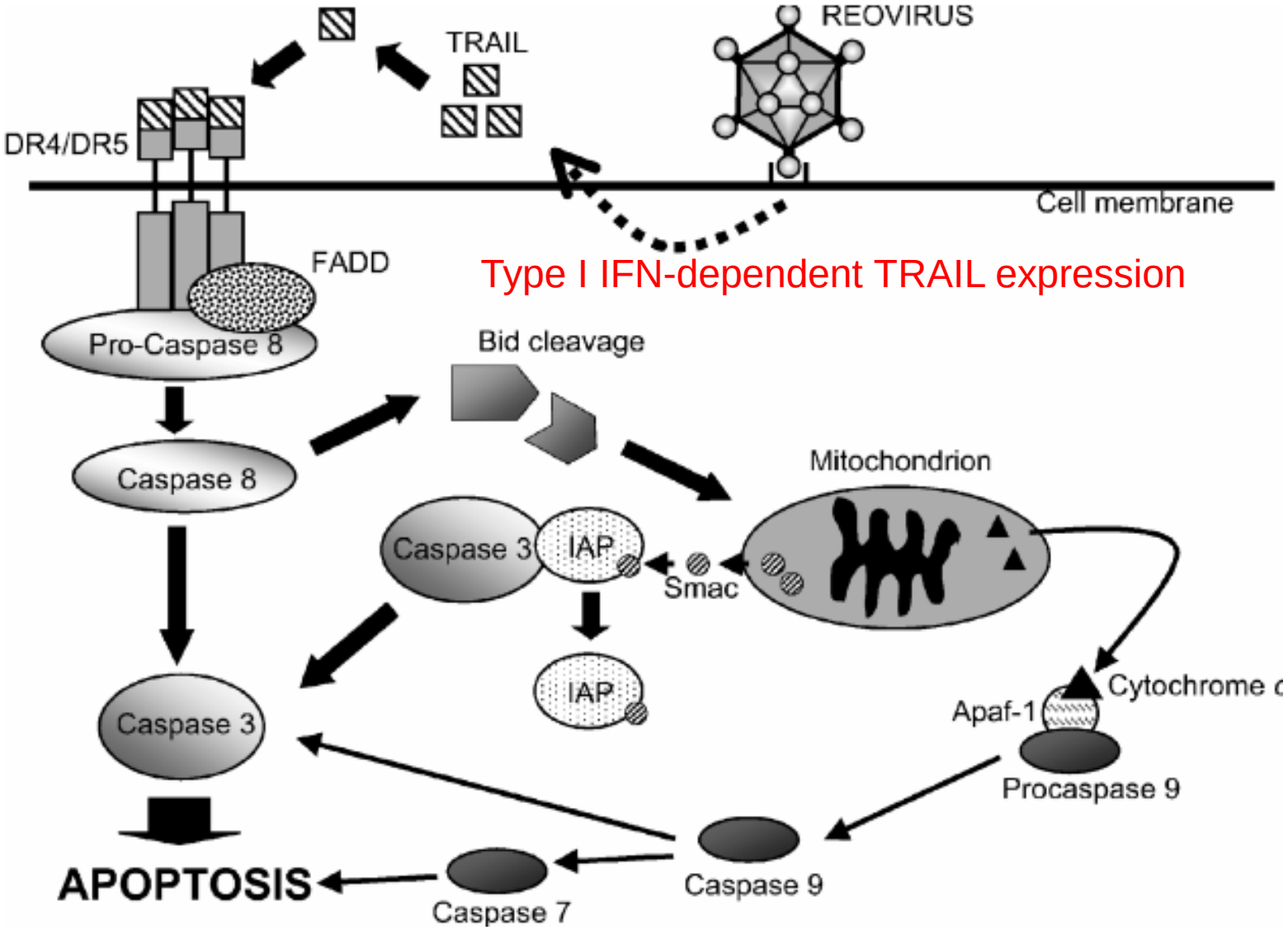
Oncolytic virotherapy, updates and future directions

- ✓ Oncolytic viruses (OVs) are viral strains that **can infect and kill malignant cells while spare their normal counterparts**.
- ✓ IMLYGIC™ (T-VEC/ Talimogene Laherparepvec), a **genetically engineered Herpes Simplex Virus**, is the first OV approved for use in the United States and the European Union for patients with **locally advanced or non-resectable melanoma**.
- ✓ Exploiting inherent tumor weaknesses, such as RAS pathway activation or by genetic modification. For example, knockdown of thymidine kinase (TK)-negative gene in HSV can lead to preferential killing of tumor cells, as **TK-negative HSV can replicate only in dividing cells**.
- ✓ Although OVs have a favorable toxicity profile and are impressively active anticancer agents in vitro and in vivo the majority of OVs have **limited clinical efficacy as a single agent**.
- ✓ **The antiviral immune response can prevent the virus** reaching the tumor tissue and having a therapeutic effect.
- ✓ **Intratumoral administration** can provide **direct access to tumor tissue** and be beneficial in reducing side effects.

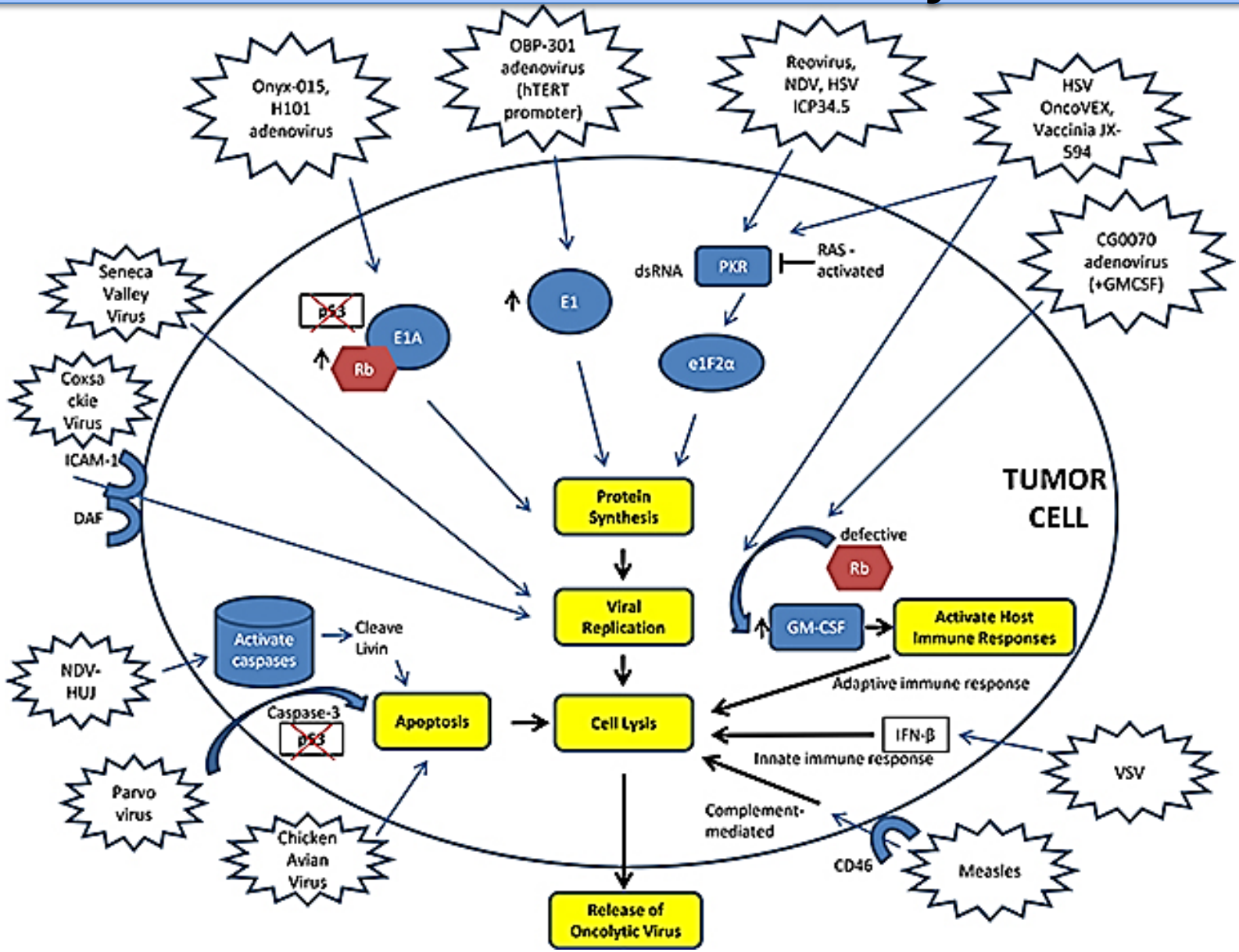
Oncolytic viruses



Onco-cytotoxic viruses



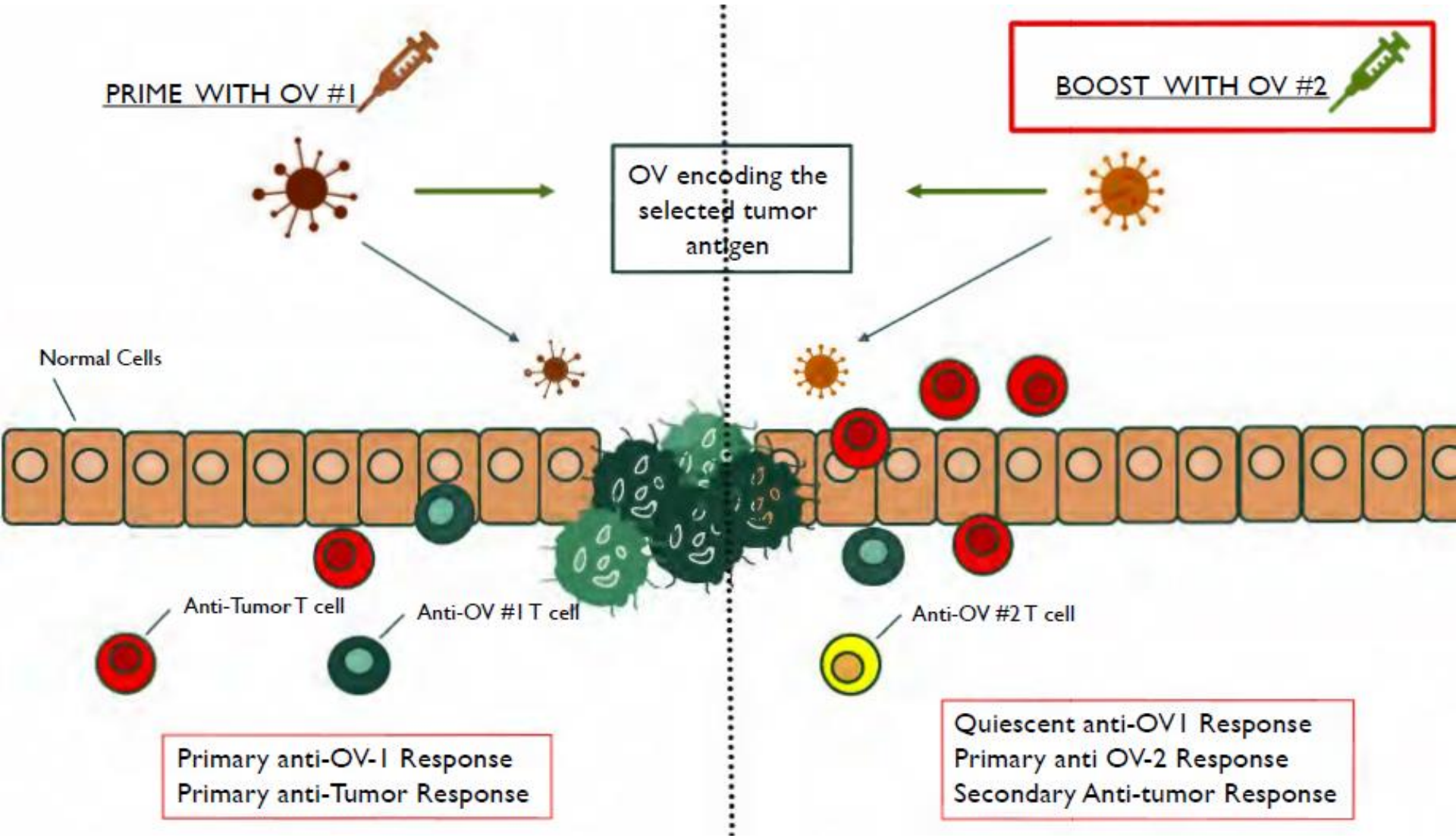
Mechanisms of action of oncolytic viruses.



DAF – Decay Accelerating Factor, GM-CSF – Granulocyte Macrophage- Colony Stimulating Factor, HSV – Herpes Simplex Virus, hTERT – Human Telomerase, ICAM-1 – Intercellular Adhesion Molecule-1, ICP – Infectious Cell Protein, INF-β – Interferon beta, NDV – Newcastle Disease Virus, VSV – Vesicular Stomatitis Virus.

Fountzilas, oncotarget, 2017

Mechanisms of action of oncolytic viruses.



DAF – Decay Accelerating Factor, GM-CSF – Granulocyte Macrophage- Colony Stimulating Factor, HSV – Herpes Simplex Virus, hTERT – Human Telomerase, ICAM-1 – Intercellular Adhesion Molecule-1, ICP – Infectious Cell Protein, INF-β – Interferon beta, NDV – Newcastle Disease Virus, VSV – Vesicular Stomatitis Virus.

Fountzilas, oncotarget, 2017

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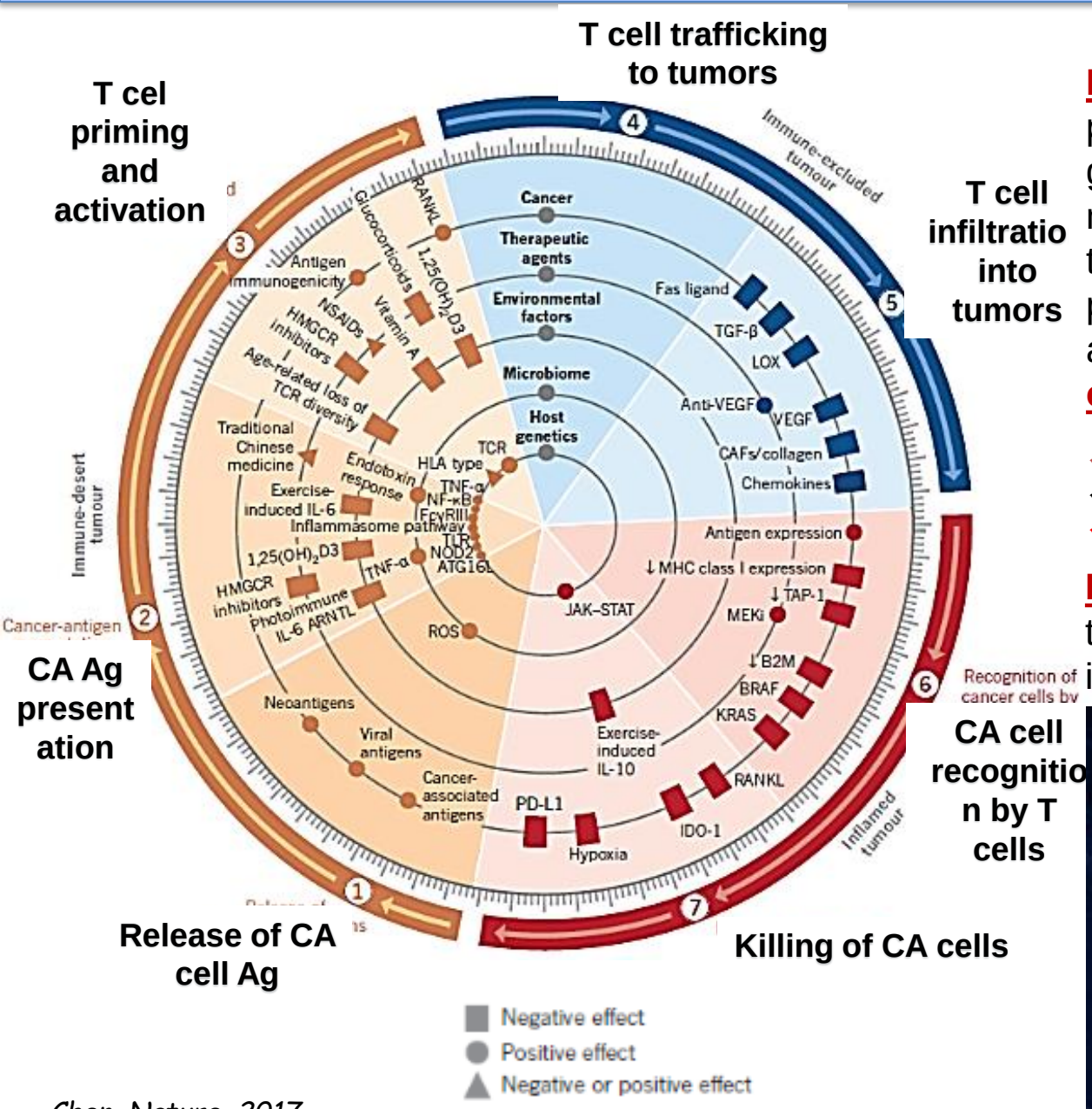
The Cancer – Immune cycle

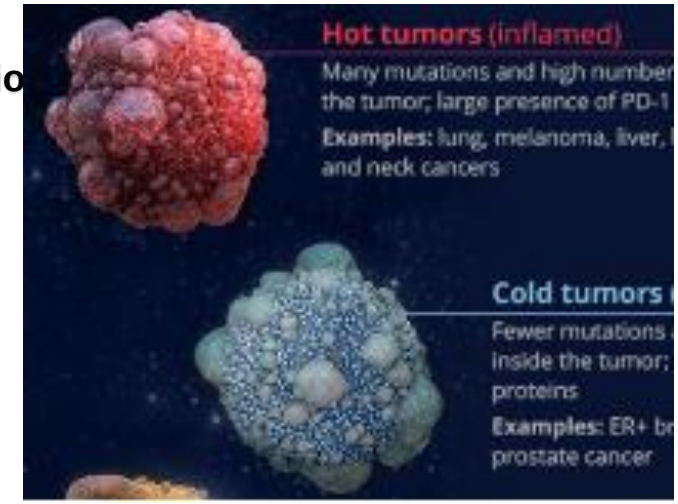


Chen, Nature, 2017

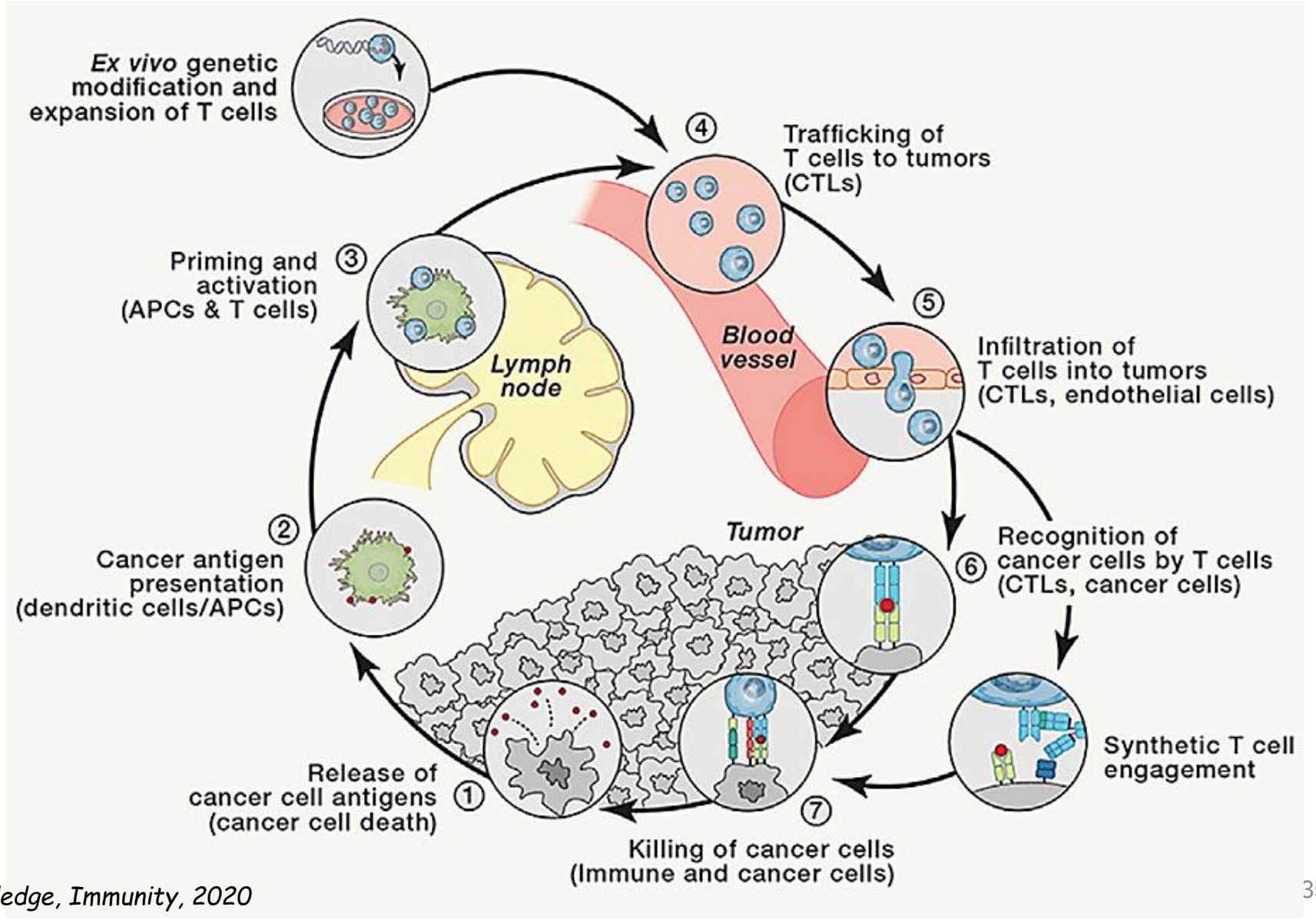
Factors proposed to establish a person's immune profile (tumor genetics, germline genetics, the microbiome, the environment and the presence of certain pharmacological agents) can be arranged in relation to the **7 steps of the cancer–immunity cycle**

- ✓ Immune desert phenotype/Cold tumor
- ✓ Immune-excluded phenotype/Warm tumor
- ✓ Immune-inflamed phenotype/Hot tumor

Biomarkers involved in 7 steps of the cancer–immunity cycle & in immune profile

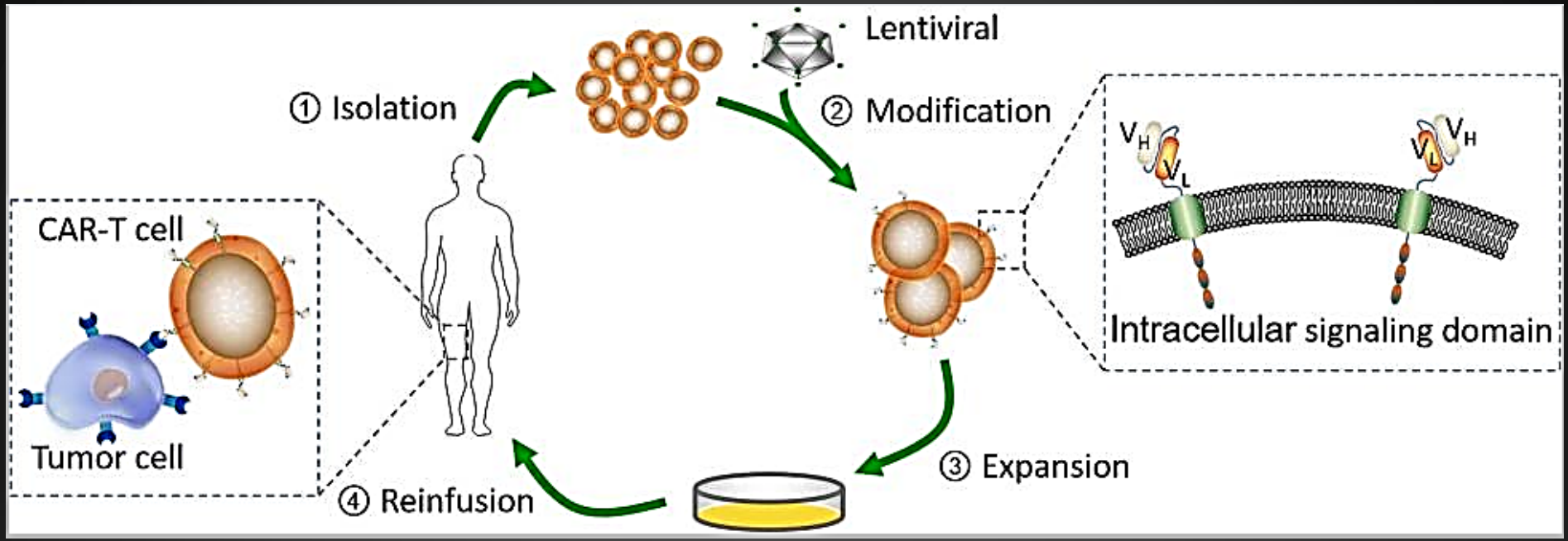


The chimeric antigen receptor (CAR) T cell inside the Cancer – Immune cycle



Hedge, Immunity, 2020

CAR-T cell therapy: ex vivo manipulation of patient T cells to create a potent, cancer-targeting therapy

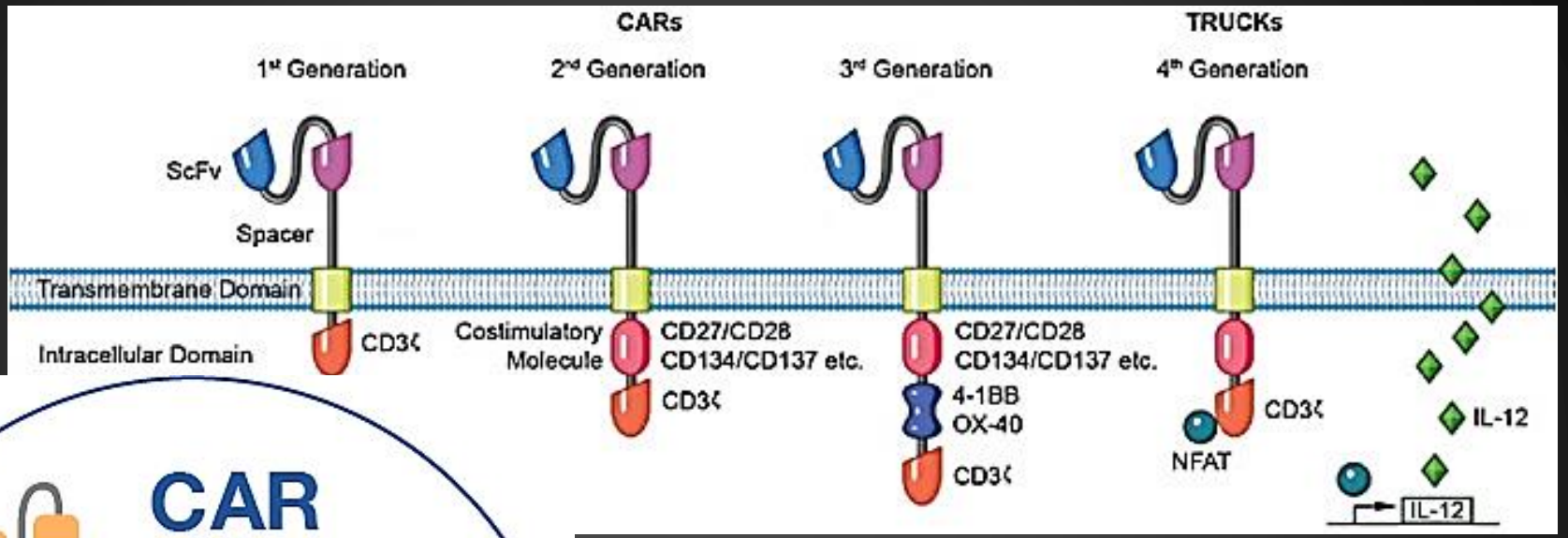


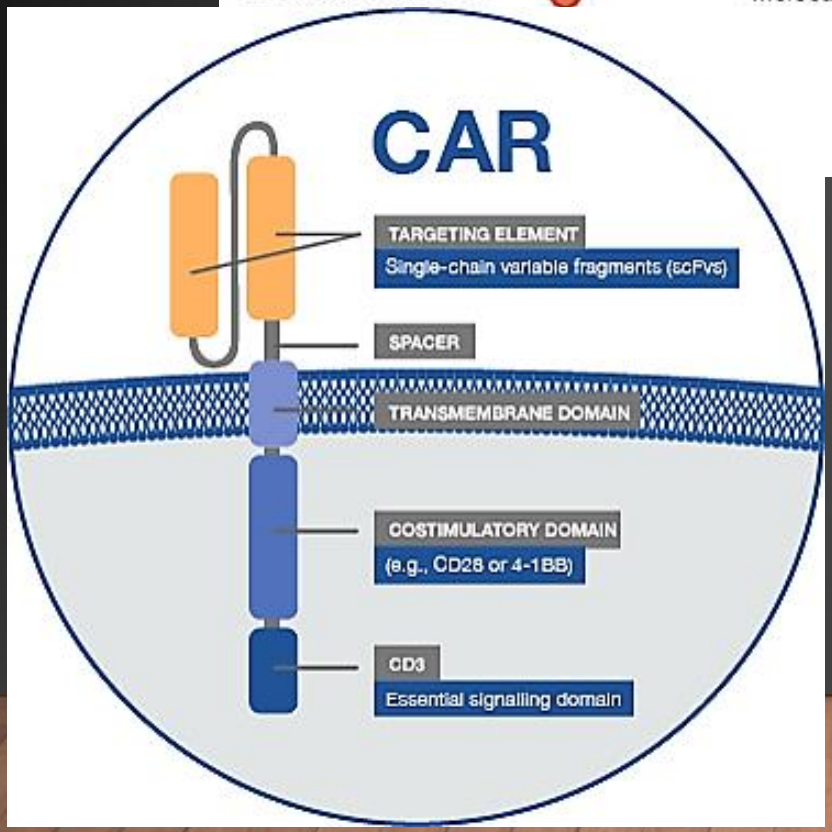
(1) **isolation**, in which PBMCs is harvested from the patient or donor's peripheral blood
(2) **modification**, in which the T cells were activated and CARs are transduced into the activated T cells by way of lentiviral
(3) **expression**, in which the **modified T cells expanded ex vivo** to obtain clinically relevant cell numbers. CARs can be divided into 3 main portions:
(4) **reinfusion**, in which the modified T cell that has reached the desired dose were reinfused into the previously lymphocyte-depleted patient.

CAR can be divided into 3 main portions:

extracellular antigen-binding domain / space region - transmembrane domain / intracellular signaling domain

CAR-T cell therapy





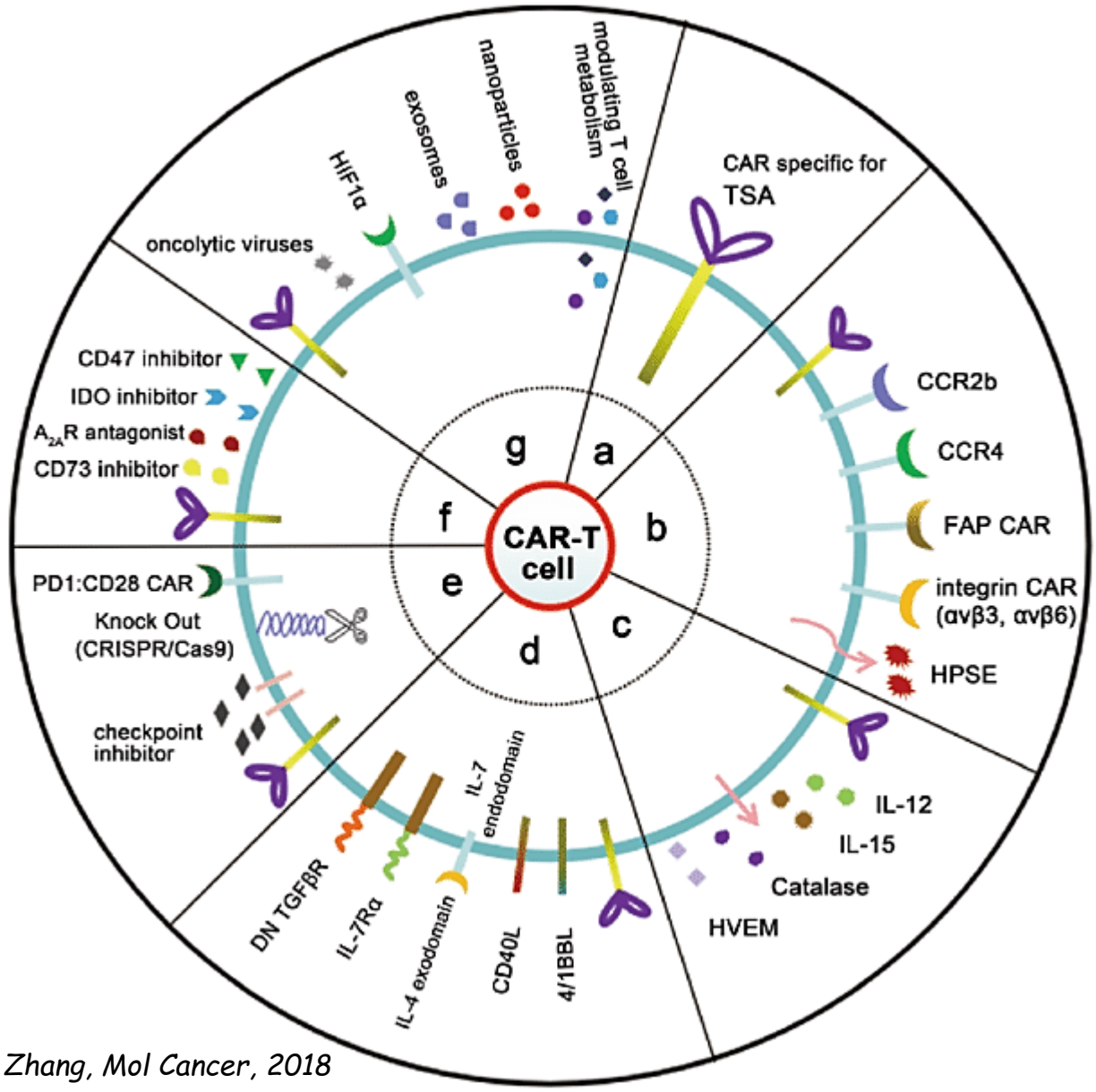
CAR can be divided into 3 main portions:

extracellular antigen-binding domain

space region - transmembrane domain

intracellular signaling domain

Novel strategies to enhance the efficacy of CAR-T cell therapy for solid tumors



Zhang, Mol Cancer, 2018

a) targeting tumor specific antigens.
b) Infiltration and homing
c) secreting cytokines or enzymes, endowed with the catalase to overcome abundant ROS
d) expressing costimulatory receptors
e) combined with the blockage of immune checkpoints using monoclonal antibodies or the CRISPR/Cas9 system.
f) Blockage of soluble tumor suppressive mediators in the solid tumor milieu (e.g., CD73, A2AR, IDO, or CD47)
g) combined with other antitumor strategies, such as oncolytic viruses, HIF-CAR, exosomes, nanoparticles, and modulating T cell metabolism

CANCER IMMUNOTHERAPY INNOVATIONS

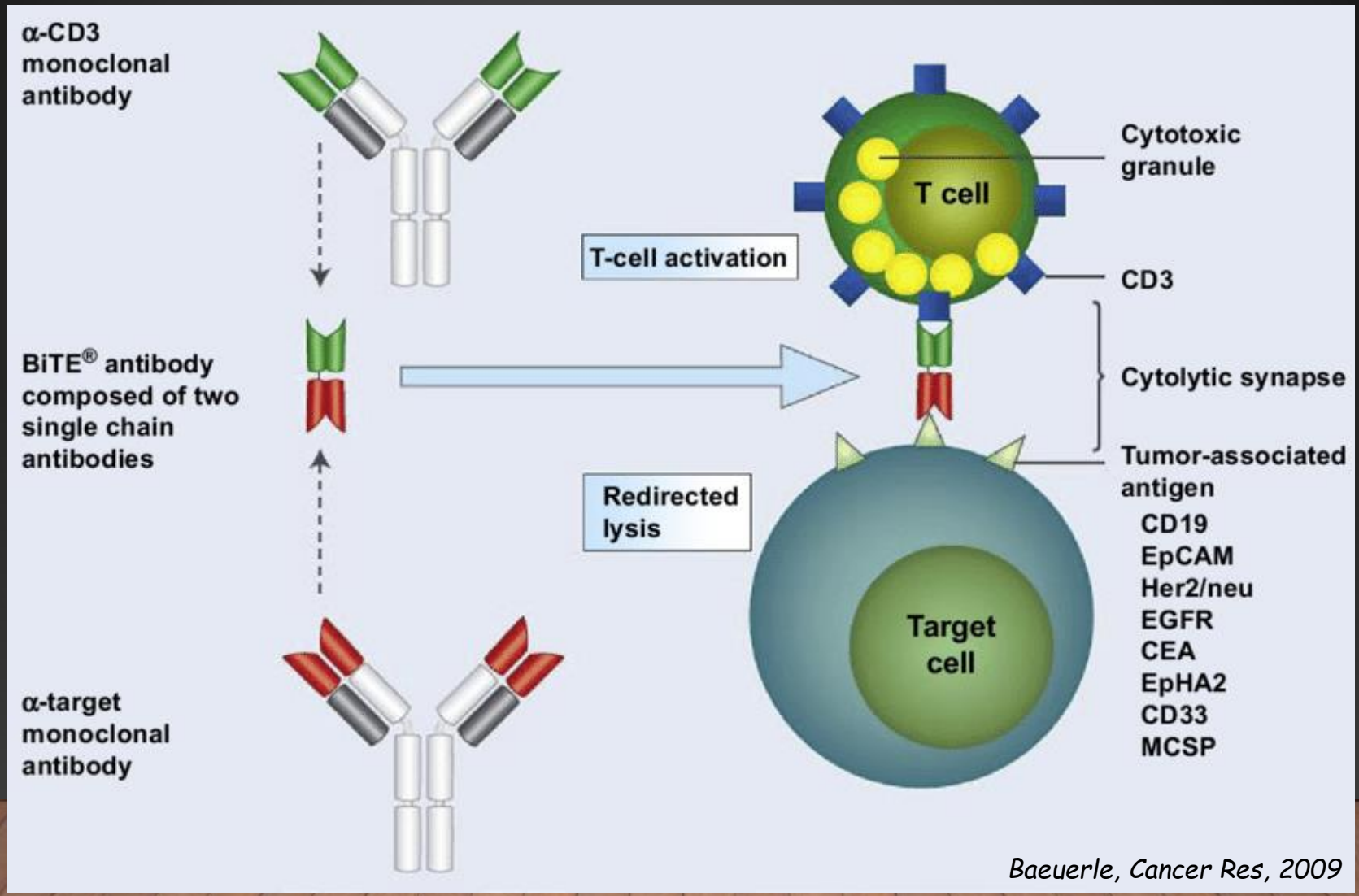
The study of interactions between the immune system & cancer cells

1. **Advanced checkpoint blockade**
2. **Oncolytic viruses**
3. **CAR T cells & novel CAR cells**
4. **BiTEs**
5. Tumor vaccines & Neo-Ag
6. Reverse vaccinology & mimotopes
7. NK-based therapy
8. Genetically engineered mouse models
9. Quantitative multivariate model of human immune cell communication

Aims: "Analyzing, understanding and manipulating interactions between tumor cells and the immune system to overcome the cancer progression.“

Bi-specific T-cell engagers (BiTEs)

A class of artificial bispecific monoclonal antibodies, investigated for the use as anti-cancer drugs. They direct a host's immune system, more specifically the T cells' cytotoxic activity, against cancer cells.



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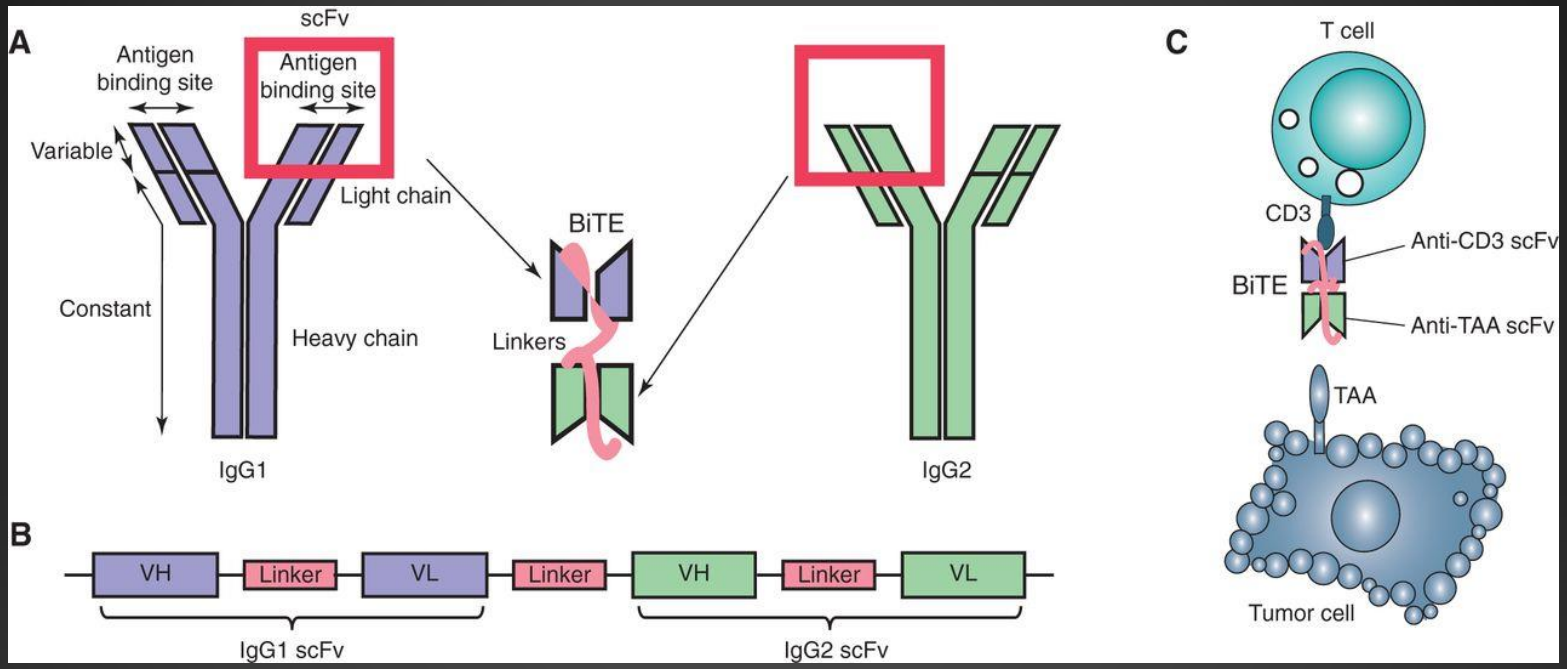
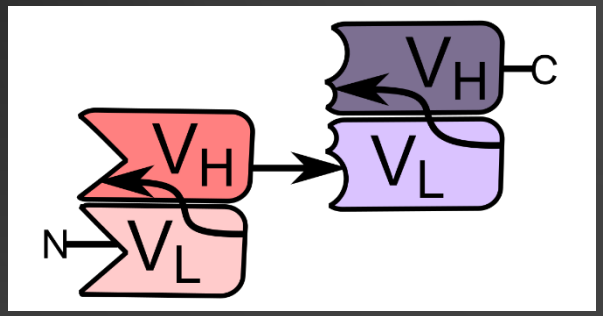
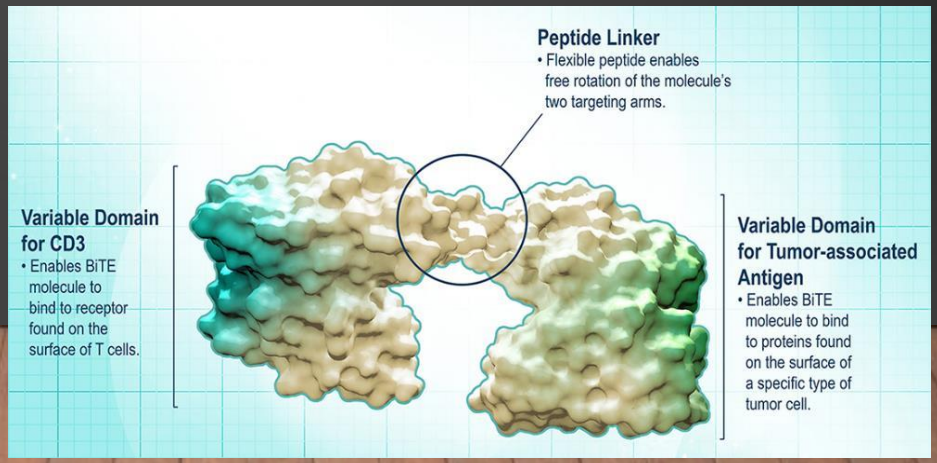


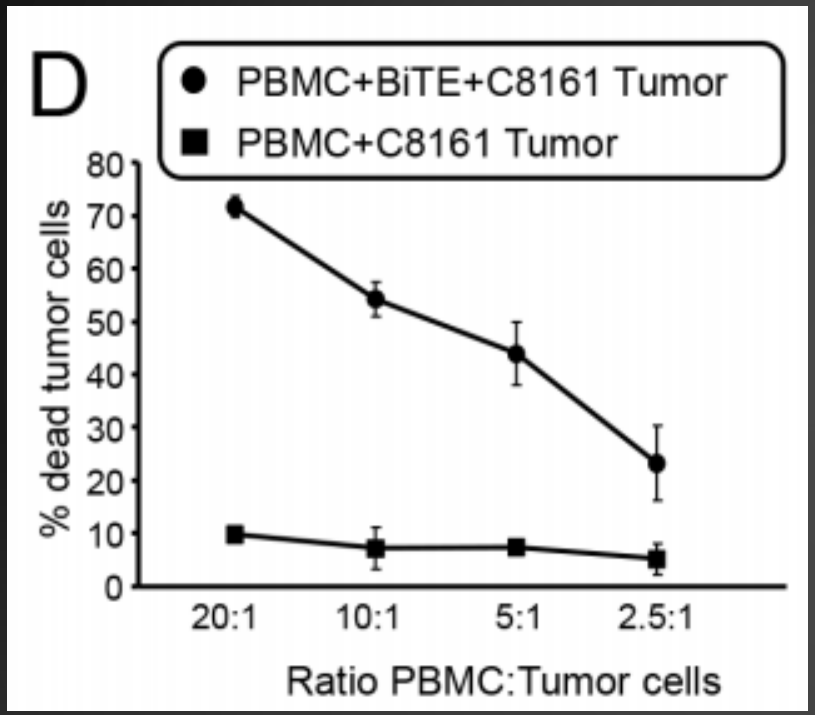


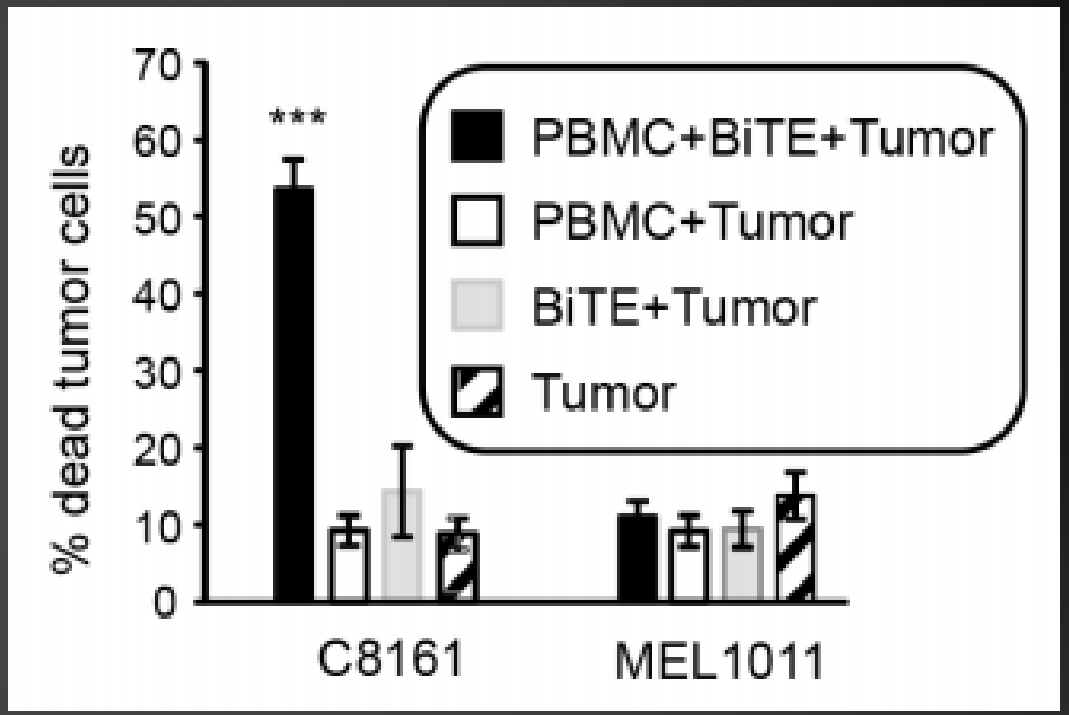


BiTE is a registered trademark of Micromet AG (fully owned subsidiary of Amgen Inc)

CD3xPDL1 BiTE activates T cells that are cytotoxic for PDL1+ tumor cells

CD3xPDL1 bi-specific T cell engager (BiTE) simultaneously activates T cells and NKT cells, kills PDL1+ tumor cells, and extends the survival of tumor-bearing humanized mice



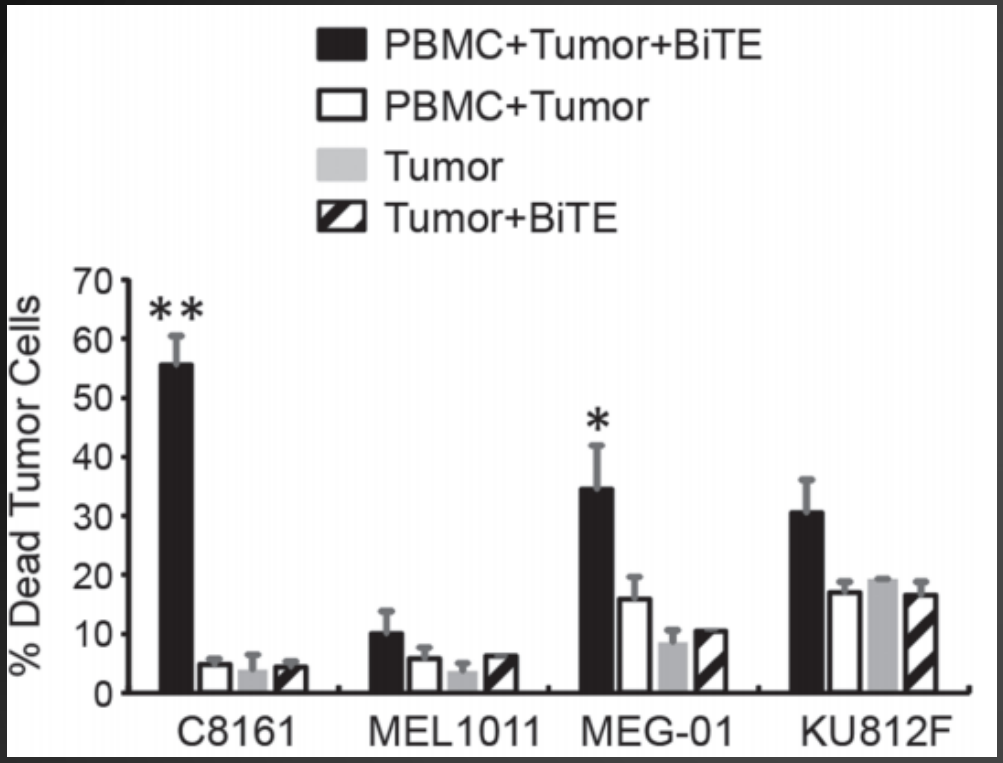


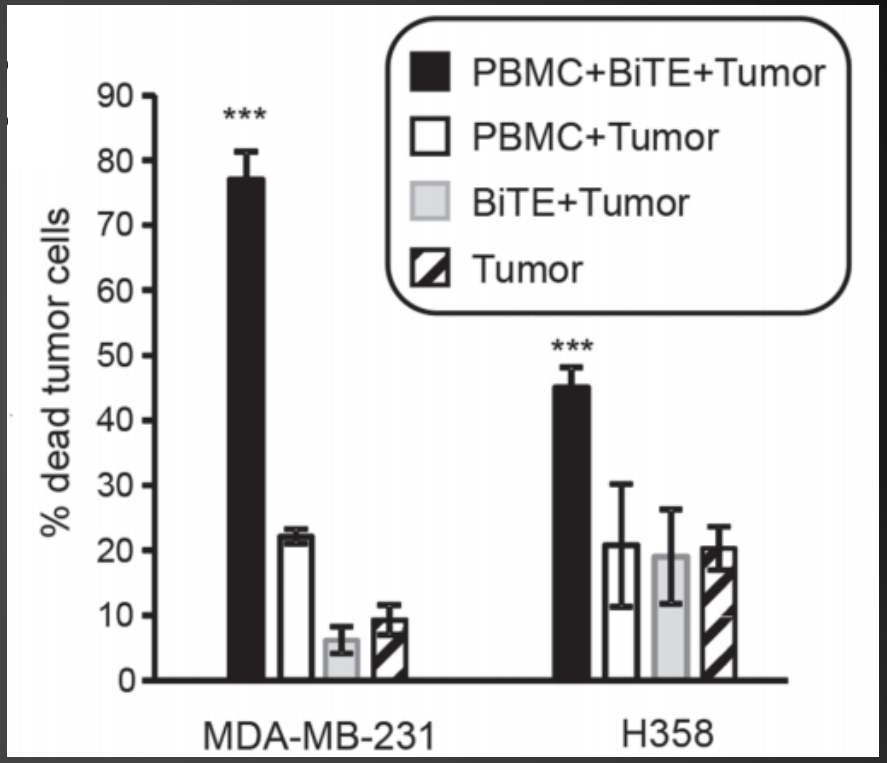
PDL1+ human melanoma C8161 cells
PDL1- human melanoma MEL1011 cells

Horn, Oncotarget, 2017

CD3xPDL1 BiTE activates T cells and is cytotoxic for PDL1+ CML, NSCLC, and breast cancer cells

CD3xPDL1 bi-specific T cell engager (BiTE) simultaneously activates T cells and NKT cells, kills PDL1+ tumor cells, and extends the survival of tumor-bearing humanized mice





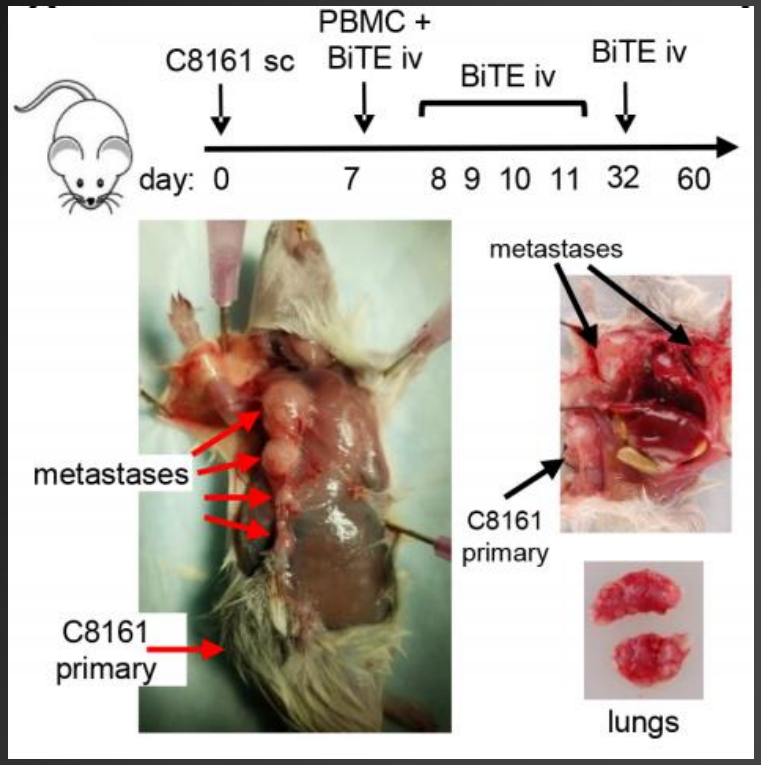
PDL1+ human melanoma C8161 cells
PDL1- human melanoma MEL1011 cells

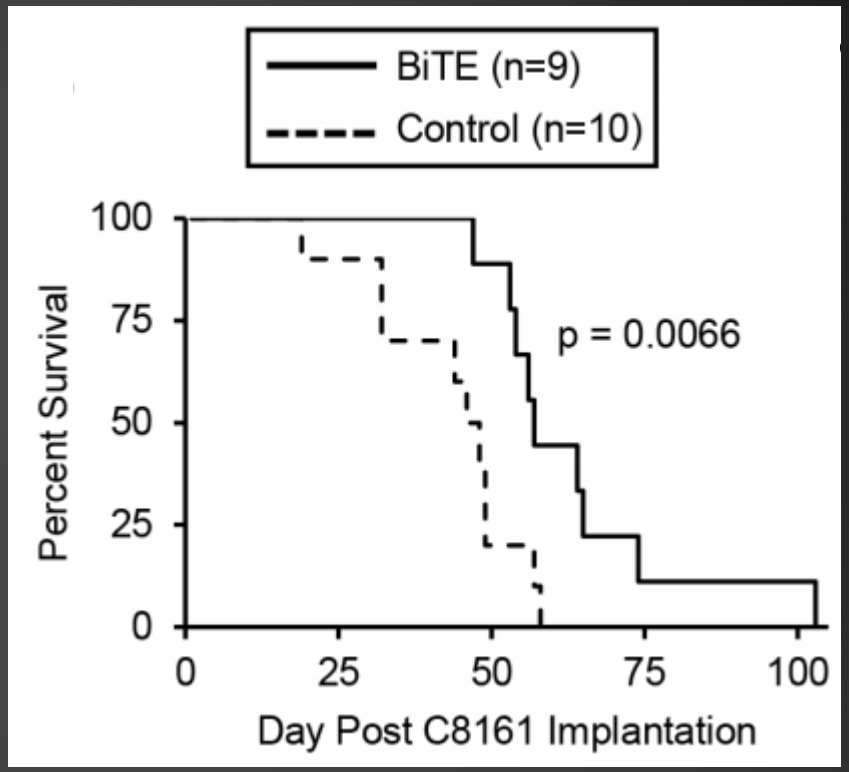
PDL1+
breast MDA-MB-231
chronic myelogenous leukemia MEG-01 and KU812F
lung adenocarcinoma H358

Horn, Oncotarget, 2017

CD3xPDL1 BiTE significantly extends the survival time of humanized NSG mice reconstituted with human PBMC and carrying established metastatic human melanoma C8161

CD3xPDL1 bi-specific T cell engager (BiTE) simultaneously activates T cells and NKT cells, kills PDL1+ tumor cells, and extends the survival of tumor-bearing humanized mice





NSG mice (NOD scid gamma mice) among the most immunodeficient described to date: lack mature T cells, B cells, and NK cells, deficient in multiple cytokine signaling pathways, many defects in innate immunity → permit the engraftment of a wide range of primary human cells

PDL1+ human melanoma C8161 cells

Horn, Oncotarget, 2017

CAR T cells versus bispecific antibodies

CAR T cells

Pros:

- Higher activity demonstrated in hematological maligancies
- Independent of receiver T cells characteristics

Cons:

- CRS concern (Cytoreductive surgery)
- Manufacturing issues (but improvements and development of allogeneic CARs)
- Immunosuppressive microenvironment : add immunomodulatory Mabs or use optimized CAR

Bispecific antibodies

Pros:

- Less manufacturing and regulatory issues
- Lower toxicity expected

Cons:

- May be more dependent on quality and/or quantity of patient T cells
- Less clinical activity demonstrated to date
- Immunosupressive microenvironment : add immunomodulatory Mabs

CAR T cells in diseases with strong qualitative or quantitative defects of T cells ?
Bispecific antibodies to induce long-term immune response after intratumoral T cell activation ?

CANCER IMMUNOTHERAPY INNOVATIONS

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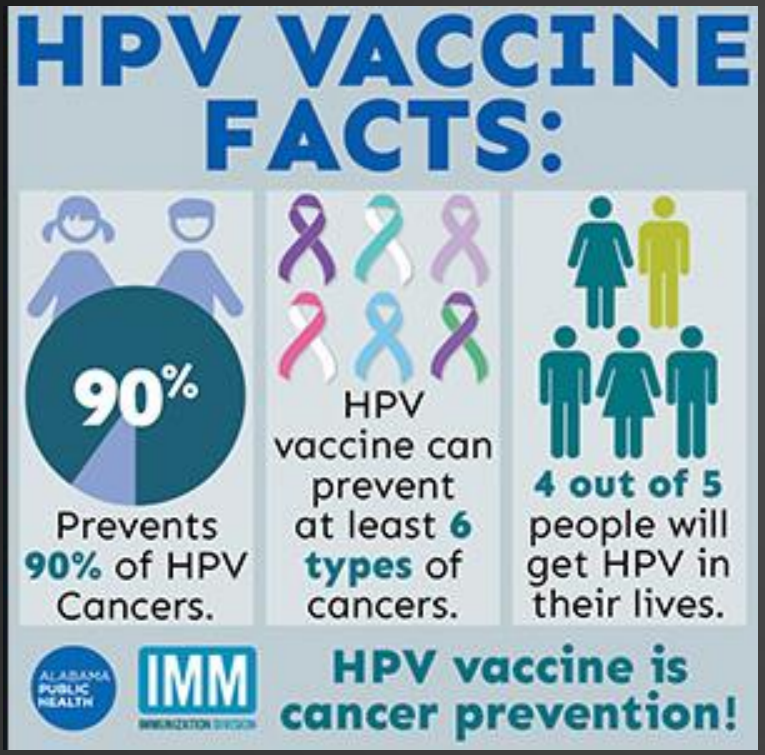
Aims: "Analyzing, understanding and manipulating interactions between tumor cells and the immune system to overcome the cancer progression.“

Already established as treatment for many years

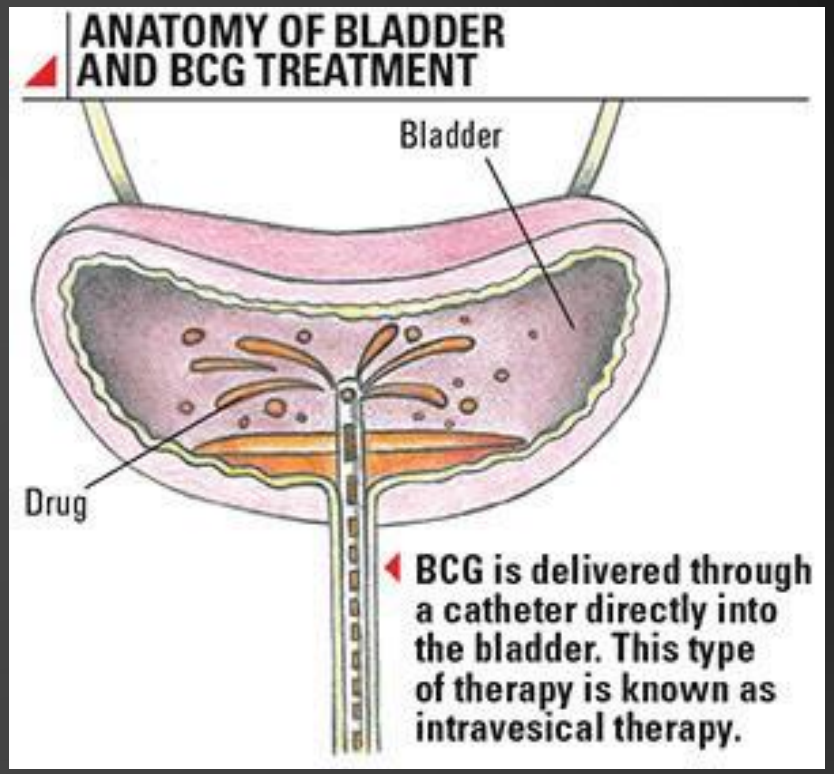
HPV vaccine against cervix

Preventive vaccine



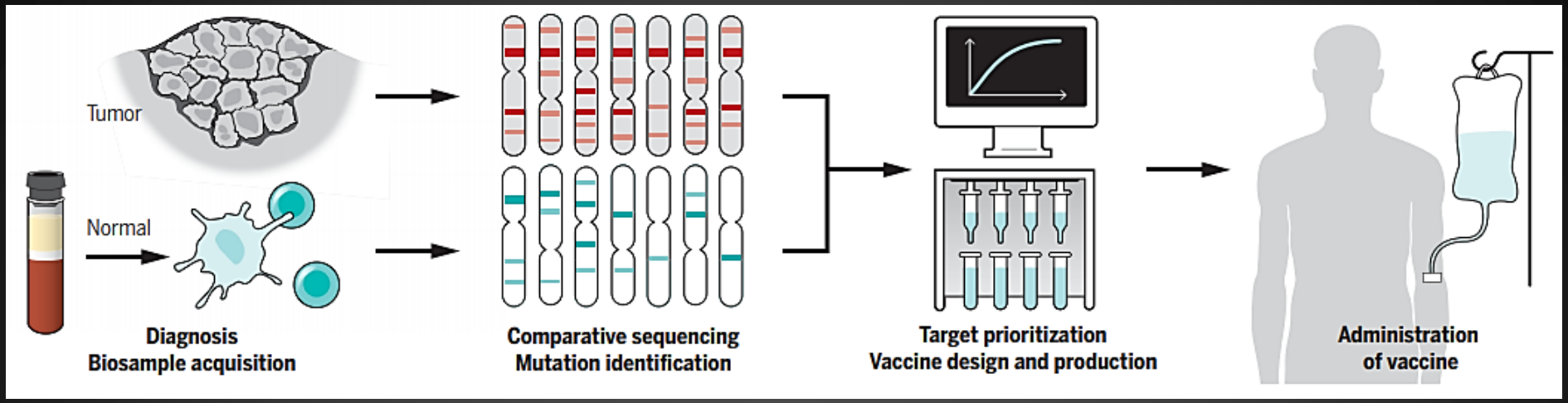
BCG-therapy of bladder CA

Therapeutic vaccine



Sahin, science, 2018

Personalized vaccines for cancer immunotherapy

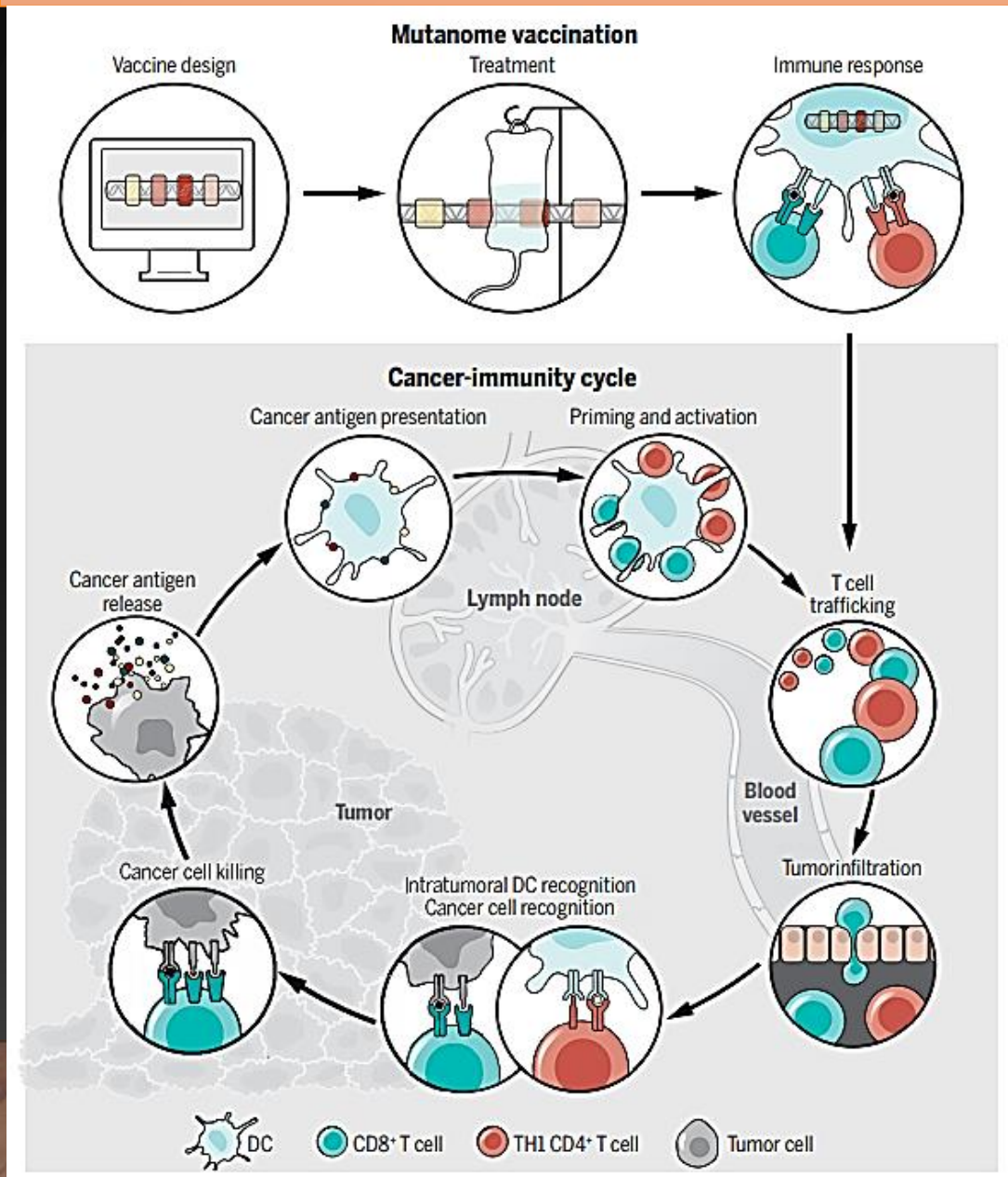


Customizing a patient-specific cancer vaccine.

1. Patient tumor biopsies and healthy tissue (e.g., peripheral blood white blood cells) are subjected to **next-generation sequencing**. By comparing the sequences obtained from tumor and normal DNA, tumor-specific nonsynonymous single-nucleotide variations or short indels in protein-coding genes are identified.
2. A computational pipeline is used to examine the mutant peptide regions for **binding to the patient's HLA alleles** (based on predicted affinity)
3. Selection of **multiple mutations to design unique neoepitope vaccines** that are manufactured under GMP conditions. "…a **personalized mutanome vaccine** has the potential to become a **universally applicable therapy** irrespective of cancer type."

Sahin, science, 2018

Neoepitope vaccines promote a functional Cancer – Immune cycle



Vaccine-induced neoepitope-specific CD4+ TH1 cells

promotion of T cell priming and expansion, proinflammatory reshaping of the tumor microenvironment

recruitment of CD4/8+ T cells for direct killing of tumor cells.

multi-neoepitope vaccines may contribute to tipping the balance from tolerance toward productive immunity against tumor cells

Sahin, science, 2018

Current vaccine formats explored for delivery of neoepitopes

Vaccine format	Advantages	Challenges
Synthetic peptides (*45*)	Cell-free manufacturing Automated synthesis established Proven clinical activity of long peptides Compatible with a wide range of formulations to improve delivery Transient activity and complete degradation	Lack of clinical-grade manufacturability of a substantial portion of sequences High variability in the physicochemical properties of individual peptides, complicating manufacturing Irrelevant immune responses against artificial epitopes created by peptide degradation in the extracellular space
Messenger RNA (*46*)	Cell-free manufacturing Inherent adjuvant function via TLR7, TLR8, and TLR3 signaling Proven clinical activity Highly efficient systemic delivery into DCs established Transient activity and complete degradation All types of epitopes can be encoded	Fast extracellular degradation of mRNA if not protected by appropriate formulation Interpatient variability of TLR7-driven adjuvant activity
DNA plasmids (*47*)	Cell-free manufacturing Inherent adjuvant activity driven by TLR9 Cost-effective and straightforward manufacturing All types of epitopes can be encoded	Potential safety risks by insertional mutagenesis Successful transfection requires entry into nucleus, thereby limiting effective delivery of vaccines into DCs
Viral vectors (*48*) (adenoviral and vaccinia)	Strong immunostimulatory activity Extensive clinical experience with vector formats in the infectious disease field All types of epitopes can be encoded	Complex manufacturing Immune responses against components of the viral vector backbone, limiting successful in vivo vaccine delivery and efficacy
Engineered attenuated bacterial vectors (*49*) (*Salmonella*, *Listeria*)	Strong immunostimulatory activity Could be combined with plasmid DNA All types of epitopes can be encoded	Complex manufacturing and "sterility" testing Immune responses against bacterial components, limiting vaccine delivery and vaccine immunogenicity Potential safety risks due to delivery of live, replication-competent bacteria
Ex vivo antigen-loaded DCs (*50*)	Strong immunostimulatory activity Proven clinical efficacy of DC vaccines Can be loaded with various antigen formats	Higher costs and resources required for adoptive cell therapy approaches

Sahin, science, 2018

Current challenges for cancer vaccine adjuvant development

- Germ line encoded receptors that play a central role in the **protection against pathogens**

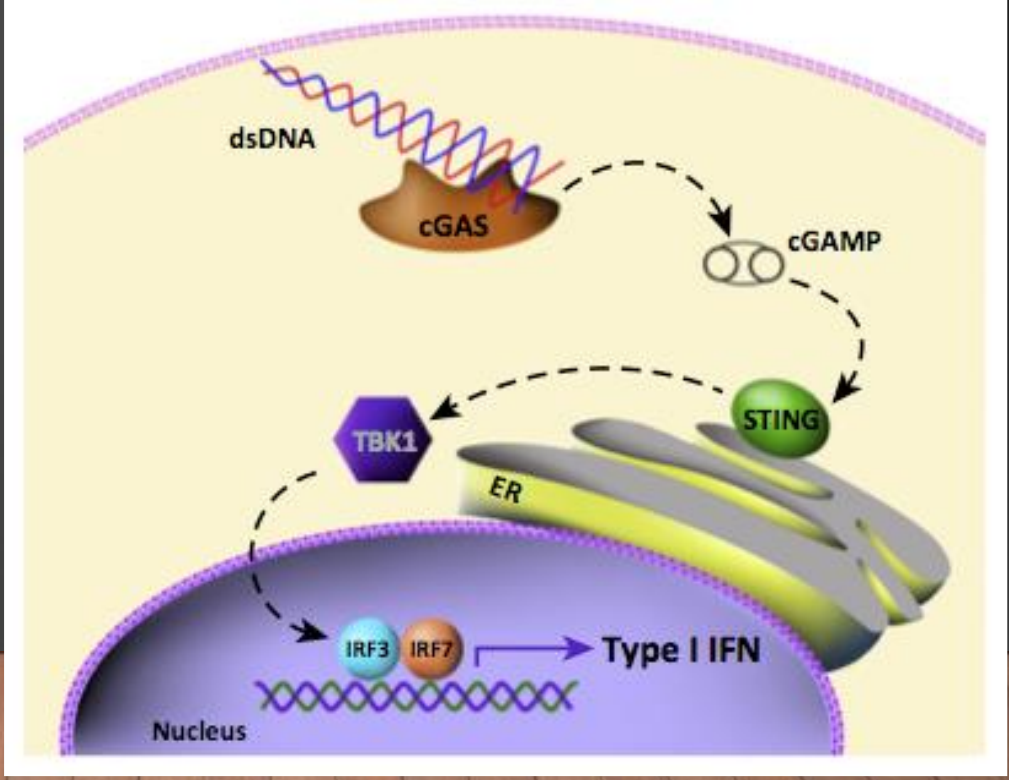
- Five families:
  - ✓ **Toll Like Receptors (TLRs)**
  - ✓ RIG-I-like Receptors (RLRs)
  - ✓ Nucleotide-binding Oligomerization Domain (NOD)-like receptors (NLRs)
  - ✓ C-type Lectin Receptors (CLRs)
  - ✓ **DNA sensors**

- Role:
  - ✓ PRRs detect Pathogen-Associated Molecular Patterns (**PAMPs**) such as LPS (Lipopolysaccharide), but also structural proteins, RNA and DNA from bacteria, virus, fungi and parasites
  - ✓ PRRs can also recognize endogenous Damage Associated Molecular Patterns (**DAMPs**) that are released upon cellular stress, apoptosis or necrosis
  - ✓ DAMPs/PAMPs recognition by PRRs leads to transient **pro-inflammatory gene expression, and immune cell activation**

Bowen, Expert Rev Vac, 2018

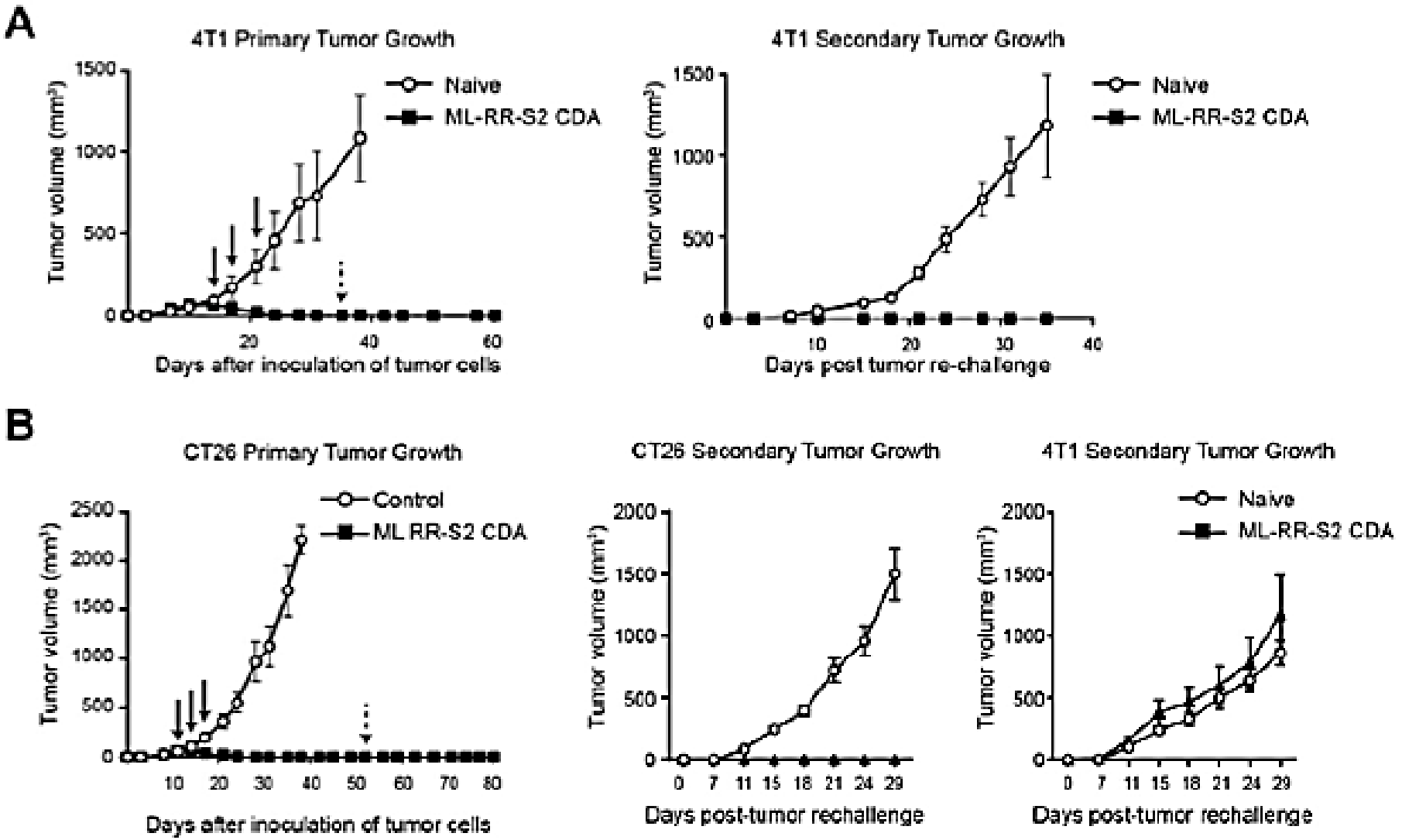
Current challenges for cancer vaccine adjuvant development

- ✓ Adjuvants **enhance the magnitude, breadth, quality and longevity of the immune response** to the antigens.
- ✓ Investigators are exploring many novel adjuvant technologies for future vaccine candidates, including **adjuvant combinations.**
- ✓ result in **complimentary and even synergistic** enhancement of immune responses
  - **dendritic-cell maturation,**
  - **T-cell expansion**
  - **relief of tumor-associated immune suppression**
- ✓ **PRR agonist: STING agonist, Stimulator of interferon Gene**



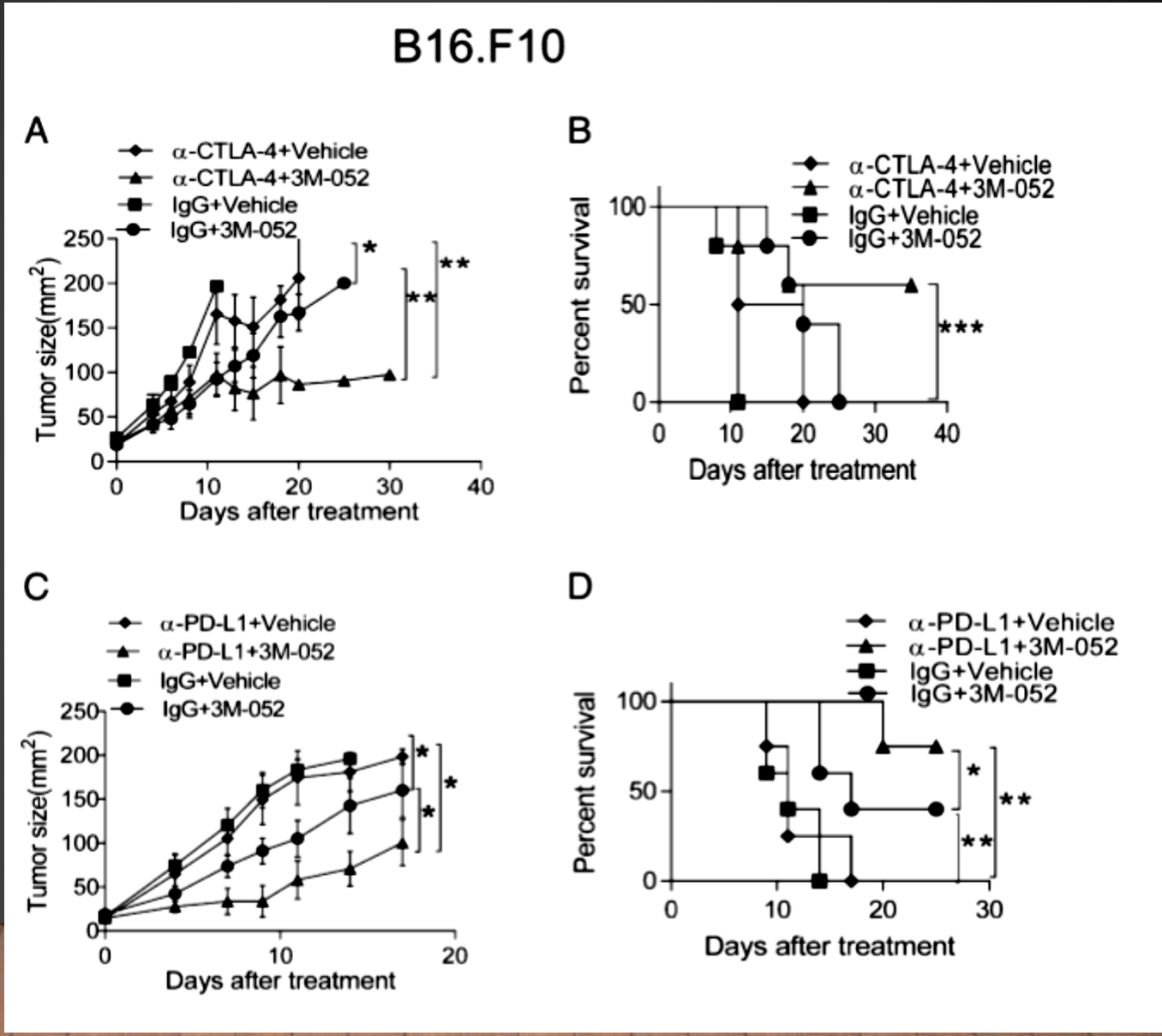
Bowen, Expert Rev Vac, 2018

In vivo effects of STING agonists



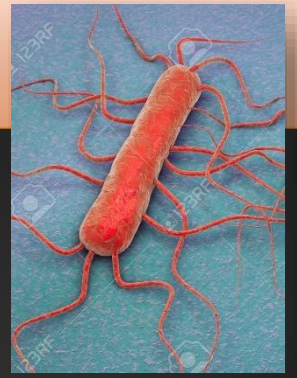
Corrales, Cell Rep 2015

PRR agonist : example of a TLR7/8 agonist, combined to checkpoint inhibitors



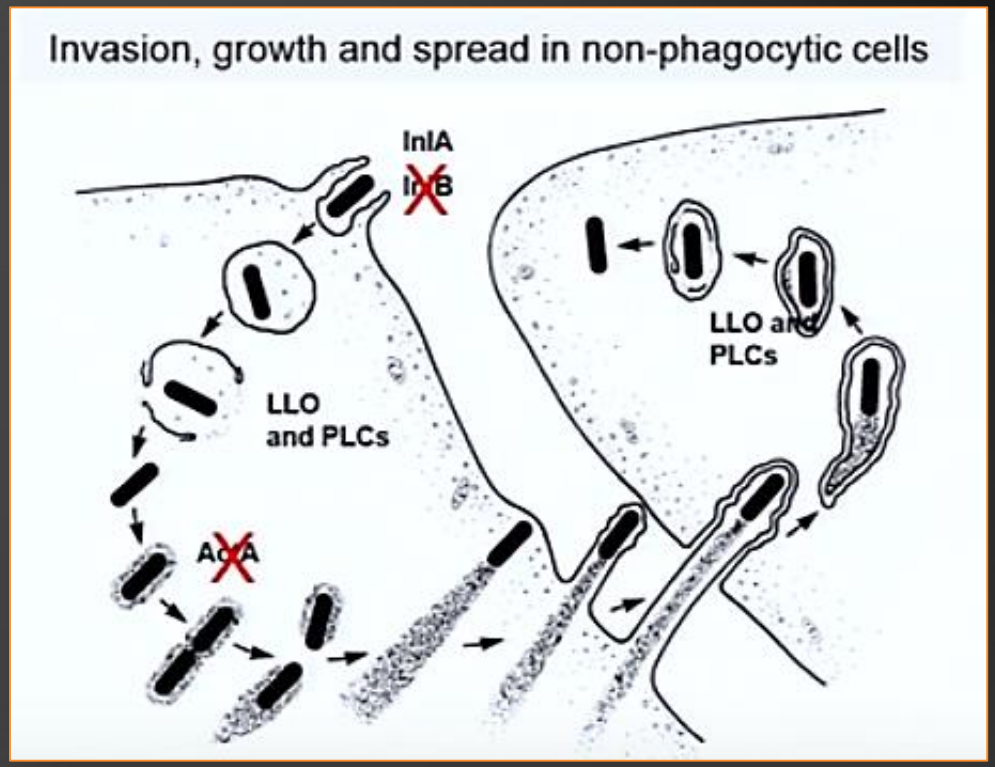
Singh, J Immunol, 2016

Listeria monocytogenes (Lm) – based cancer immunotherapy



- ✓ **Wild-type Lm:**
  - invasion, growth and spread in epithelial non-phagocytic or phagocytic cells
  - Sub-lethal doses provides long-lasting protection against lethal challenge
  - Protection both depends on CD4 and CD8 T cells
  - LLO-dpt Lm cytosolic access is required to induce protective immunity
- ✓ **Recombinant Lm:**
  - **LADD** = live-attenuated double deficient ΔactA ΔinlB
  - expressing tumor-associated antigens (TAAs)
  - activate tumor-specific CTLs: bacteria-induced acute inflammation & long-lasting anti-tumor immunity

- ✓ **Lm-based immunotherapies:** impressive therapeutic efficacy in preclinical models of cancer for two decades and are now showing promise clinically

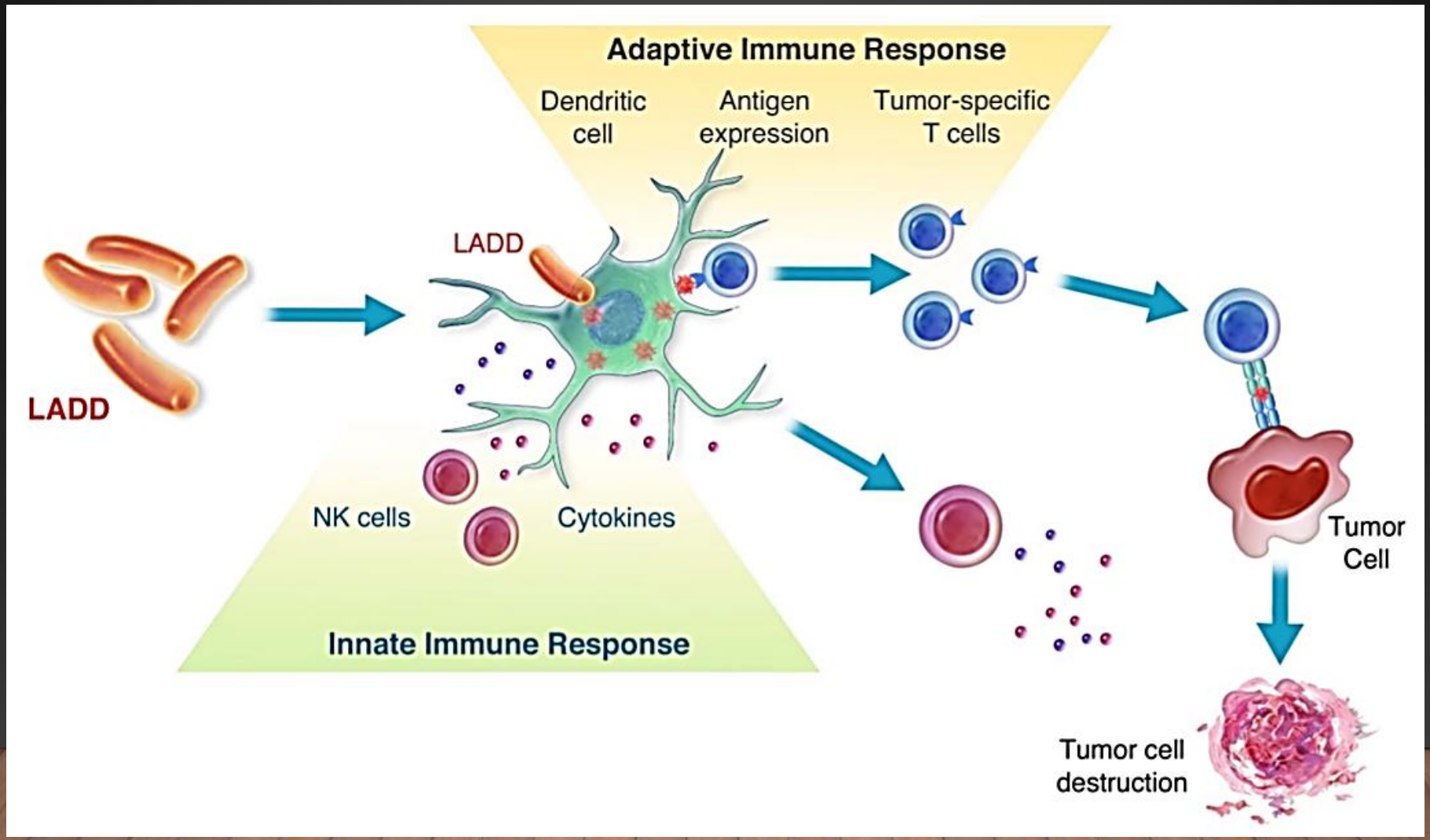


LLO, listeriolysine

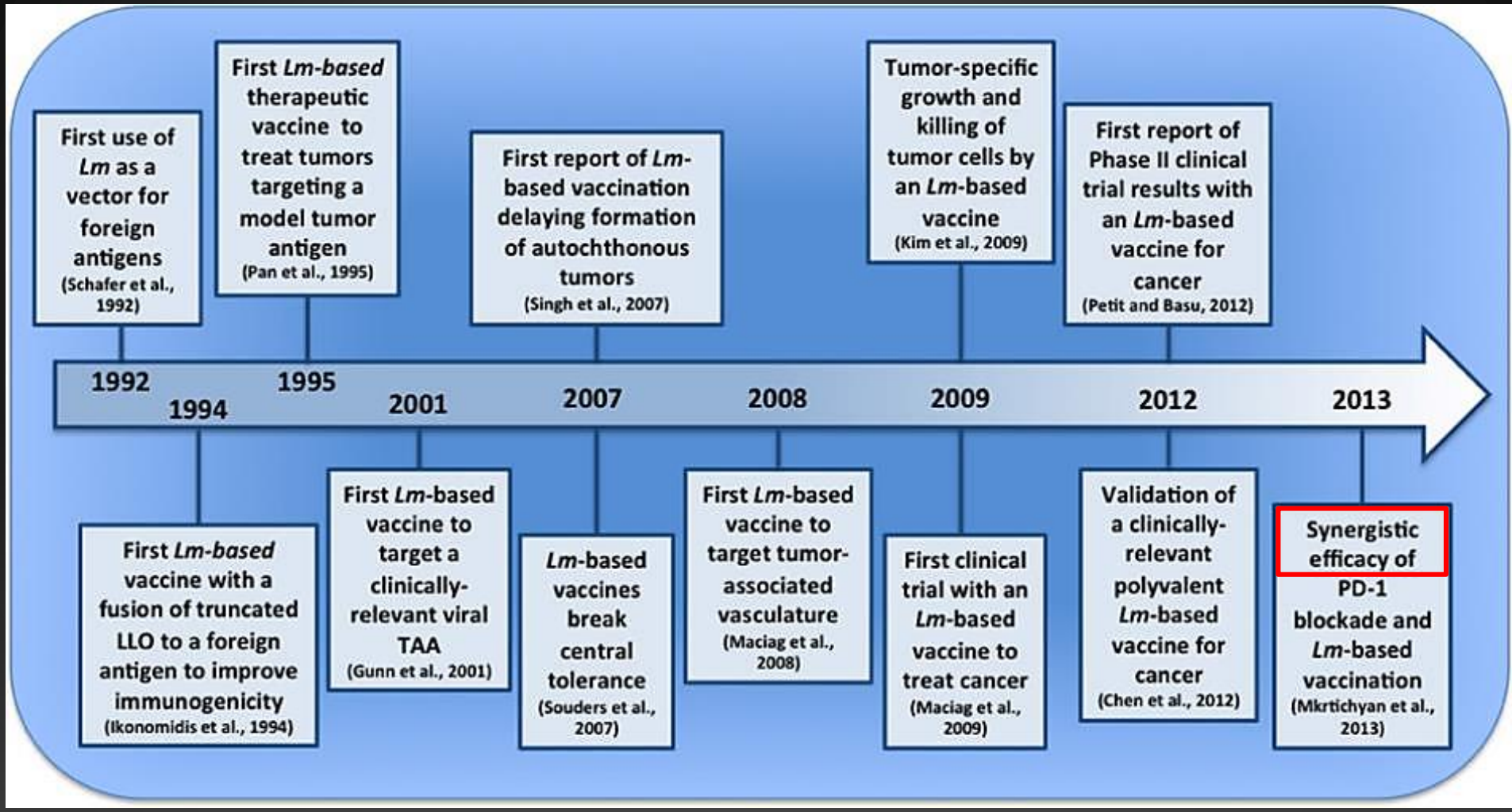
Travier, Cur Op Microbiol, 2014

Listeria monocytogenes (Lm) – based cancer immunotherapy

- ✓ Endogeneous tumor-initiated T cell priming: target immune checkpoint blockade
- ✓ Exogeneous T cell priming: recombinant live-attenuated Lm:



Pivotal events in the development of Lm-based vaccines for tumor immunotherapy



Wood, Front Cell Infect Microbiol, 2014

Lm-based vaccines in development that target clinically-relevant tumor-associated antigens

Target	Target antigen	*Lm*-based Vaccine	*Lm*-strain
Cervical cancer	HPV16 E7	*Lm*-E7	10403S (wt)
	HPV16 E7	*Lm*-LLO-E7 (ADXS-HPV)	XFL-7 (*prfA*-)
	HPV16 E7	r*Lm*-E7	10403S (wt)
	HPV16 E7	*Lm*-ActA-E7	XFL-7 (*prfA*-)
	HPV16 E7	*Lm*-PEST-E7	XFL-7 (*prfA*-)
	HPV16 E7	*Lm*-v1 and v2	*Lm*dd (*dal- dat-*)
	CRPV E1	E1-r*Lm*	10403S (wt)
Breast cancer	Rat Her2/neu	*Lm*-LLO-EC1, EC2, EC3, IC1, and IC2	XFL-7 (*prfA*-)
	Human Her2/neu	*Lm*-hHer2/neu chimera	XFL-7 (*prfA*-)
	Human Her2/neu	*Lm*-cHer2 (ADXS-cHER2)	*Lm*ddA (*dal- dat- actA-*)
	Mouse ISG15	*Lm*-LLO-ISG15	XFL-7 (*prfA*-)
	Mouse MAGE-b	*Lm* LLO Mage-$b_{311-660}$	XFL-7 (*prfA*-)
	Human p53	*Lm*ddA-LLO-p53	*Lm*ddA (*dal- dat- actA-*)

Wood, Frontiers Cell Infect Mucrobiol, 2014

Lm-based vaccines in development that target clinically-relevant tumor-associated antigens

Target	Target antigen	*Lm*-based Vaccine	*Lm*-strain
Tumor-associated vasculature	Mouse VEGFR-2 (Flk-1)	*Lm*-LLO-Flk-E1, E2, and I1	XFL-7 (*prfA*-)
	Human HMW-MAA	*Lm*-LLO-HMWMAA-C	XFL-7 (*prfA*-)
	Mouse CD105 (endoglin)	*Lm*-LLO-CD105A and B	XFL-7 (*prfA*-)
Melanoma	Mouse TRP2, LCMV NP	*Lm*-TRP2-NP	10403S (wt)
	Mouse TRP2	*Lm*-TRP2	10403S (wt)
	Human HMW-MAA	*Lm*-LLO-HMWMAA-C	XFL-7 (*prfA*-)
Prostate cancer	Human PSA	*Lm*-LLO-PSA	XFL-7 (*prfA*-)
	Human PSA	ADVX-31-142 (ADXS-PSA)	*Lm*ddA (*dal- dat- actA*-)
Hepatocellular carcinoma	HBc, HBV-X, Human alpha-Fetoprotein, and Human MAGE-A	*Lm*-MPFG	*Lm*dd (*dal- dat*-)

Wood, Frontiers Cell Infect Mucrobiol, 2014

Lm-based vaccine clinical trials pipeline

Vaccine	Indication	Antigen	Preclinical	Phase I	Phase II	Phase III
ADXS-HPV[a]	Cervical cancer	HPV16 E7				
	Head and neck cancer	HPV16 E7				
	Anal cancer	HPV16 E7				
CRS-207[b]	Pancreatic Cancer	Mesothelin				
	Mesothelioma	Mesothelin				
ADXS-cHER2[a]	Breast cancer	HER2				
	Canine Osteosarcoma	HER2				
ADXS-PSA[a]	Prostate cancer	PSA				
ADU-214[b]	Ovarian Cancer	Mesothelin and EGFRvIII				
	Non-small cell lung cancer	Mesothelin and EGFRvIII				
ADU-623[b]	Glioblastoma multiforme	NYESO-1 and EGFRvIII				
Lm Prostate[b]	Prostate	Multiple undisclosed				

[a] http://www.advaxis.com/clinical-pipeline
[b] http://www.adurobiotech.com/pipeline.aspx

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7. NK-based therapy
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9. Quantitative multivariate model of human immune cell communication

Aims: "Analyzing, understanding and manipulating interactions between tumor cells and the immune system to overcome the cancer progression.“

Reverse vaccinology objectives

- To minimize the Laboratory based research.
- To develop a computational analysis of Antigens using Bioinformatics tools.
- To design a molecule that can replace an antigen in detection process.
- For the development of immunodiagnostic tests and vaccines.
- For detection of antibodies produced as a result of infections, allergies, autoimmune diseases, or cancers.

Reverse Vaccinology: identifying the proteins that are exposed on the surface by using genome instead of the

Conventional Vaccinology

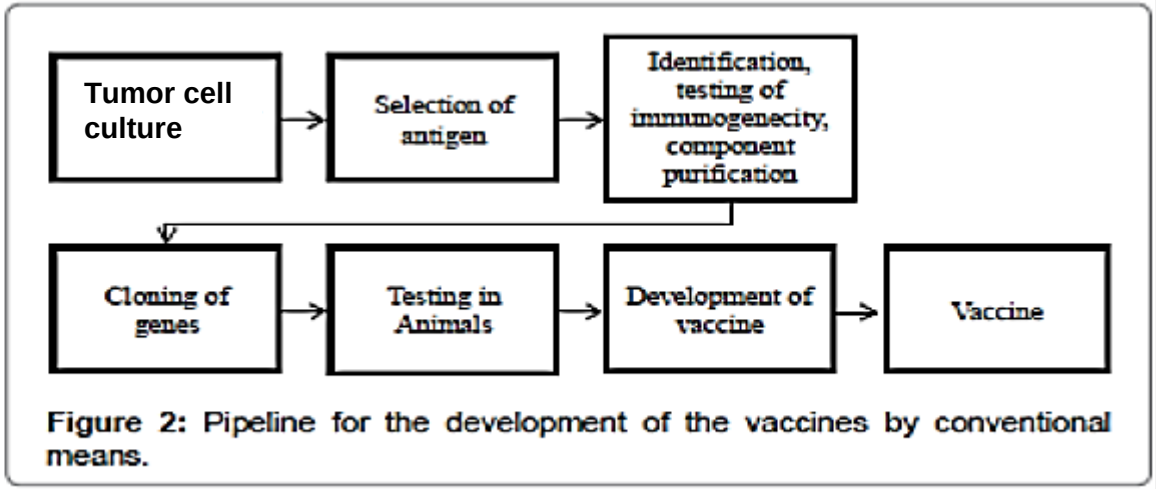


Figure 2: Pipeline for the development of the vaccines by conventional means.

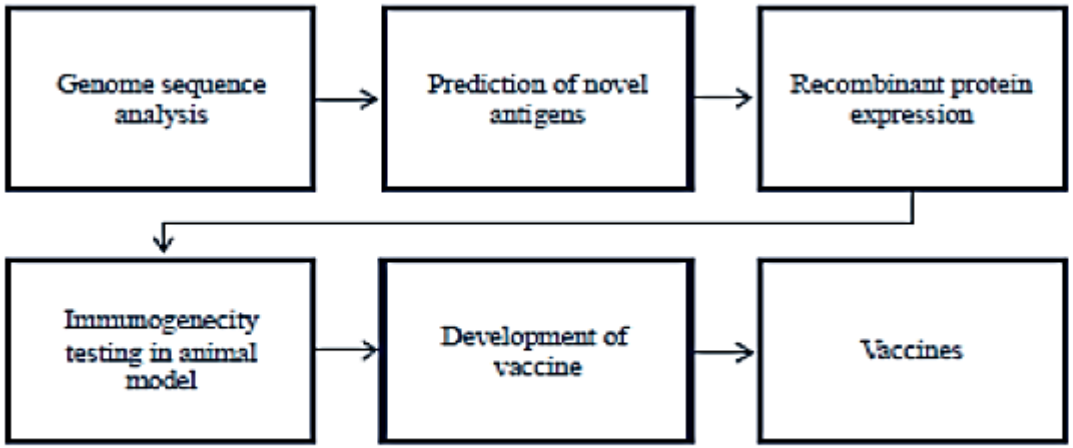
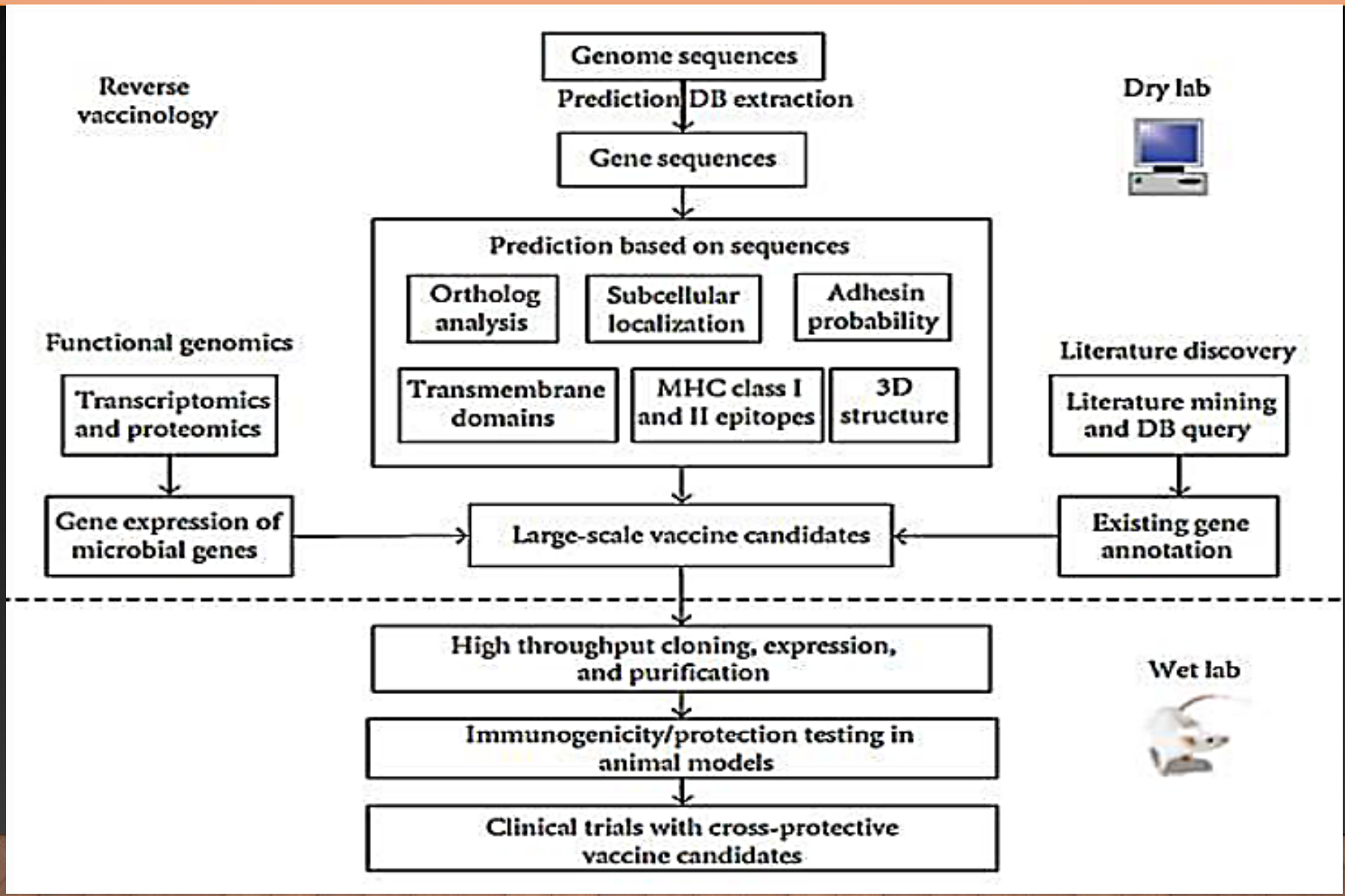


Figure 3: Pipeline for the development of the vaccines by reverse vaccinology.

Reverse Vaccinology

Aashi, 2018

Reverse Vaccinology: identifying the proteins that are exposed on the surface by using genome instead of the cancer cell



Aashi, 2018

Reverse vaccinology: epitope prediction

- Epitope prediction means to discover peptides that could mimic protein epitopes and possess the same immunogenicity as the whole protein.

- Based on the knowledge of the protein three-dimensional structure.
- Discontinuous epitopes with a known 3D structure can be reconstituted from the antibody binding peptides selected from randomized peptide libraries.
- Several bioinformatics tools address the convenience for structural studies-
- 3D-Epitope-Explorer (3DEX), MIMOX, Epitope Mapping Tool (EMT)), EPIMAP, MIMOP, PepSurf and Mapitope.
- The MEPS server facilitates a structure-based design of peptides representing the whole surface or a particular region of a protein.

Aashi, 2018

Targeting tumor-associated carbohydrate antigens (TACAs) with ... mimotopes

- ✓ Cancer cells can be distinguished from normal cells by displaying aberrant levels and types of carbohydrate structures on their surfaces: TACAs.
- ✓ Unfortunately, carbohydrates alone are poorly immunogenic
- ✓ How to overcome these obstacles:
  - covalently coupling TACAs to proper carriers to improve immunogenicity, including clustered or multivalent conjugate vaccines
  - coupling TACAs to T-cell peptide epitopes or the built-in adjuvant to form multicomponent glycoconjugate vaccines
  - Use TACAs as mimotopes: generation of a peptide mimotope library that reflects the common TACAs... with amino acid sequence, same 3D shape as TACAs, BUT made with aminoacids → immunogenicity!

Feng, acschembio, 2016

CANCER IMMUNOTHERAPY INNOVATIONS

The study of interactions between the immune system & cancer cells

1. **Advanced checkpoint blockade**
2. **Oncolytic viruses**
3. **CAR T cells & novel CAR cells**
4. **BiTEs**
5. **Tumor vaccines & Neo-Ag**
6. **Reverse vaccinology & mimotopes**
7. **NK-based therapy**
8. Genetically engineered mouse models
9. Quantitative multivariate model of human immune cell communication

Aims: "Analyzing, understanding and manipulating interactions between tumor cells and the immune system to overcome the cancer progression.“

NK-based immunotherapy: why?

- ✓ **NK cells inhibit tumor development** in mouse models
- ✓ NK presence in tumors **correlates with patient survival**.
- ✓ lung adenocarcinoma,
  - NK cells localized to tumor **stroma** with immature phenotypes and low functional capacity.
  - After stimulation, NK cells localized **inside tumors, with increased cytokine production capacity.**
  - Strikingly, **T cells** were also recruited to tumors in an NK cell-dependent manner, and exhibited higher functionality.
  - even in established disease **NK cells can be activated to contribute to antitumor immunity**,

  → **NK = important target in cancer immunotherapy.**

Feng, acschembio, 2016

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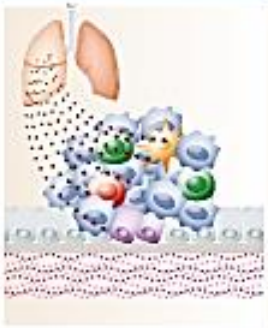
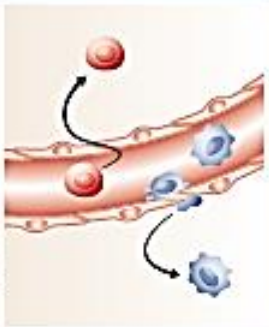
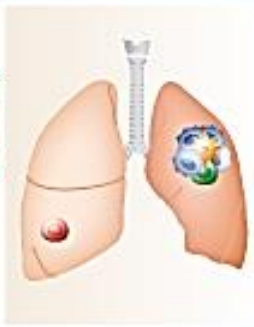
GEMMs

- ✓ **Genetically engineered** mouse models (GEMMs)
- ✓ In contrast to cancer cell inoculation models, GEMMs **develop de novo tum**ors in a natural immune-proficient microenvironment.
- ✓ Tumors arising in advanced GEMMs closely **mimic the histopathological and molecular features of their human counterparts**, display genetic heterogeneity, and are able to spontaneously progress toward metastatic disease.

Feng, acschembio, 2016

GEMMs

	Intratumoral heterogeneity	Priming metastatic niche	Invasion	Circulation/ Extravasation	Seeding/ Colonization	Clinically overt multi-organ metastasis
						
Cell line inoculation*						
• Tail vein	✗	✗	✗	✓	✓	✓
• Orthotopic	✗	✓	✓	✓	✓	✓
PDTX*	✓	?	✓	Model-dependent	Model-dependent	Model-dependent
Conventional GEMM	✓	✓	✓	Model-dependent	Model-dependent	Model-dependent
Next generation GEMM	✓	✓	✓	?	?	?
GEMM-based orthotopic transplantation model for metastatic disease	✓	✓	✓	✓	✓	✓

Kersten, embo Med, 2019

CANCER IMMUNOTHERAPY INNOVATIONS

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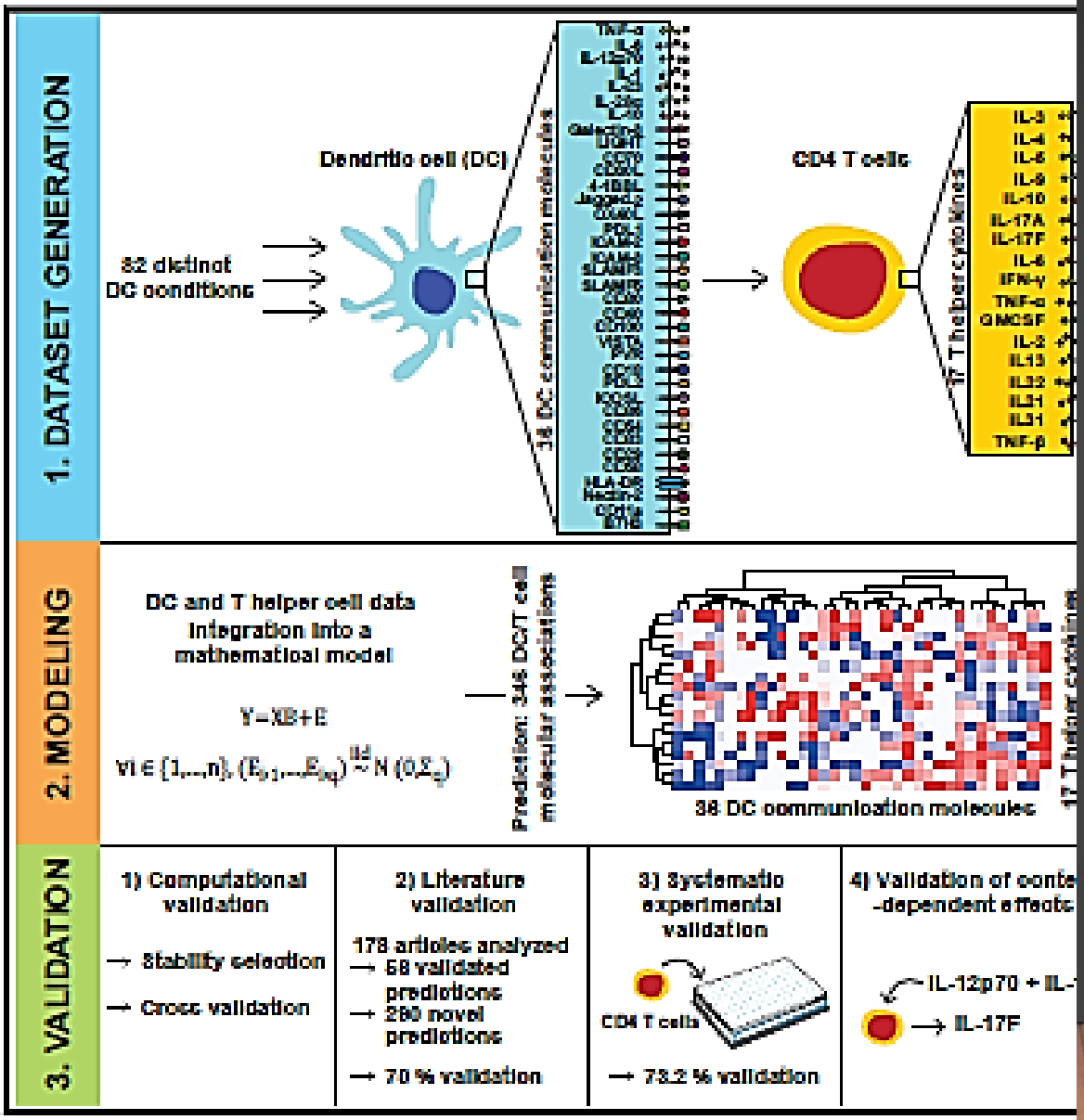
A Quantitative Multivariate Model of Human Dendritic Cell-T Helper Cell Communication



They measured

- 36 DC-derived signals
- 17 Th cytokines
- broadly covering Th diversity in 428 observations.

They developed a data-driven, computationally validated model capturing 56 already described and **290 potentially novel mechanisms** of Th cell specification.

A unique resource to decipher the complex combinatorial rules governing DC-Th cell communication and guide their manipulation for vaccine design and immunotherapies.

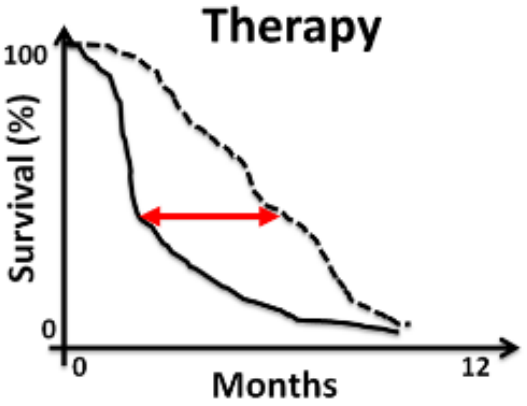
The ultimate aim is to predict context-dependent behaviors.

Gandclaudon, Cell, 2019

blood 2012 120: 4454-4455
doi:10.1182/blood-2012-09-455105

Combined targeted and immunotherapy: the future of personalized medicine

Aurélien Marabelle and Christophe Caux



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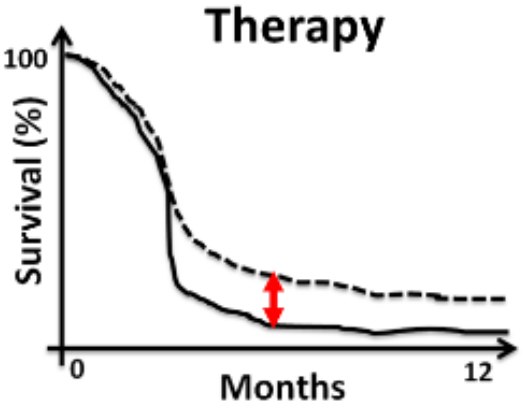

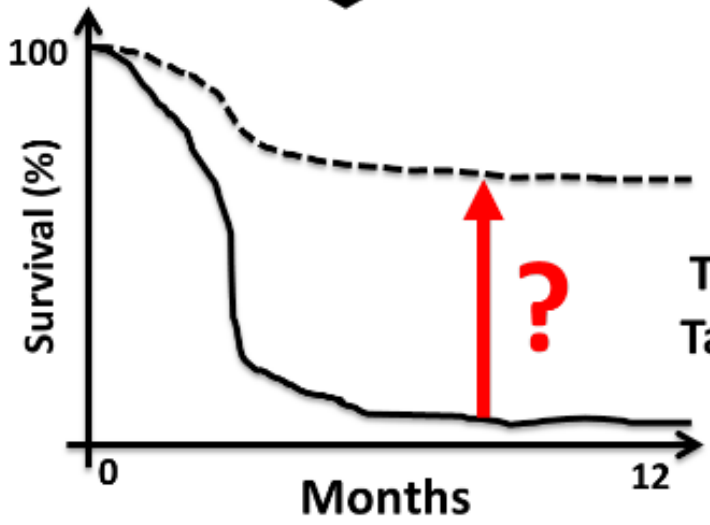

Tumor+ Immune
Targeted Therapy

Best wishes!

Long live TTHH

Twenty Twenty

Healthy & Happy!