Can Cancer Vaccines Really Work? Vaccination Strategies and Identification of Neoantigens

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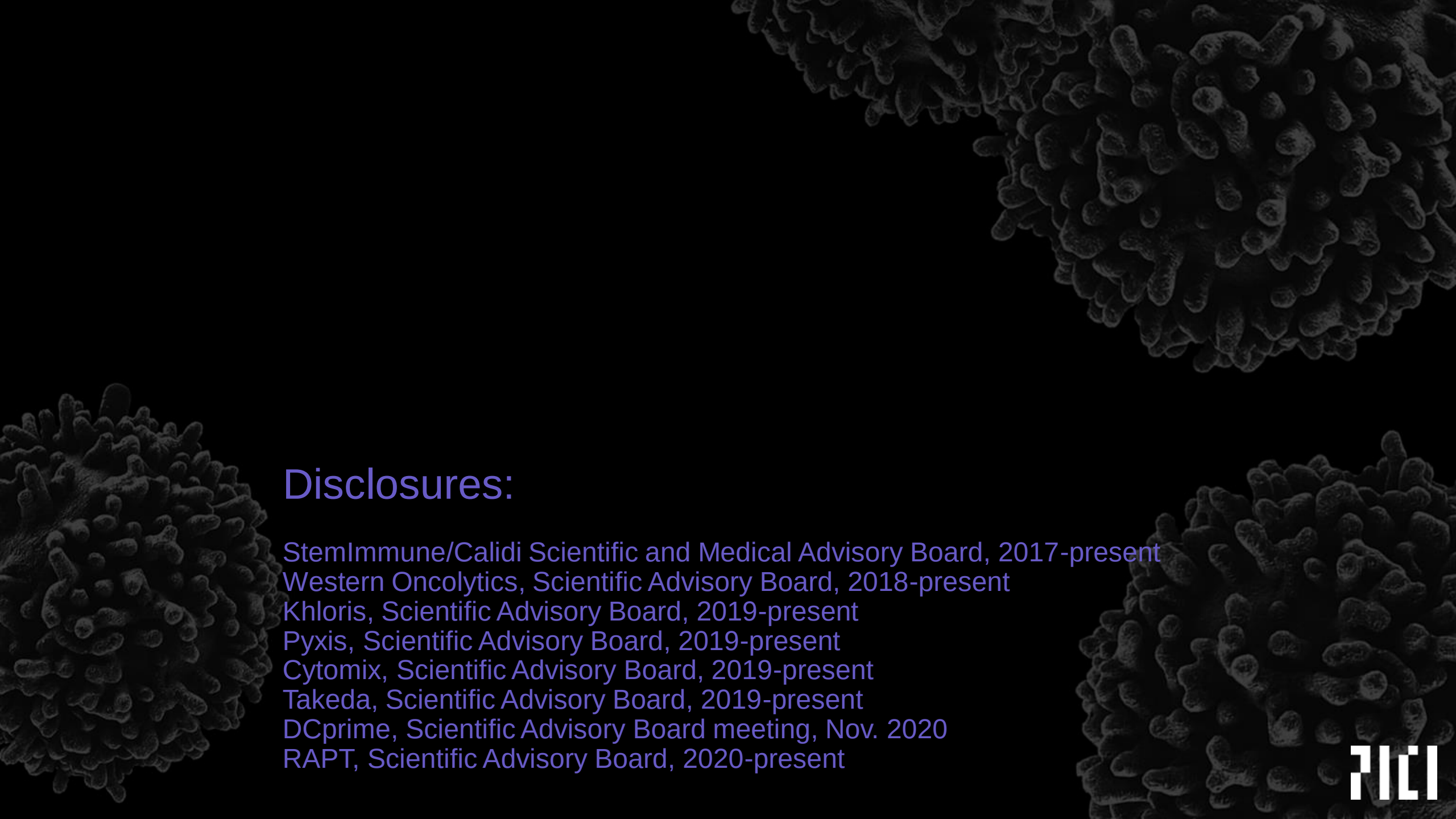
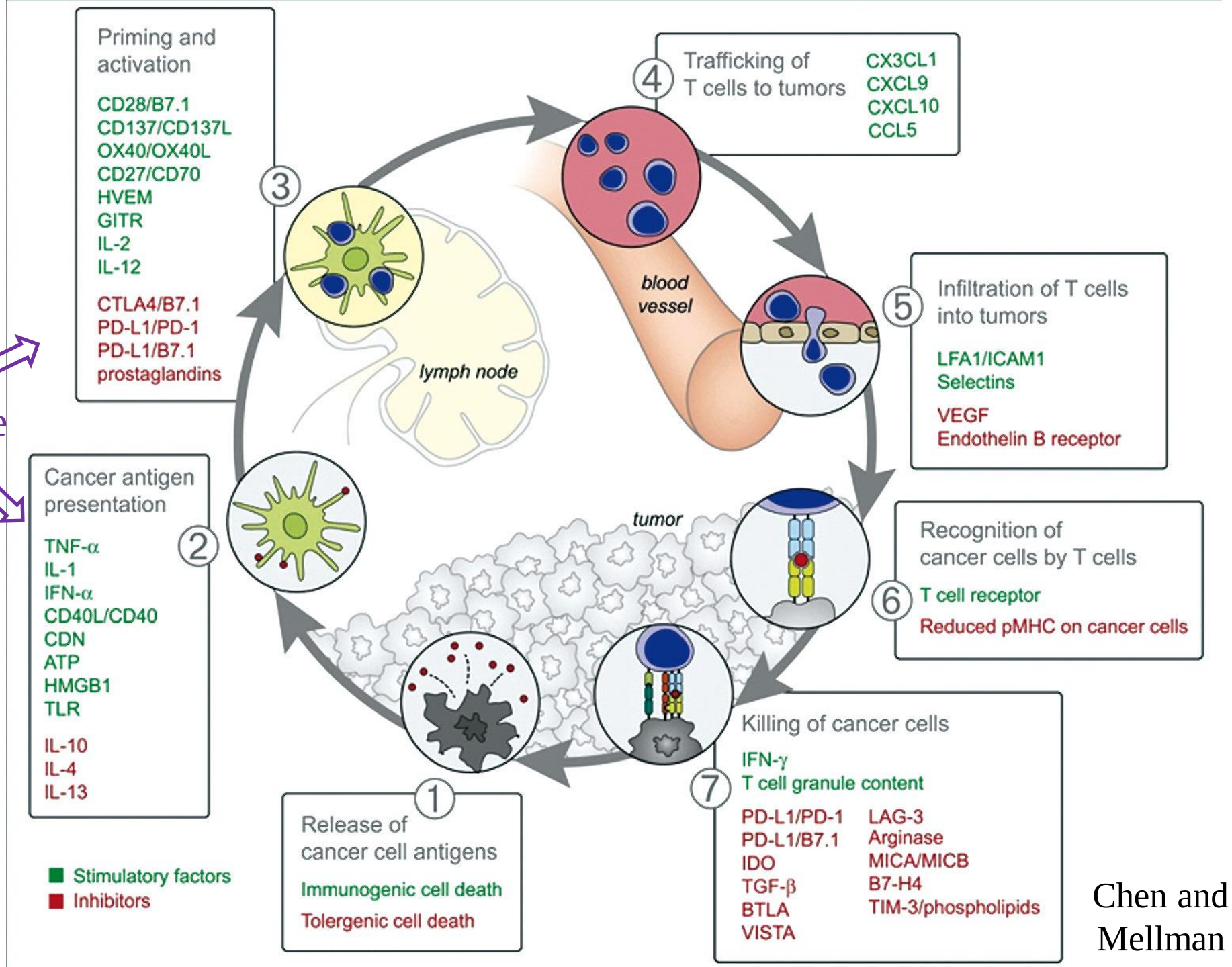
Past President, SITC

PARKER INSTITUTE FOR CANCER IMMUNOTHERAPY



Disclosures:

StemImmune/Calidi Scientific and Medical Advisory Board, 2017-present
Western Oncolytics, Scientific Advisory Board, 2018-present
Khloris, Scientific Advisory Board, 2019-present
Pyxis, Scientific Advisory Board, 2019-present
Cytomix, Scientific Advisory Board, 2019-present
Takeda, Scientific Advisory Board, 2019-present
DCprime, Scientific Advisory Board meeting, Nov. 2020
RAPT, Scientific Advisory Board, 2020-present



You are here

Chen and Mellman

Common Cancer Drivers

Cell Growth Genes: cell division

Angiogenesis-related Genes: obtain nutrients from blood

Metastasis-related Genes: escape tissue of origin and continue growth

Immune Suppression: remain invisible to immune system surveillance

Tumor Associated Antigens

What is Different about the Tumor?

<u>How to identify a tumor antigen:</u>

Use TIL (tumor infiltrating lymphocytes) which can “recognize” the tumor to screen a cDNA library:

1. Which cDNA transfected into an unrelated (but HLA-matched) cell line confers TIL recognition?

2. Identify gene encoded by plasmid in cDNA library

The Classics: Commonly Targeted Shared Tumor Antigens

1) MAGE-1, -2 and –3, BAGE and RAGE, which are non-mutated “cancer-testes” antigens expressed in a variety of tumor cells

2) lineage specific tumor antigens, like the melanocyte/melanoma lineage antigens MART-1/Melan-A (**MART-1**), *gp100, gp75, mda-7*, tyrosinase and tyrosinase-related-protein (TRP-1 and -2), or the prostate antigens PSMA and PSA

3) proteins derived from genes mutated in tumor cells compared to normal cells, like mutated *ras*, *bcr/abl* rearrangement or mutated *p53*

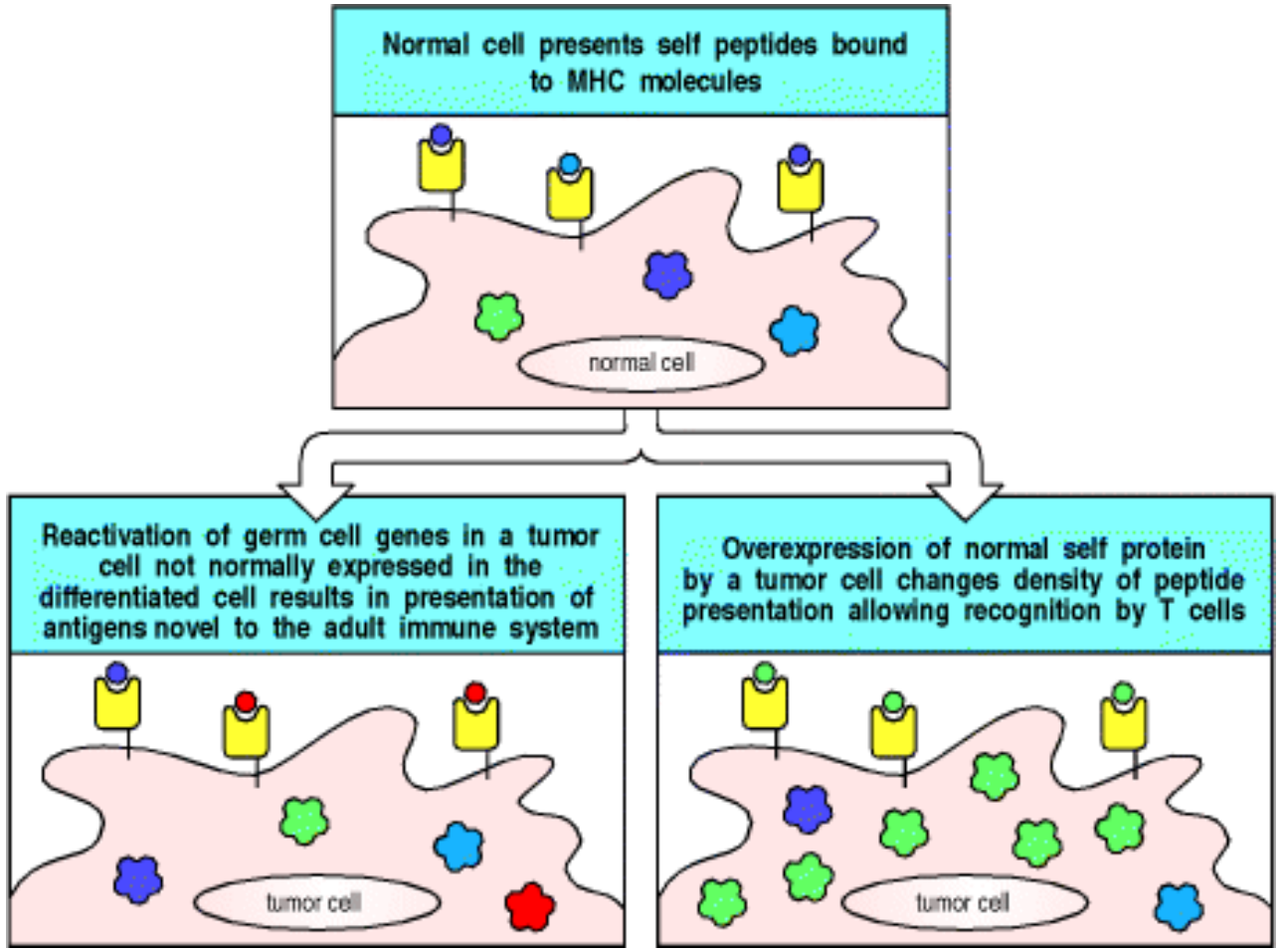
4) proteins derived from oncoviruses, like Human Papilloma Virus (HPV) proteins E6 and E7, HBV, HCV, MCPV

5) non-mutated proteins with a tumor-selective, increased expression, including CEA, PSA, Her2/neu and alpha-fetoprotein (**AFP**), and differentially glycosylated MUC-1

Tumor Antigens

onco-fetal antigens, over-expressed proteins

Sort of "new"



Much more

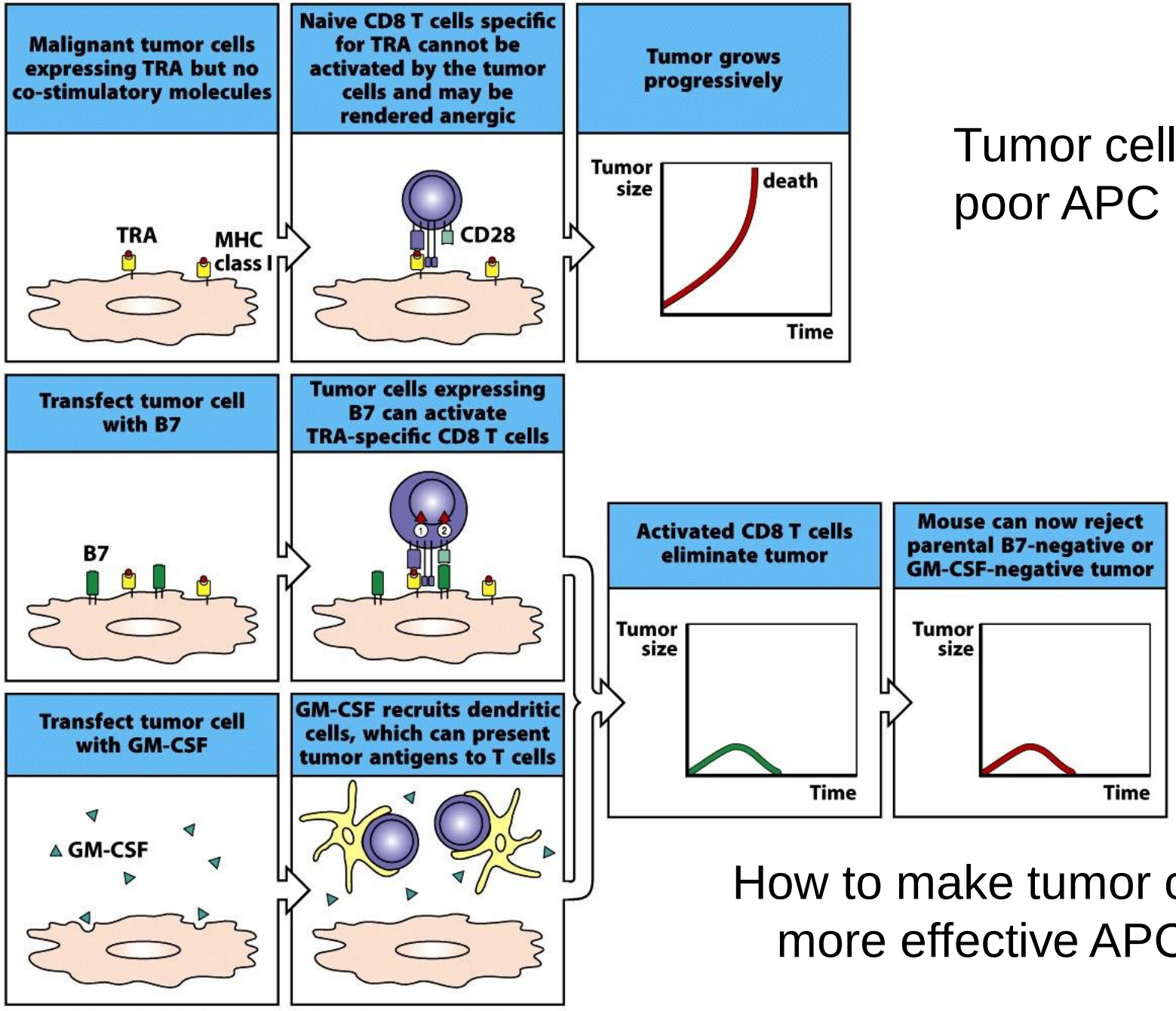
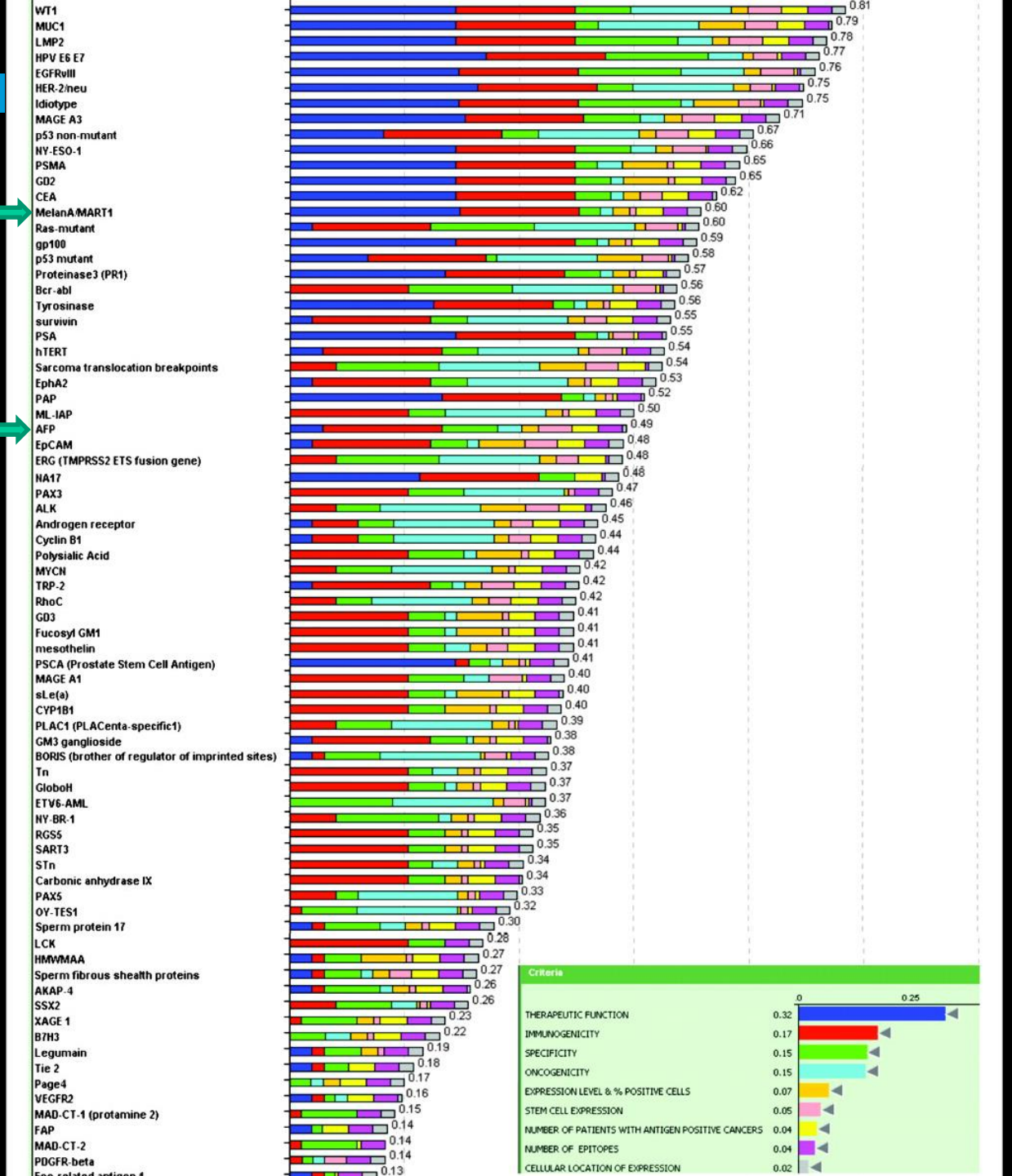


Figure 15-24 Immunobiology, 7ed. (© Garland Science 2008)

Tumor cells are poor APC

How to make tumor cells more effective APC?

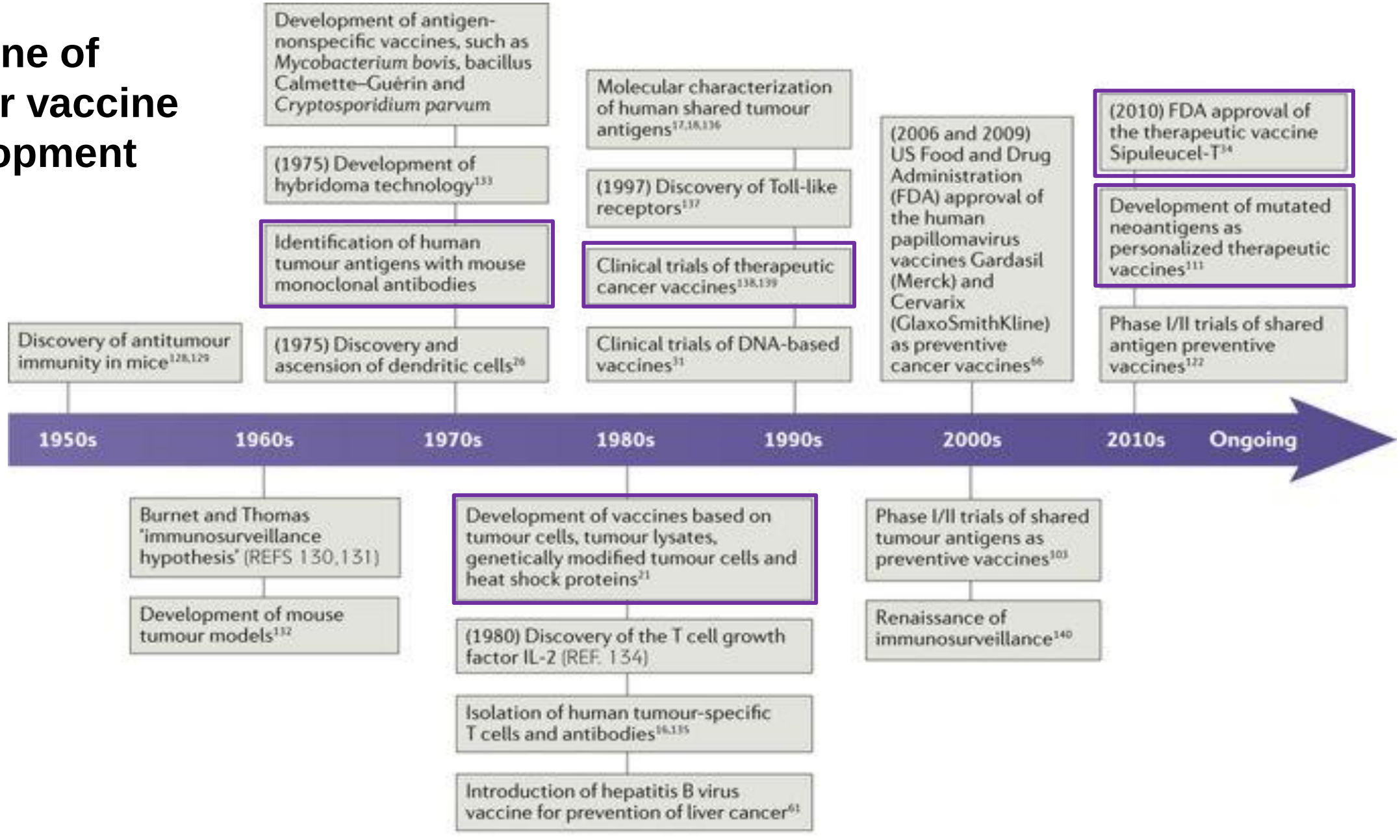


The Prioritization of Cancer Antigens:

A National Cancer Institute Pilot Project for the Acceleration of Translational Research

Cheever, CCR 2009

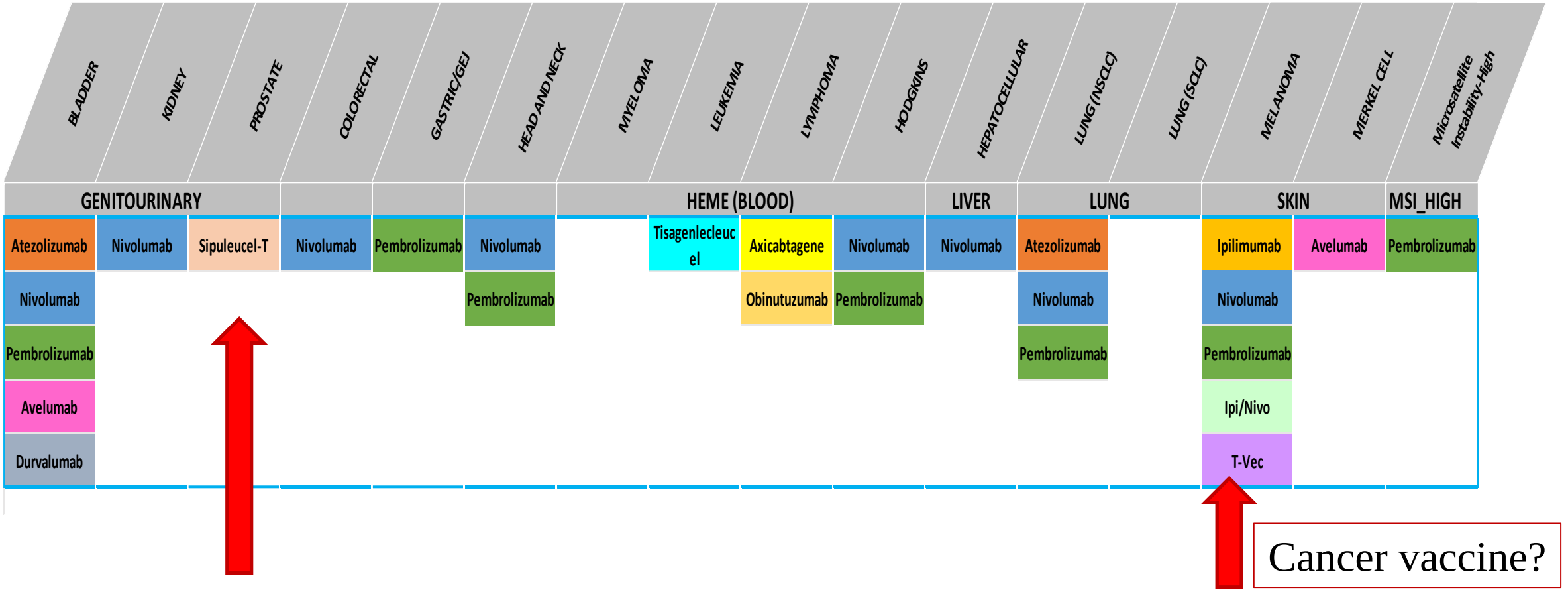
Timeline of cancer vaccine development

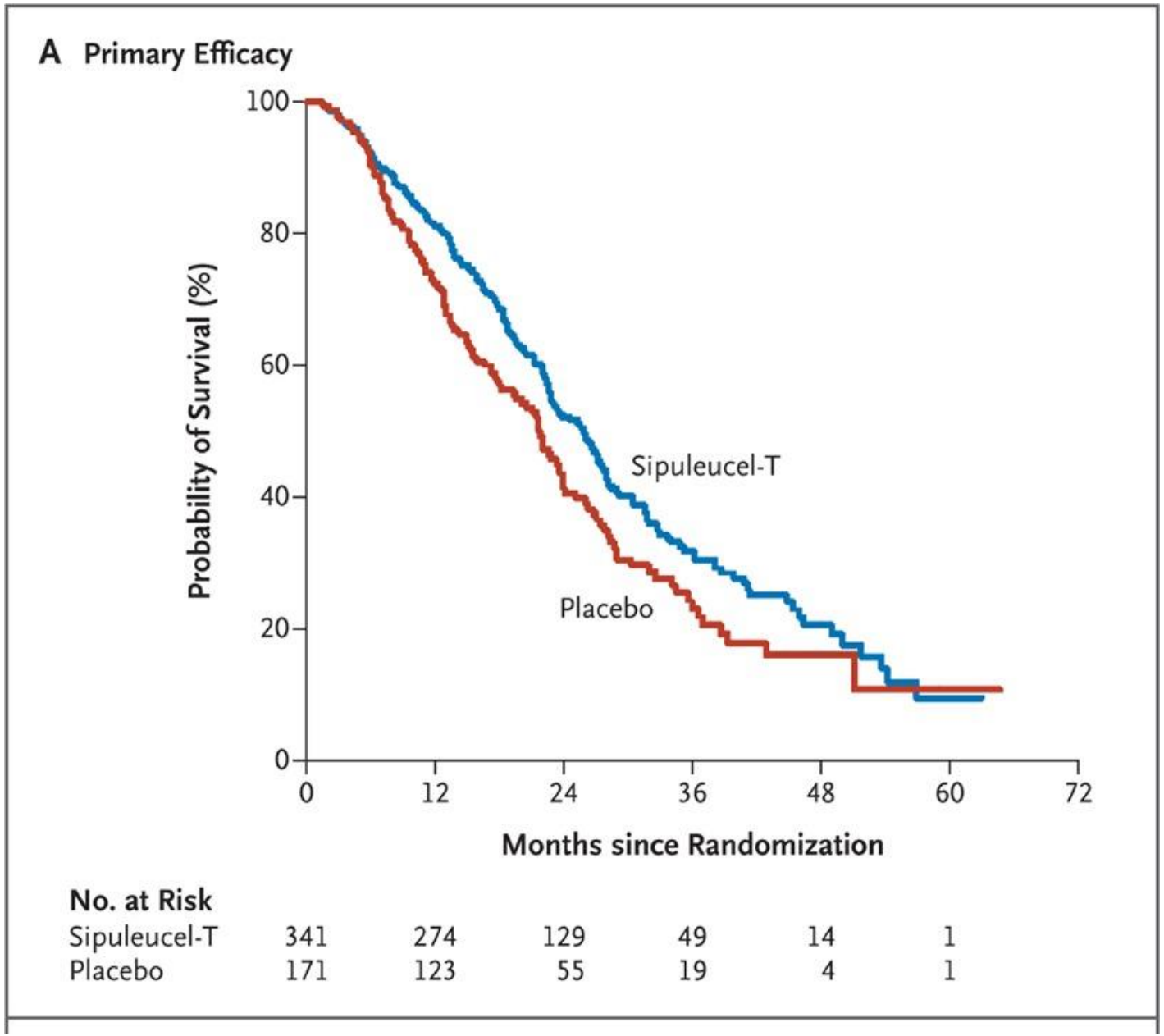


O. Finn

Nature Reviews | Immunology

US Immunotherapy Approvals by tumor





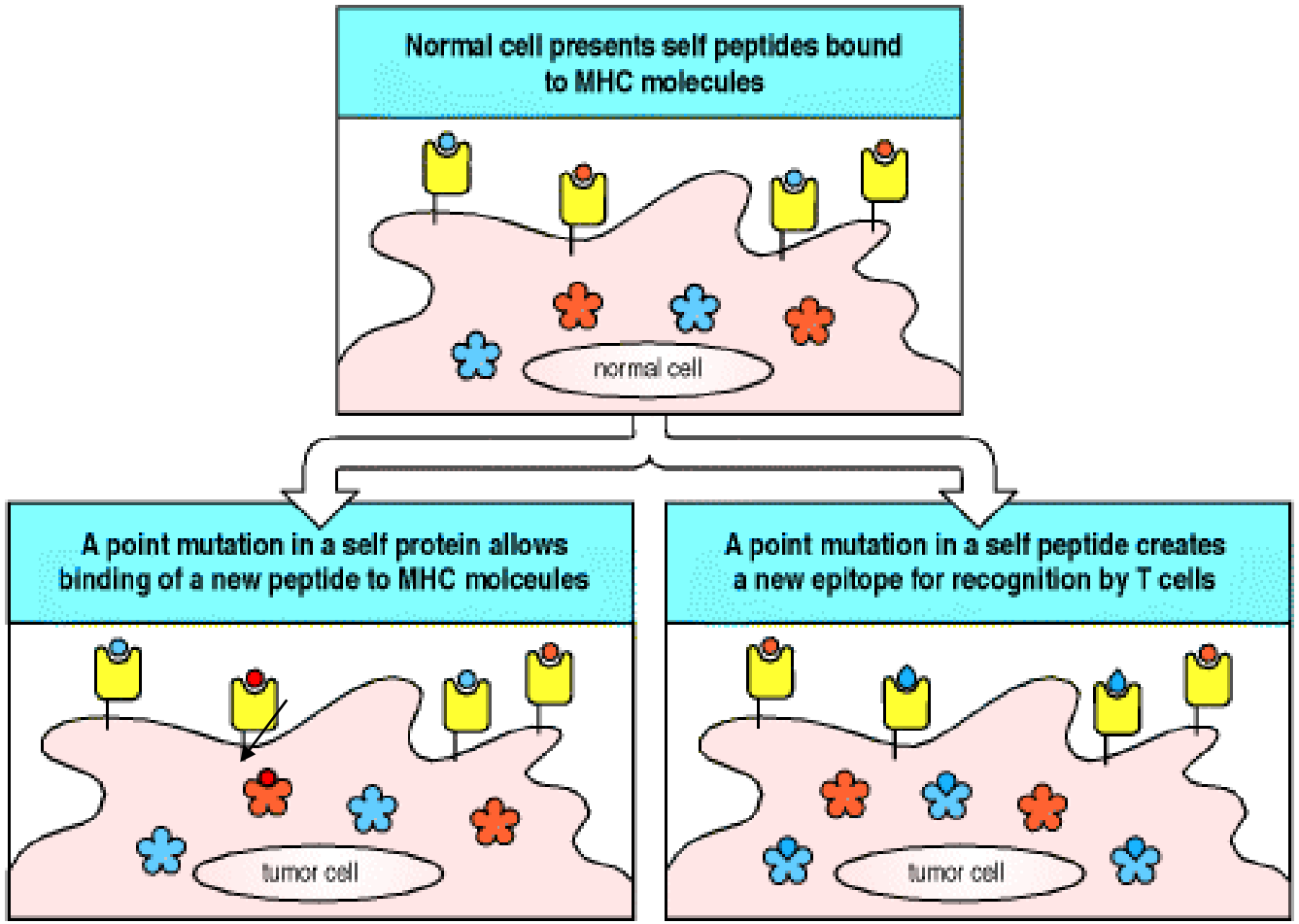
In April 2010, the U.S. Food and Drug Administration (**FDA**) **approved** (sipuleucelT), an autologous cellular immunotherapy, for the treatment of patients with asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer (mCRPC)

Cold tumor to hot?

Activated T cell trafficking to tumor (Fong, 2014)

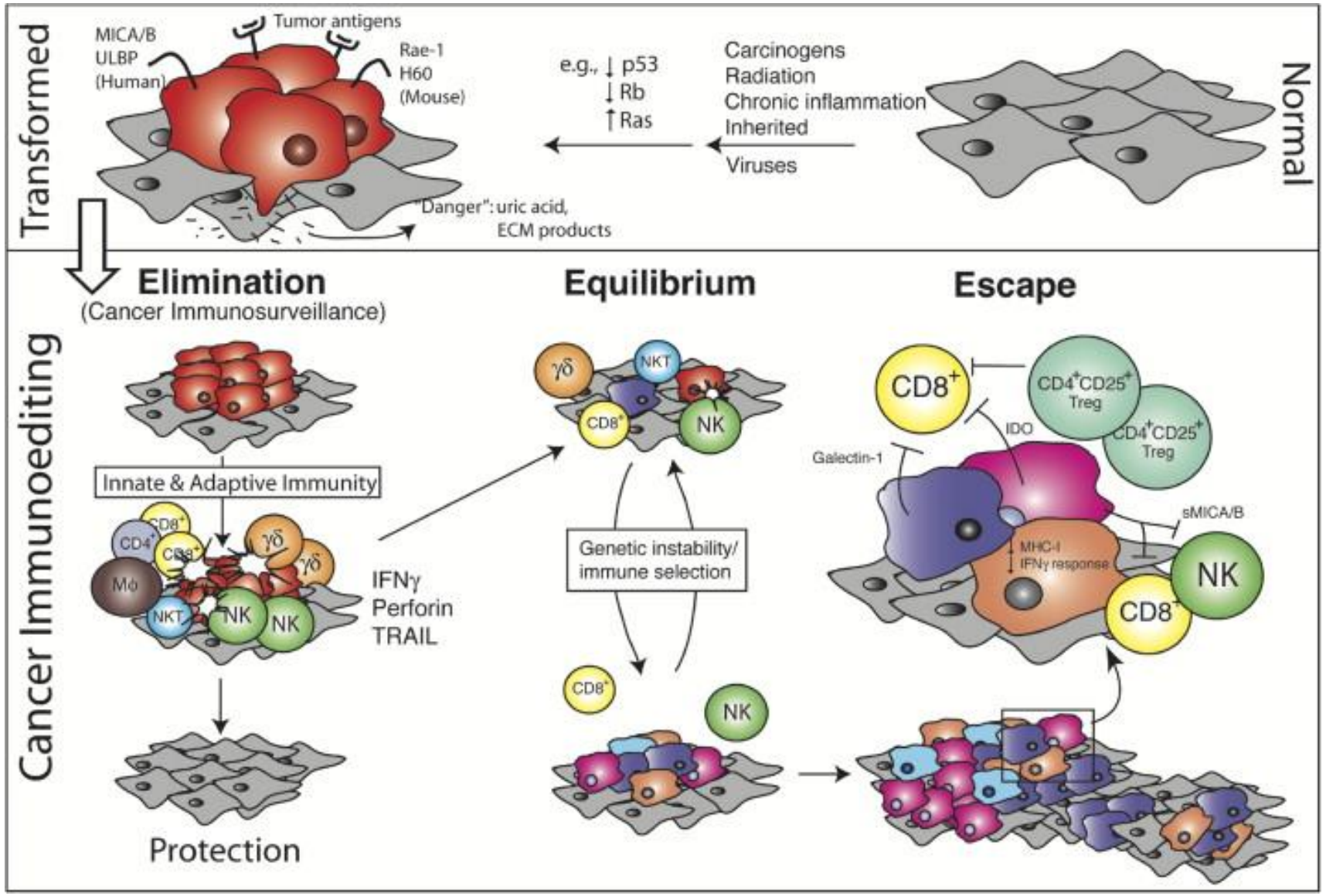
2020-2021: +/- Anti-CTLA-4
+/- IL-7

Tumor Antigens
“private” or patient-specific



Mutation: processed and presented? In which MHC? How to identify for each patient?

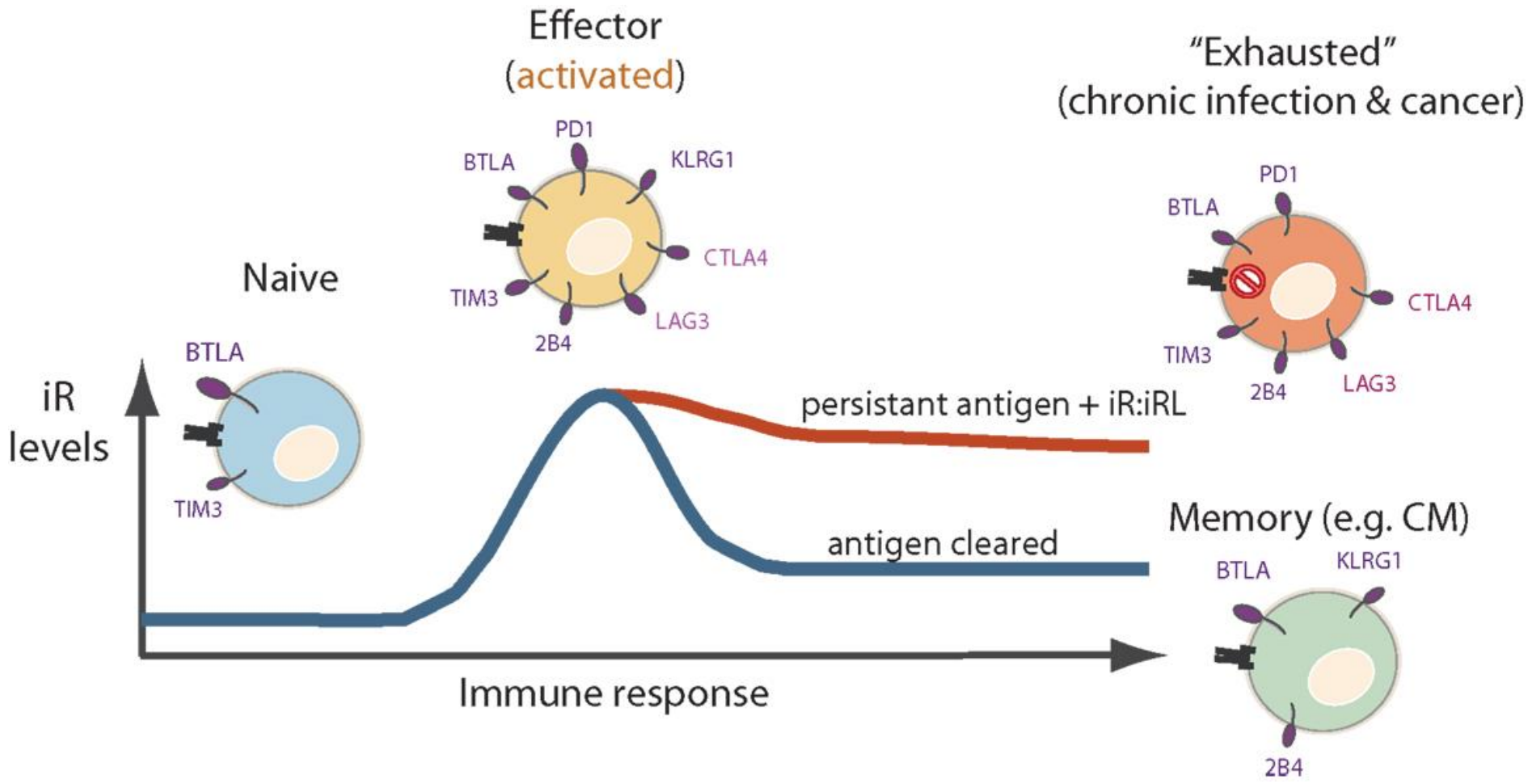
Three Phases of the Cancer Immuno-editing



Did we already get rid of the “easy” tumor cell targets?

Gavin P. Dunn , Lloyd J. Old , Robert D. Schreiber

The Immunobiology of Cancer Immunosurveillance and Immunoediting
Immunity, Volume 21, Issue 2, 2004, 137 - 148

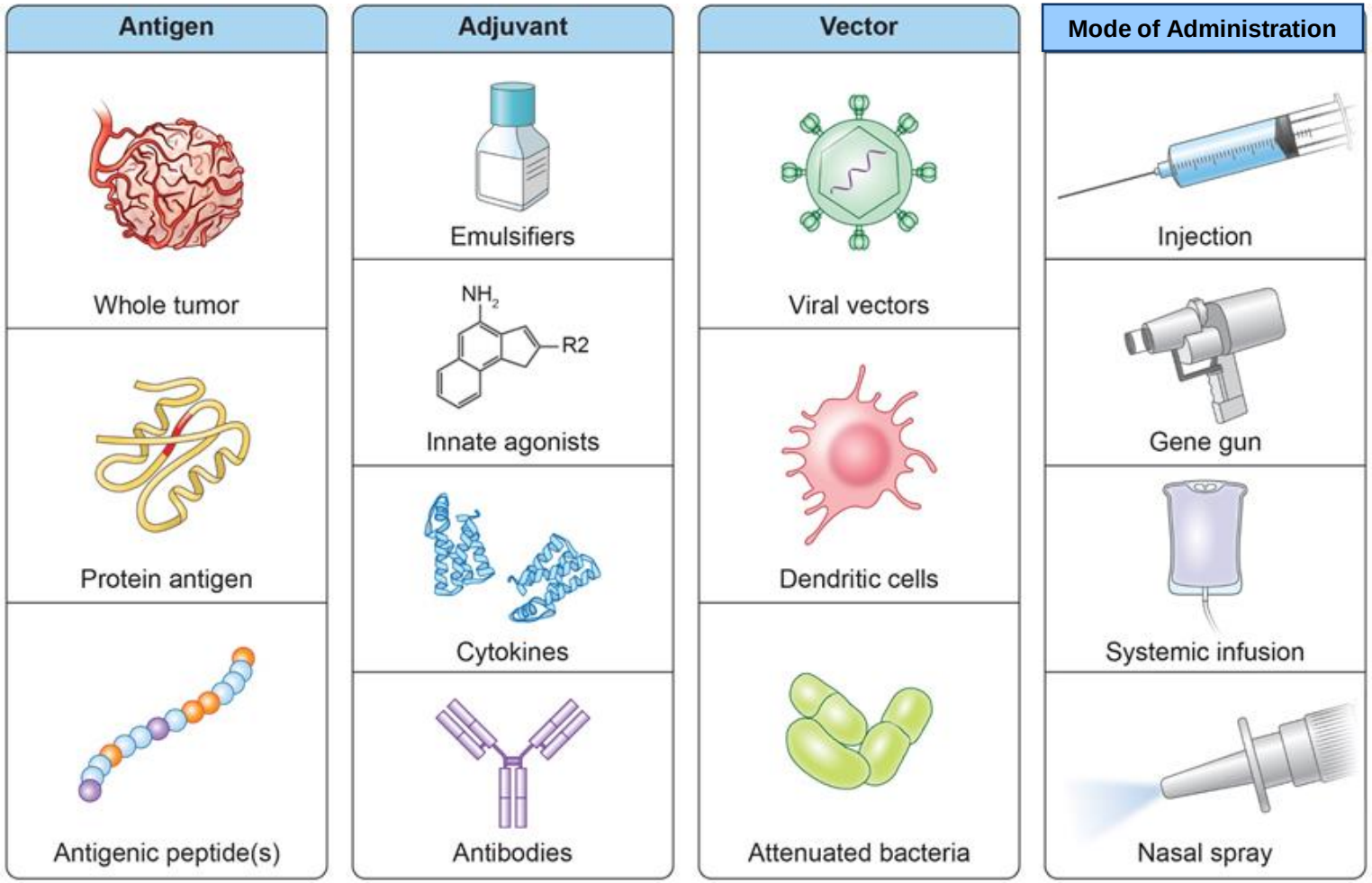


T Cell Exhaustion. Naïve cells express mainly BTLA and low levels of TIM3. Effector cells express a wider variety of **inhibitory receptors**. The levels of certain inhibitory receptors such as PD1, CTLA-4, LAG3, and TIM3 may peak at the effector phase. Thereafter, expression differs in chronically stimulated cells ("exhausted cells") where inhibitory receptors are relatively maintained, as opposed to memory cells after clearance of an acute infection where inhibitory receptors are down-modulated.

Front. Immunol., 26 June 2015 Fuertes, Speiser

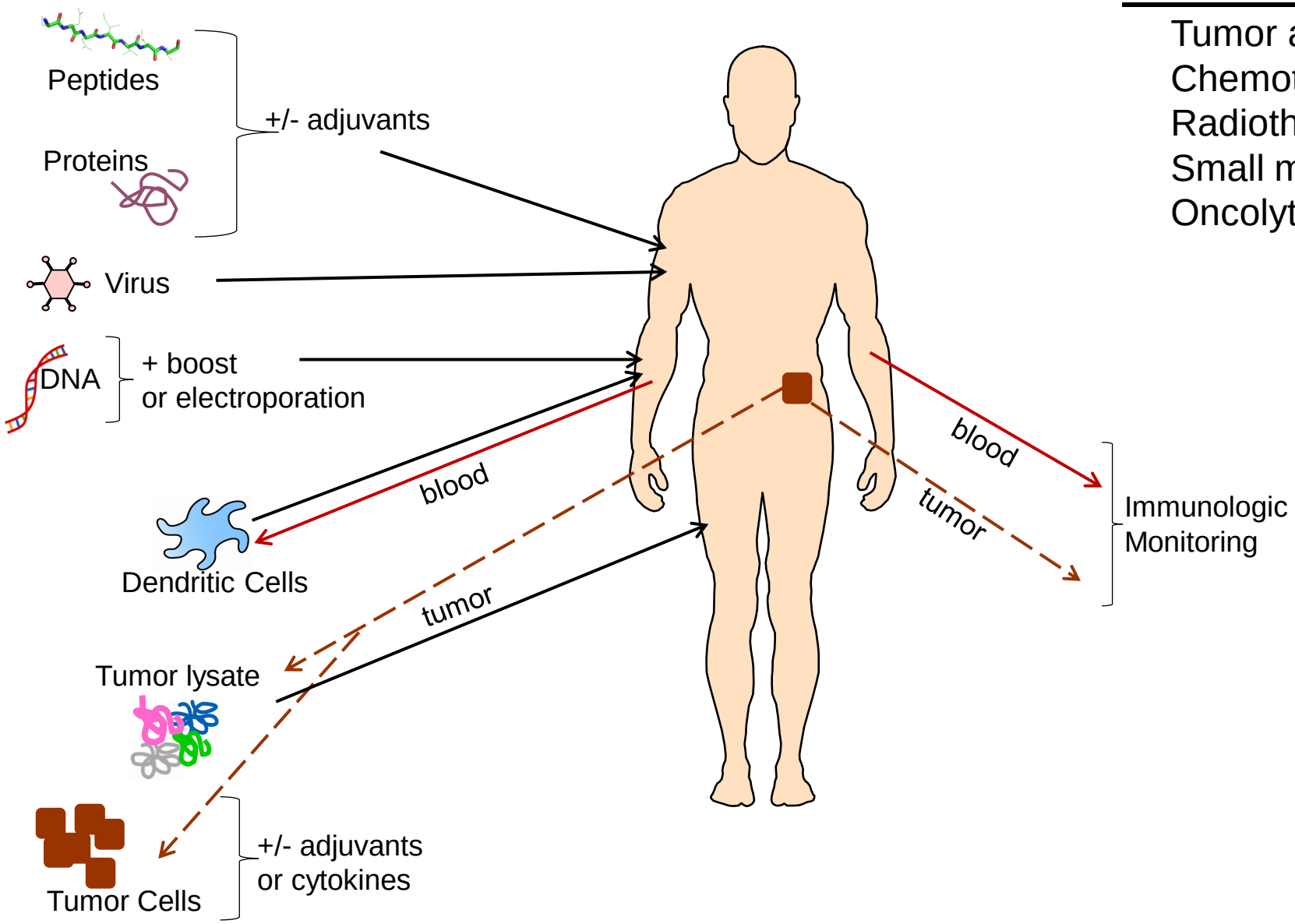
ADVANCES IN Cancer IMMUNOTHERAPY™

Components of a cancer vaccine



And RNA/DNA

Vaccine platforms

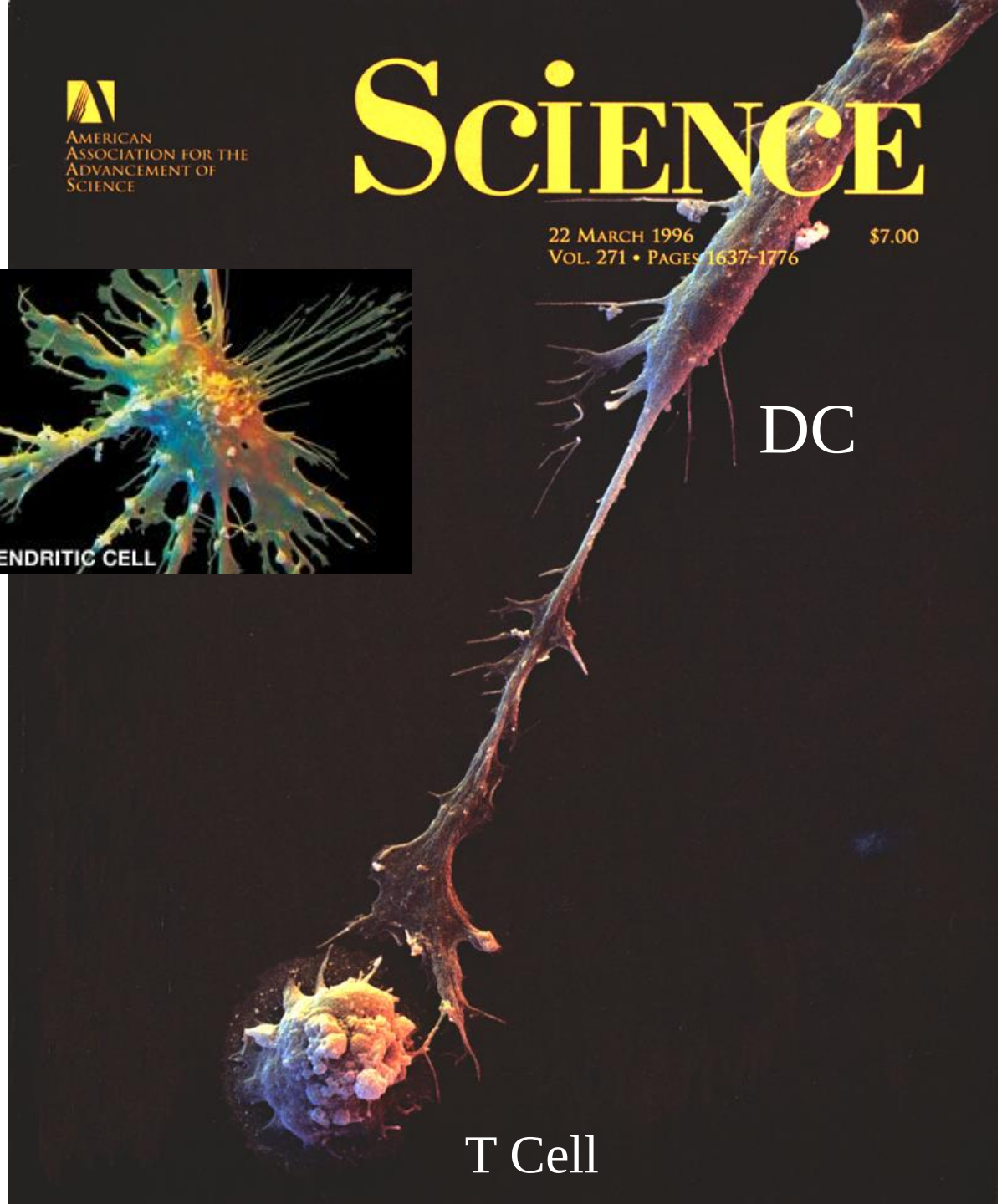


Vaccine Effects

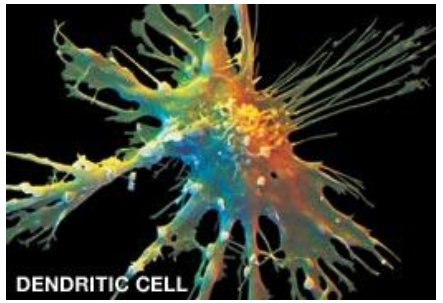
- Tumor ablation
- Chemotherapy
- Radiotherapy
- Small molecules
- Oncolytic virus

Dendritic Cells at the center of the immunological universe:

1. Sampling their environment
2. Sensing pathogens
3. Trafficking from the periphery to lymph nodes
4. Presenting antigen and shaping the adaptive immune response
5. Inhibiting unwanted responses (tolerance) and activating needed responses
6. Many different types of DC



DC Vaccines



- ➢200 DC trials since 1996
- ➢5 current phase III trials recruiting
- ➢5 current phase II trials of DC **+ *anti-PD-1***

Dendreon Sipuleucel T: **>**$80,000/patient; Pittsburgh: $6,500/pt.

Historically, 5-10% CR+PR in late stage patients in some trials, 0% in other trials.

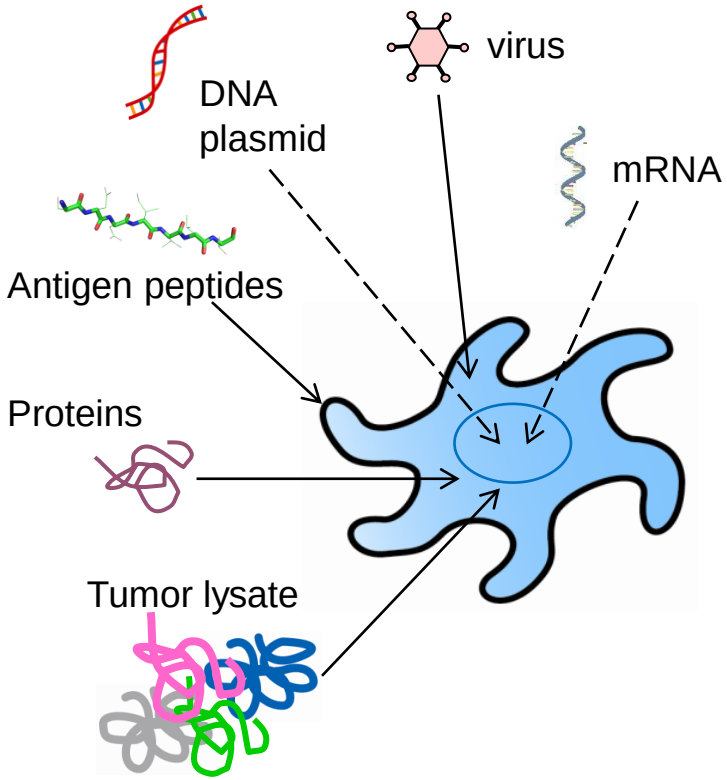
Recent DC vaccine studies (combinations, *author conclusions*):

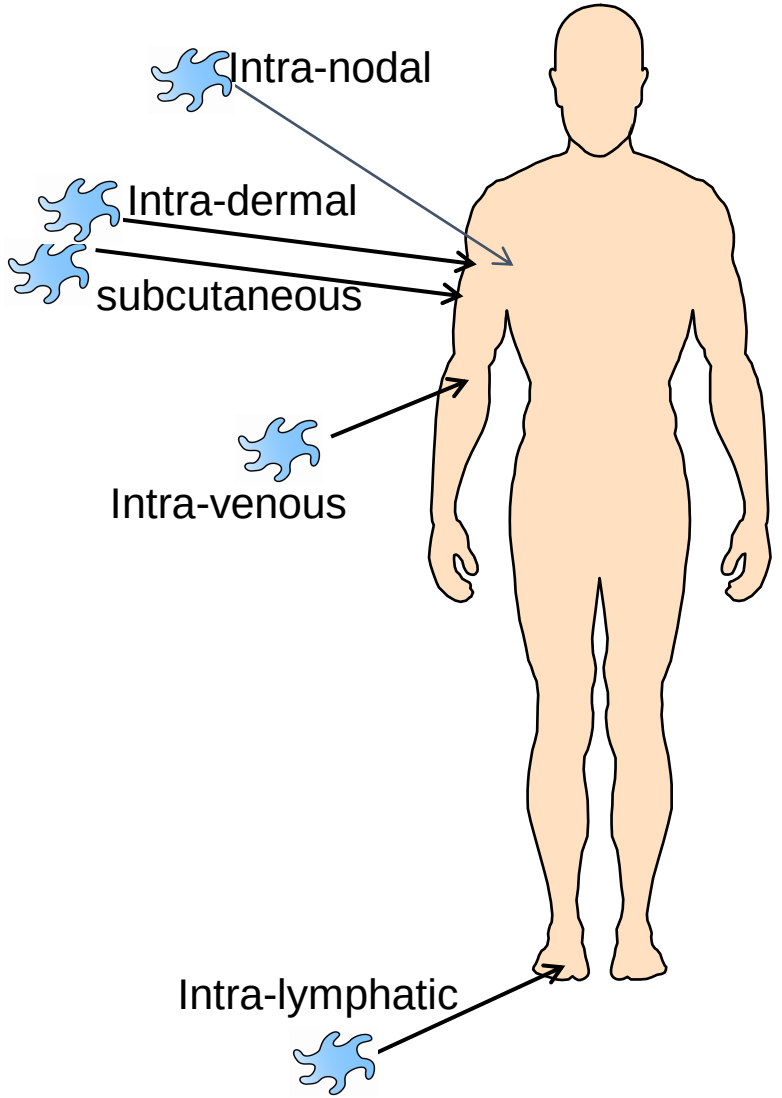
1. Kongstad, Svane: **Cytotherapy 2017**: DC + chemo in 43 prostate cancer pt. (*safe and immunogenic*)
2. Schreibelt, De Vries: **CaRes 2016**: 14 stg. IV melanoma pt., CD1c+ isolated blood DC, 16 hour culture, + gp100 and tyrosinase. *4/14 pt. PFS 12-35 mo*.
3. Wilgenhof, Neyns: **JCO 2016**: 39 “adv. Melanoma” pt., mRNA: gp100, tyrosinase, MAGE-A3, MAGE-C2/DC + ipi. *“Encouraging” ORR, 8 CR+7 PR/39.*
4. Greene, Peoples: **CII 2016**: DC/tumor fusions + low dose IL-2 in 25 melanoma pt. *Benefit for some?*
5. Carreno, Linette: **Science 2015**: 3 stg. III melanoma pt., DC+ neoAg peptides, *some + immune responses (proof of principle).*
6. Chodon, Ribas: **CCR 2014**: DC + MART-1 ACT, 14 melanoma pt.*, objective responses, needs improvement for durability*
7. Ribas, Gomez-Navarro: **CCR 2009**: DC + anti-CTLA-4, 16 melanoma pt., *combo not better.*

Why DC Vaccines?

- Originally considered a stand-alone therapeutic approach to promote regression of tumors.
- After being proven “safe and immunogenic” over years, testing in earlier stage patients and in the prevention setting in high risk patients is being pursued.
- With the success of checkpoint blockade and data supporting the need for a pre-existing immune response in the tumor for checkpoint response, *vaccines may be critical to promote antitumor immunity in those who lack it spontaneously.*

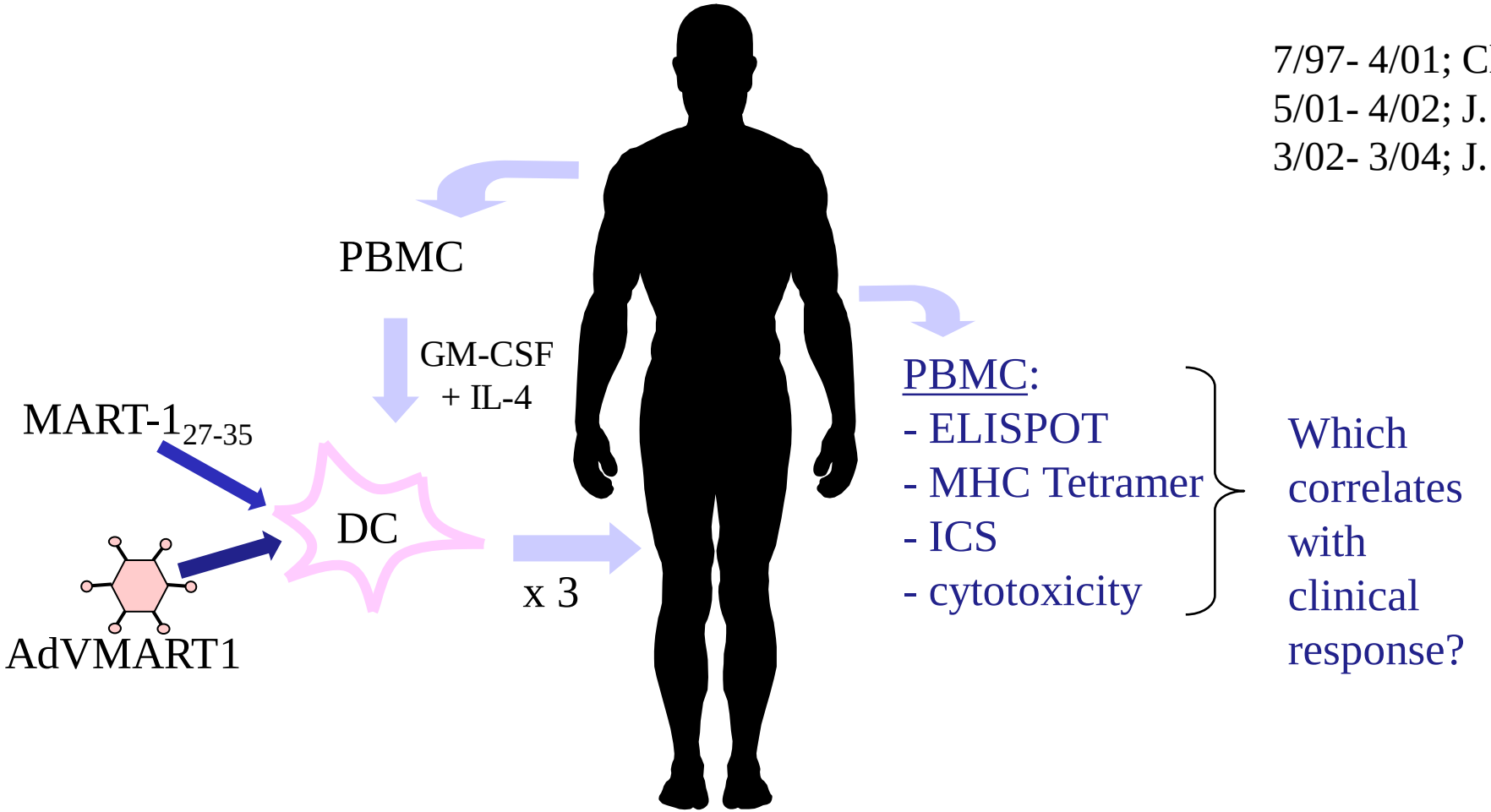
Antigen delivery to DC





MART-1 loaded-DC Clinical Trials

7/97- 4/01; Clin.Ca.Res., 3/03
5/01- 4/02; J. Immunother., 9/04
3/02- 3/04; J. Immunother., 4/08



Peptide/DC Phase I: 10^5, 10^6, 10^7 DC/injection
i.v. vs. i.d. at each dose (18 pt.)

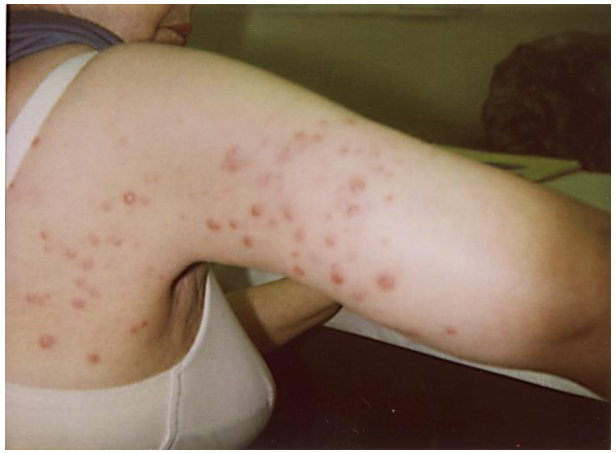
Peptide/DC Phase II: 10^7 DC/injection, i.d. (10 pt.)

AdV/DC Phase I/II: 10^7 DC/injection, i.d. (23 pt.)

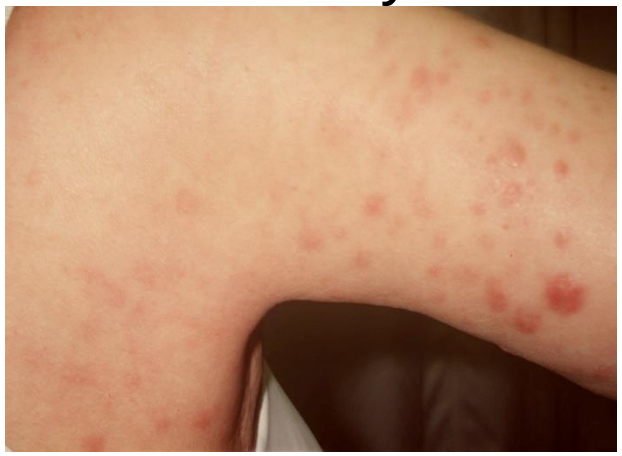
PI: J.S. Economou

Patient E1 (10^7 DC, i.d.) post: 6 surgeries, 32 doses radiation, 6 infusions IFNα. >10 yrs NED

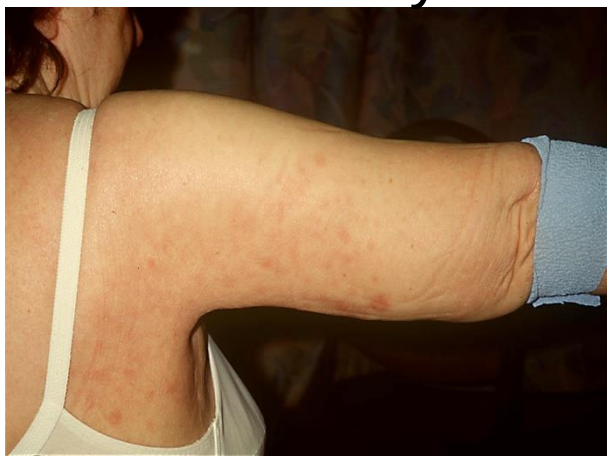
Pretreatment

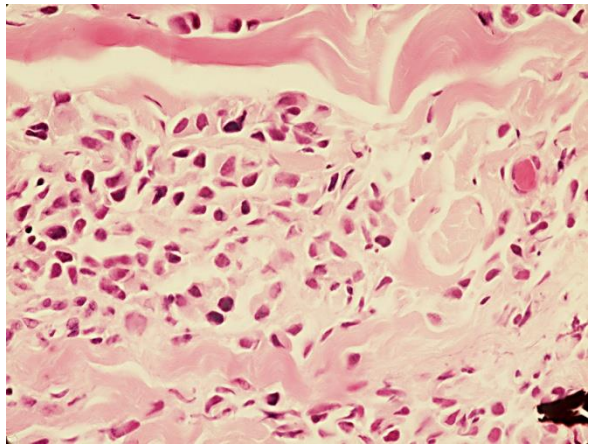


+56 days

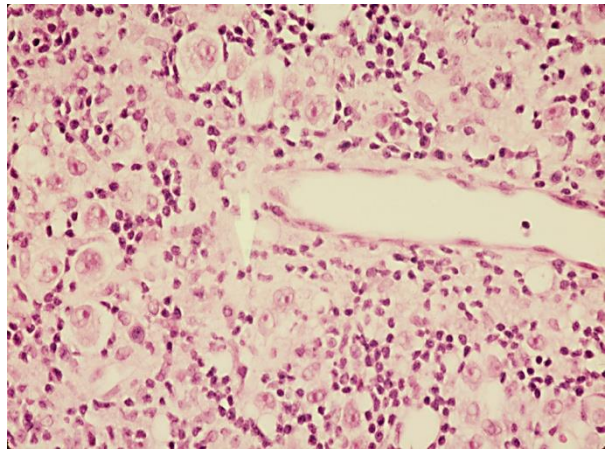


+130 days

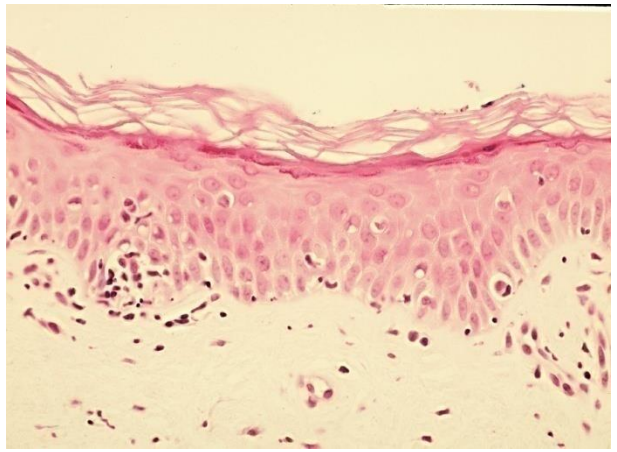




Melanoma Tumor



Lymphocytic Infiltrate
(largely CD8+, also CD4+)



Absence of Melanoma

Summary of Completed MART-1-based Melanoma Clinical Trials

Phase I MART-1_{27-35} pep/DC:

- 10^5, 10^6, 10^7 DC/injection; routes: i.v. vs. i.d. (18 pt., stg. III-IV)
- 13/16 immune responses by MHC tetramer; and 13/15 by IFNγ ELISPOT
- 10 pt. w/disease: 2 SD (4, 12 mo.), 1 CR
- 8 pt. NED: 5/8 remained NED (18+ to 27+ mo.)

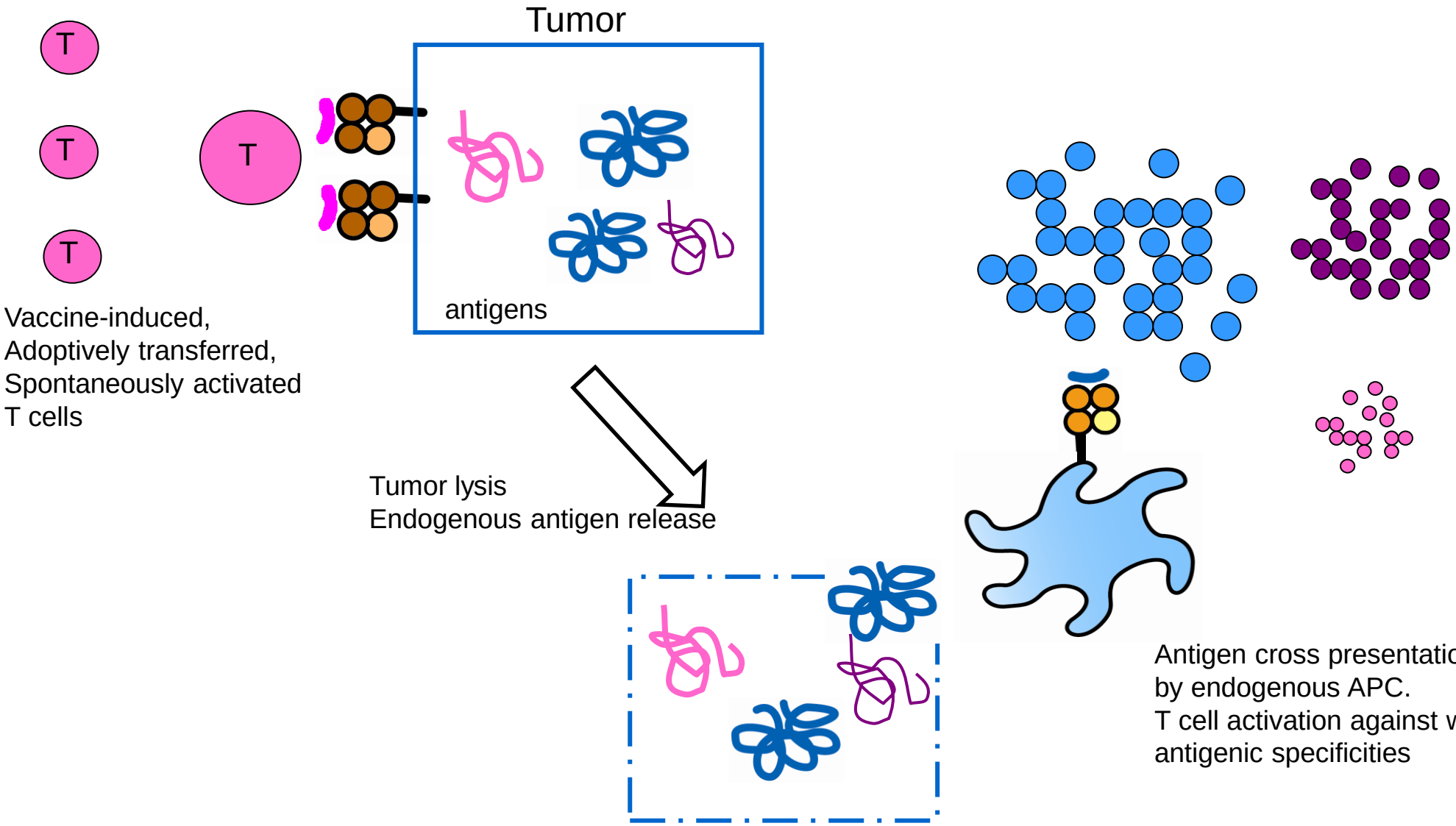
Phase II MART-1_{27-35} pep/DC:

- 10^7 DC/injection, i.d. (10 pt., stg. II-IV)
- 9/10 MART-1 immune responses by MHC tetramer and/or IFNγ ELISPOT
- 5 pt. w/disease: 1 MR, 1 SD (6 mo.), 1 CR (+ ipi).
- 4/5 NED remained NED (20+ to 27+ mo.)

AdVMART1/DC:

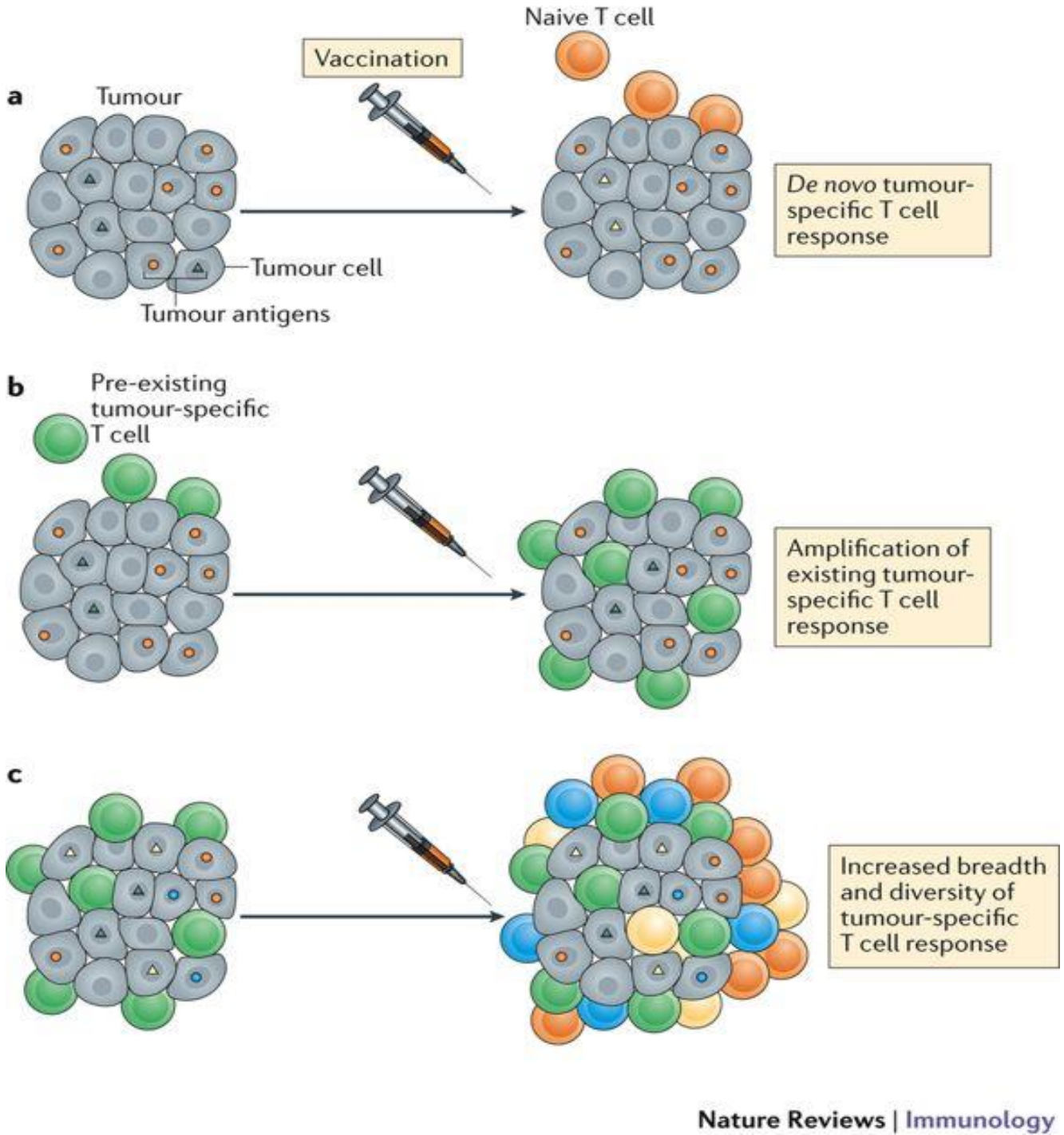
- 3/02-3/04 (23 enrolled); 14 received all 3 vaccines (all metastatic)
- 12/13 MART-1 immune responses by IFNγ ELISPOT; 9/14 MHC Tetramer+
- 1 “unevaluable” (54+ mo.),
- 4 SD (27, 33, 36, 42 mo.), 1 became resectable/NED (56+ mo.)

Determinant/Epitope/Antigen Spreading



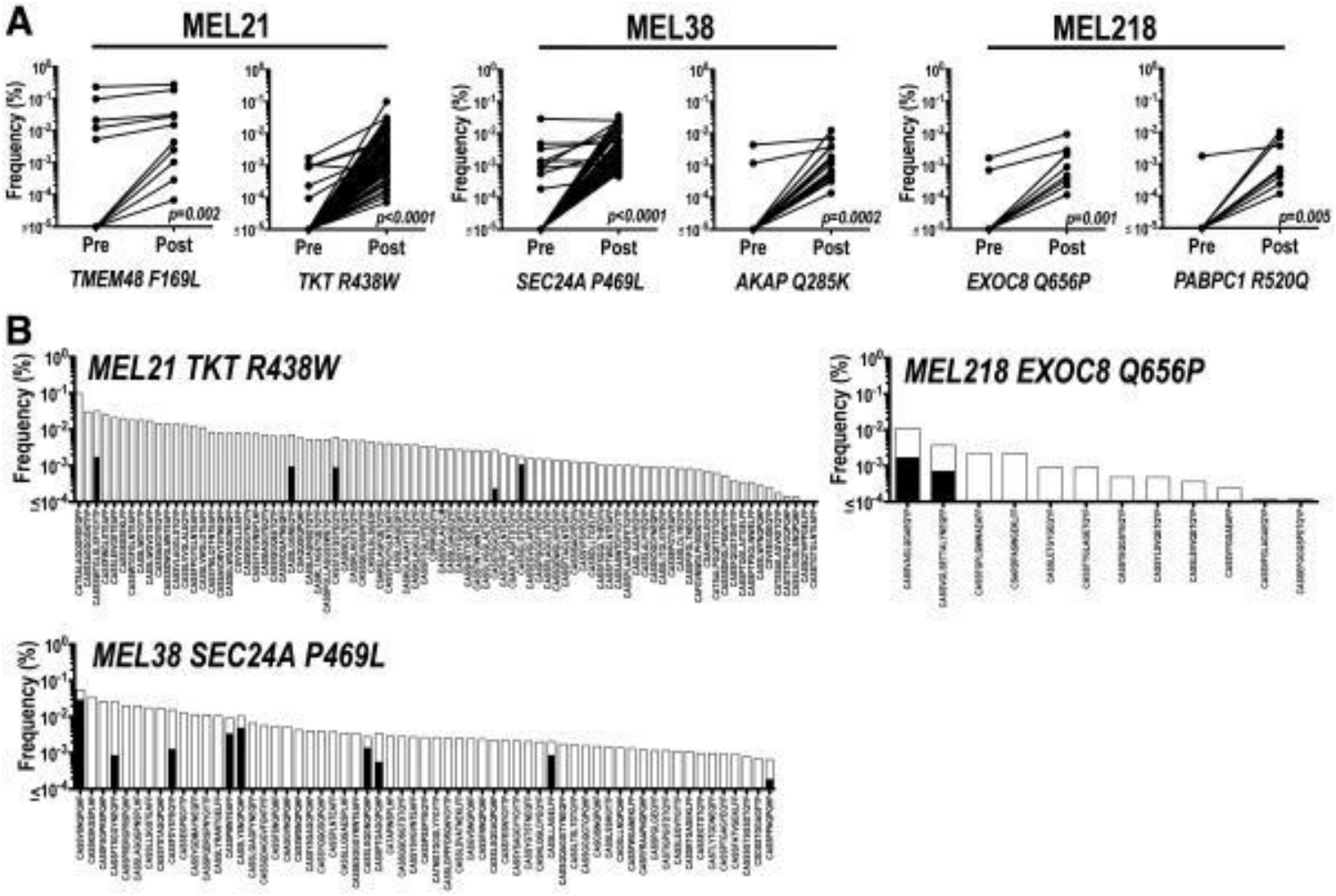
Ranieri ‘00; Disis ’02; Butterfield ‘03; Ribas ‘04; Wierecky ’06, Butterfield ’08

What have vaccines been shown to do?



Z. Hu, P. Ott, C. Wu *Nat Rev Immunol 2018*

Vaccination promotes a diverse neoantigen-specific T cell repertoire.
Summary of TCRβ clonotypes identified, using neoantigen-specific TCRβ CDR3 reference libraries in CD8+ T cell populations isolated from PBMC obtained before and after vaccination.



More diversity in the blood = better outcome
Expansion of good clones in the tumor = better outcome

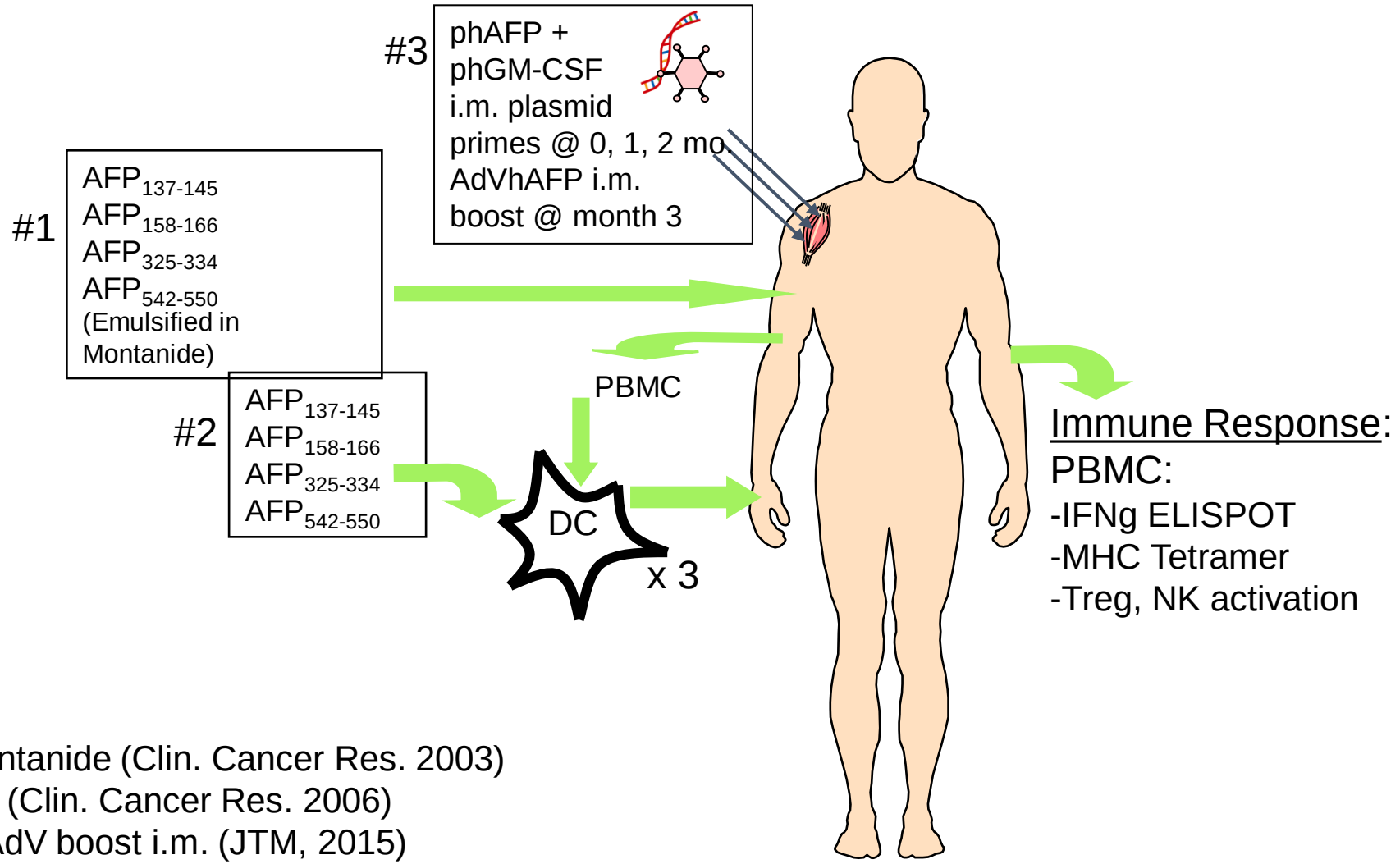
Science. 2015 May 15 Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells.
Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, Ly A, Lie WR, Hildebrand WH, Mardis ER, Linette GP

The antigen matters: Alpha Fetoprotein (AFP)

1. 1.8 kb cDNA, 15 exons/14 introns over 22 kb of genomic DNA, chromosome 4, 18aa leader sequence for secretion.
2. Transcriptionally regulated, cell-type specific promoter and enhancer, silencers utilized after birth.
3. 609 aa glycoprotein (591aa mature size), synthesized in fetal liver and yolk sac, major serum protein before birth.
4. **Possible roles in serum component transport (esp. fatty acids), binds hormones including estrogen, possible breast cancer prevention role, binds TNFα, possible immunoregulatory role.**
5. Serum levels in fetus: maximum at 10-13 weeks (3 mg/ml), decreases to 30-100 ug/ml at birth, adult levels 1-3 ng/ml.
6. 50% to 80% HCC express AFP (serum AFP up to 1 mg/ml).
7. 14 HLA-A2.1-restricted peptides were characterized (4 immuno-dominant, 10 sub-dominant) and the 4 immunodominant were found to be immunogenic *in vivo*, in HCC pt. with high serum AFP.

(Cancer Res. ’99, Molec. Immunol. ’00, J. Immunol. ’01, Clin. Cancer Res. ’03)

AFP Based Immunotherapy Clinical Trials for HCC



Trials

1. Peptides/Montanide (Clin. Cancer Res. 2003)
2. Peptides/DC (Clin. Cancer Res. 2006)
3. DNA prime/AdV boost i.m. (JTM, 2015)

Summary of Completed AFP-based Clinical Trials

AFP peptides/Montanide:

6 patients, Stage IVa, IVb,
Four AFP peptides in Montanide ISA adjuvant
100 ug, 500 ug each peptide, 3 intradermal injections (skin toxicity only)
6/6 immune responses by MHC tetramer and/or IFNγ ELISPOT
No objective clinical responses or AFP decreases, OS = 2-17 months

AFP peptides/DC:

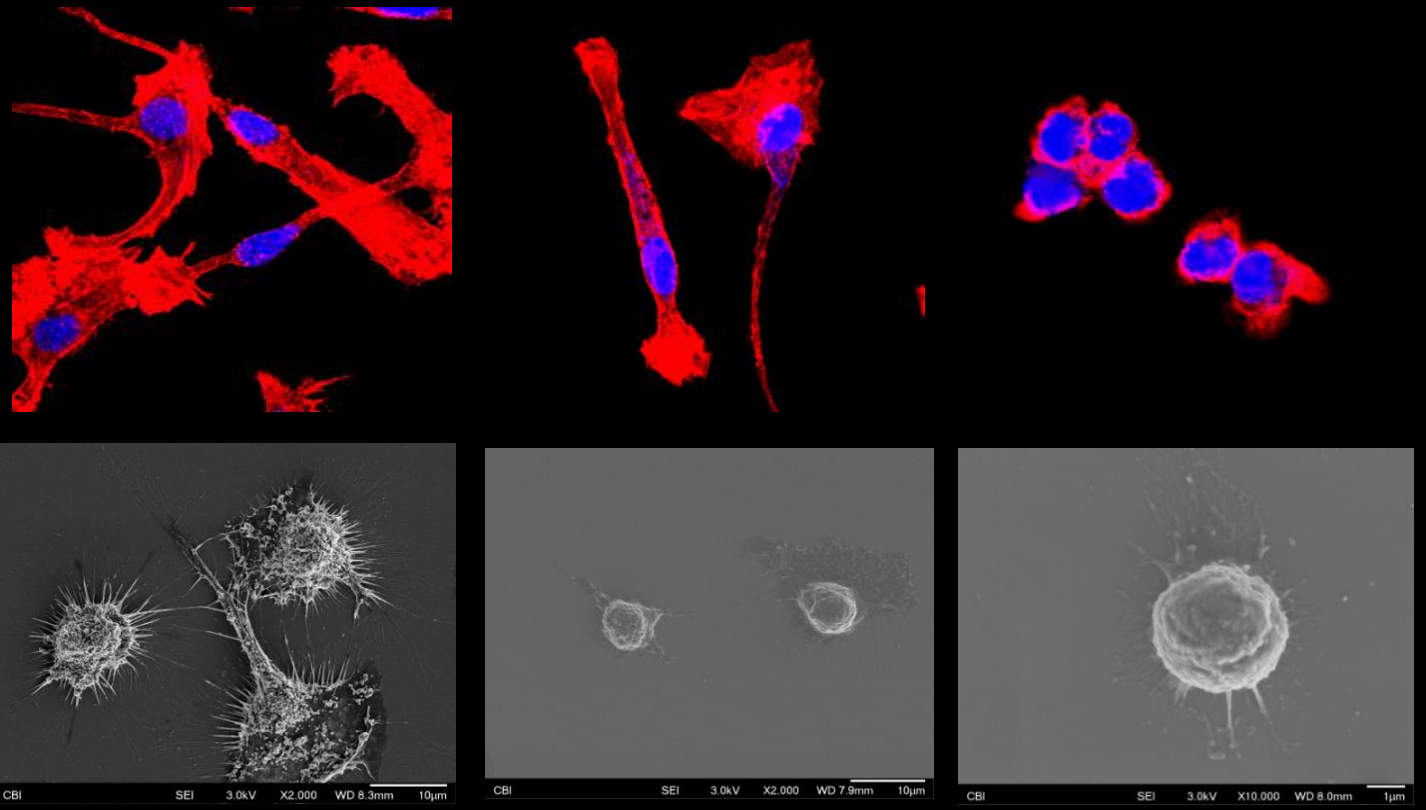
10 patients, stage III-IVb
Four AFP peptides pulsed onto autologous GM-CSF/IL-4 DC
3 injections, intradermal, no toxicities
8/10 immune responses by MHC tetramer and/or IFN γ ELISPOT
No objective clinical responses, 2 serum AFP decreases, OS = 2-35 months

AFP DNA prime/AFPAdV boost:

2 patients, stage II
AFP + GM-CSF plasmids x 3, then AdVhAFP x 1; monthly i.m.
Pt. #1 Minimal AFP-specific T cell immunity and low anti-AdV neutralizing antibodies.
9 mo. AFP positive recurrence.
Pt. #2 *Strong* AFP-specific T cell immunity and + anti-AdV neutralizing antibodies.
18 mo. AFP-negative suspected recurrence.

Monocytes cultured +/- normal AFP or tumor-derived AFP during DC culture: antigen matters

OVA nAFP tAFP

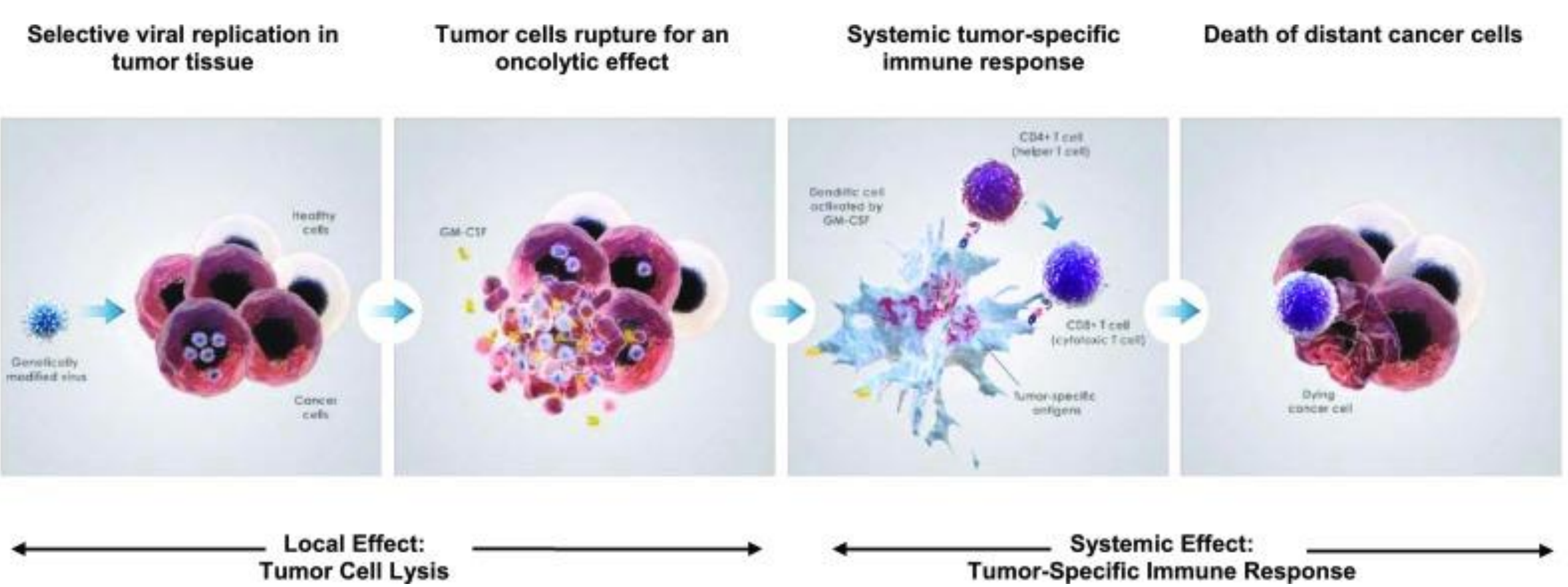


AFP alters DC phenotype to an immature phenotype that cannot be reversed by maturation, AFP inhibits DC metabolic function and T cell stimulatory capability (Pardee 2014, Santos 2019)

Other effective platforms: Synthetic and Viral Vaccines

1. TVEC (Amgen) ***FDA approved 2015**
   - Oncolytic virus: HSV-1 + GM-CSF transgene
   - Metastatic melanoma, 26% response rate (vs. 6% in control arm)
2. ISA101 (Immune System Activation)
   - HPV16 Synthetic long peptide (SLP, 24-32mer) in Montanide
   - Cervical cancer
   - Appears to synergize with cisplatin chemotherapy
3. STINGVAX (Aduro)
   - Cyclic dinucleotides (CDN) are recognized by Stimulator of Interferon Genes (STING): TLR-like mechanism
   - STINGVAX = CDN with a GM-CSF secreting tumor cell vaccine
4. Prostvac
   - Vaccinia (prime) and fowlpox (boost) viruses encoding PSA and three costimulatory molecules
   - Overall survival in advanced prostate cancer increased by 9 months

Presented at SITC annual meeting 2013



Talimogene laherparepvec proposed mechanism of action. *CMV* cytomegalovirus, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *hGM-CSF* human GM-CSF, *pA* poly-adenosine, *TDA* tumor-derived antigen

Cancer Immunol Immunother. 2017; 66(10): 1249–1264.

Oncolytic Viruses

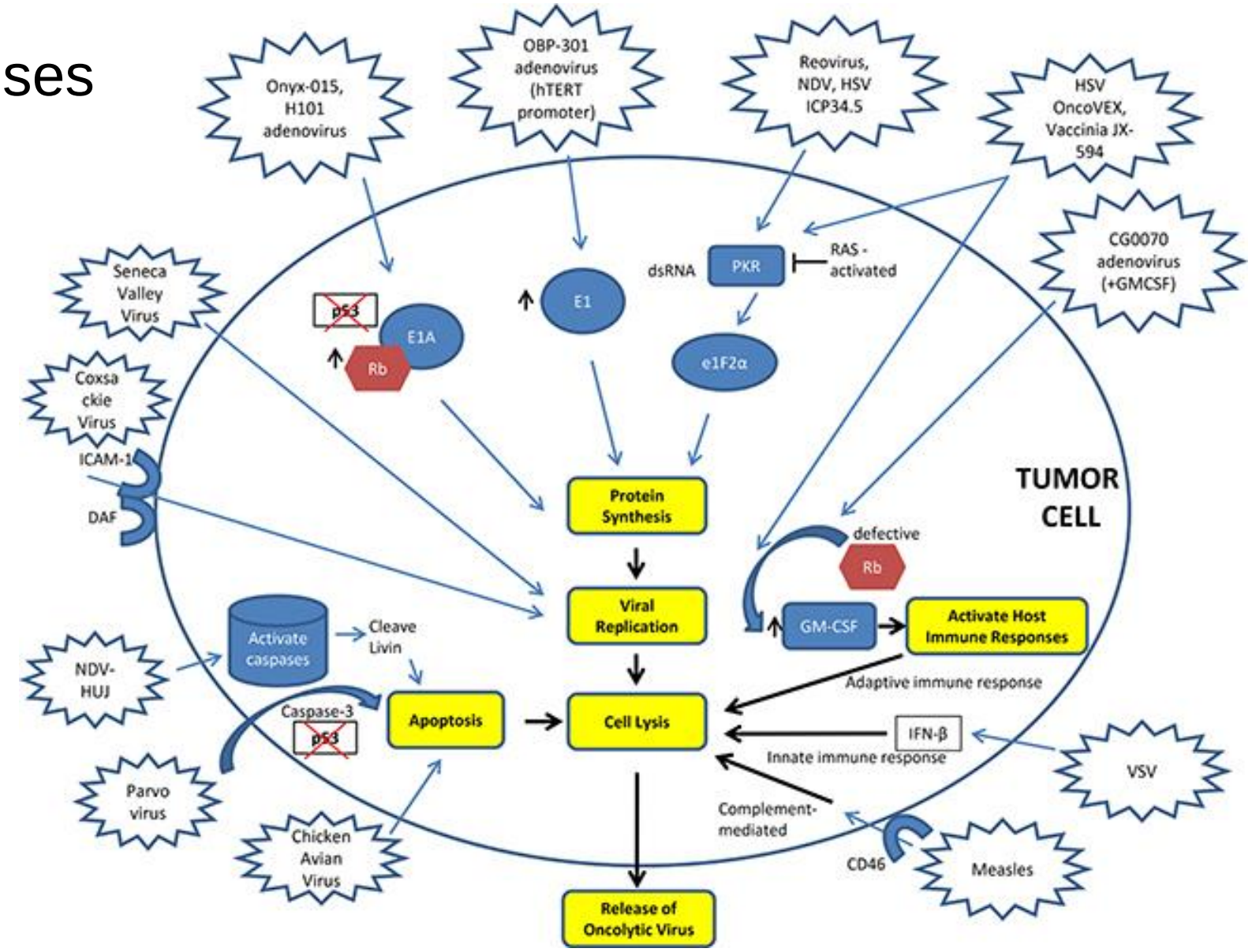


Figure 1: Mechanisms of action of oncolytic viruses. DAF – Decay Accelerating Factor, GM-CSF – Granulocyte Macrophage-Colony Stimulating Factor, HSV – Herpes Simplex Virus, hTERT – Human Telomerase, ICAM-1 – Intercellular Adhesion Molecule-1, ICP – Infectious Cell Protein, INF-β – Interferon beta, NDV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

Tumor Mutations

Malignant transformation of cells depends on accumulation of DNA damage.

The immune system frequently responds to the neoantigens that arise as a consequence of this DNA damage.

Recognition of neoantigens appears an important driver of the clinical activity of both T cell checkpoint blockade and adoptive T cell therapy as cancer immunotherapies.

Neoantigens can be targeted by therapeutic vaccines

Published in final edited form as:
Science. 2015 May 15; 348(6236): 803–808. doi:10.1126/science.aaa3828.

A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells

Beatriz M. Carreno[1,*], **Vincent Magrini**[2], **Michelle Becker-Hapak**[1], **Saghar Kaabinejadian**[3], **Jasreet Hundal**[ ], **R. Mardis**[2], and

LETTER

doi:10.1038/nature23003

Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer

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LETTER

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An immunogenic personal neoantigen vaccine for patients with melanoma

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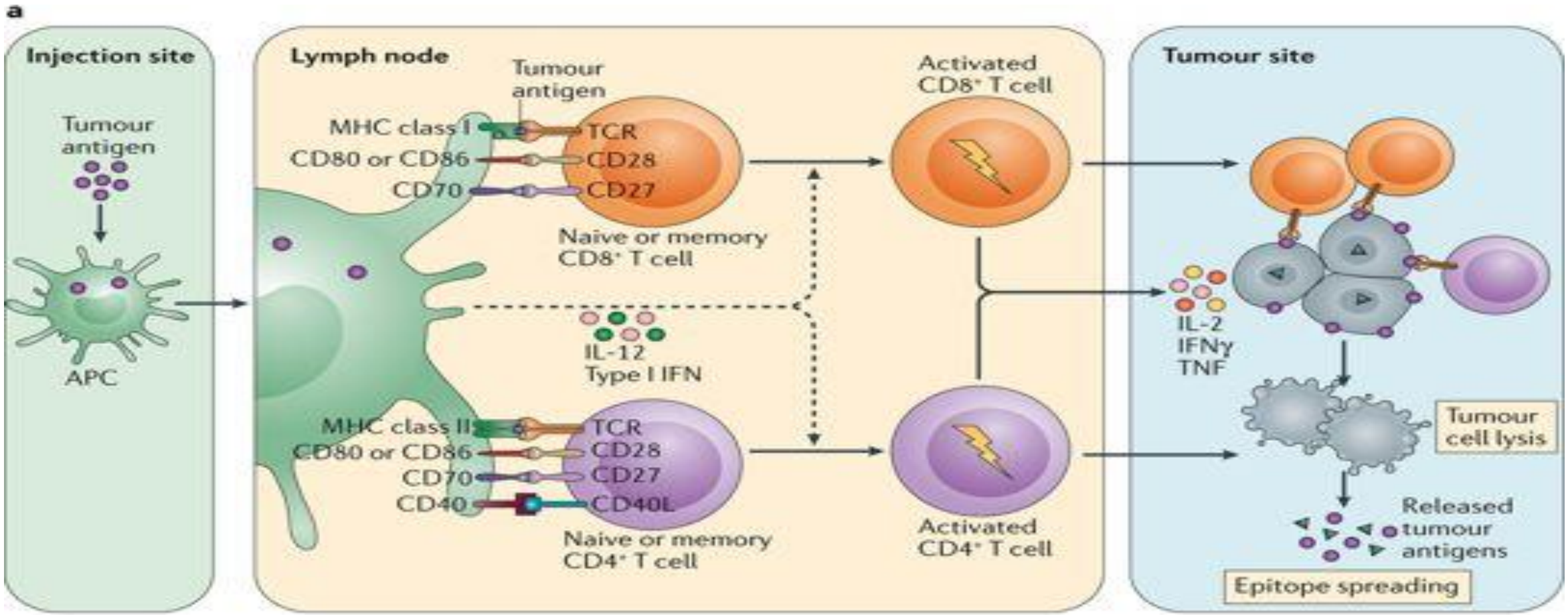
•Neoantigens have emerged as targets of effective tumor-directed T cell responses. Increased neoantigen load is associated with improved patient outcomes.

•**Three clinical trials** of neoantigen-based vaccines in patients with melanoma, using dendritic cells loaded with short peptides, long peptides or RNA, have shown the **safety, feasibility and robust immunogenicity** of this approach.

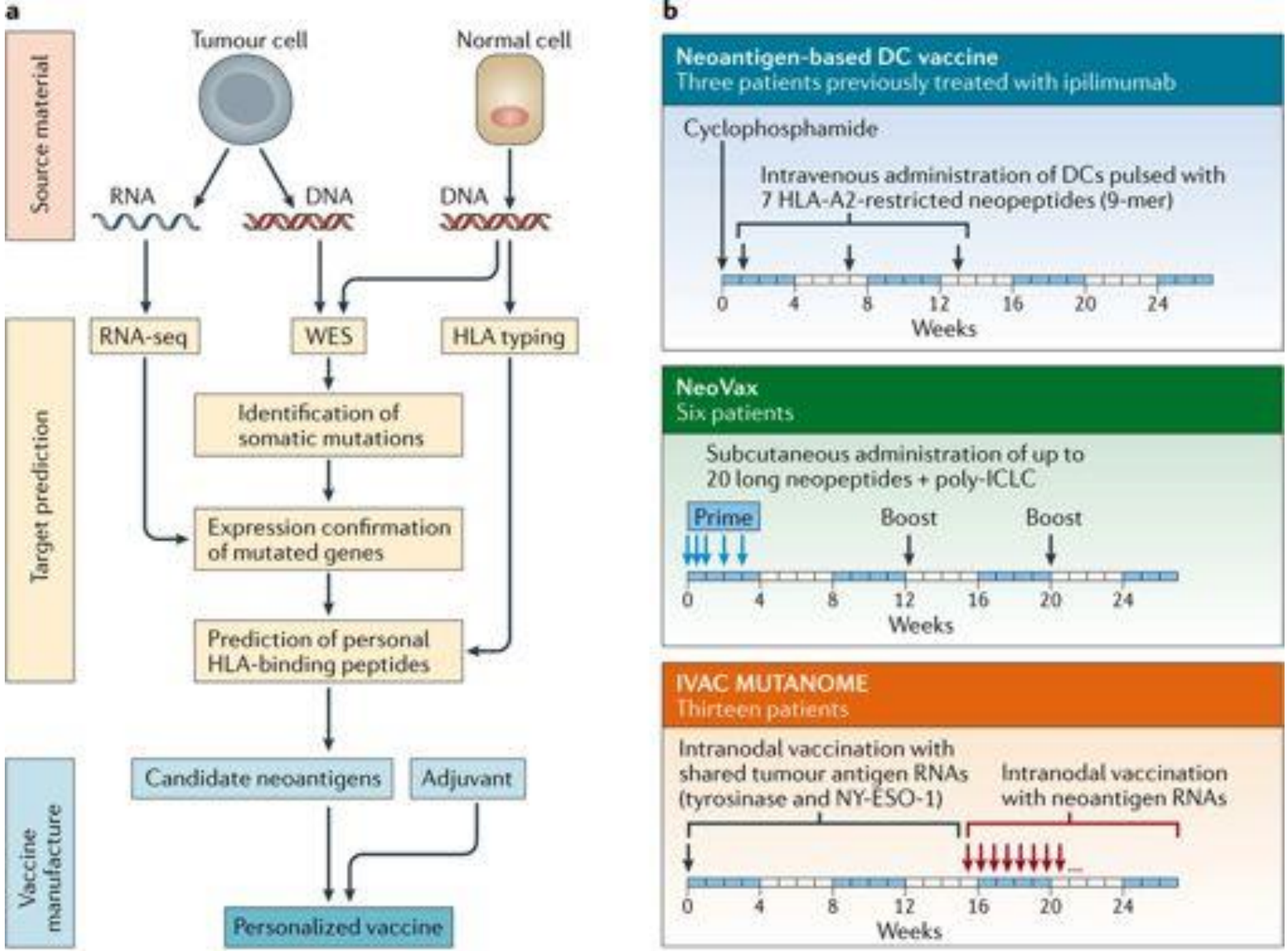
•A crucial aspect of a vaccine targeting neoantigens is the selection of epitopes that can be presented *in vivo* by tumor or antigen-presenting cells. HLA-binding prediction, high-resolution mass spectrometry and understanding of antigen processing are important research areas for further discovery.

•Optimal neoantigen delivery — use of the most effective formulations, immune adjuvants, delivery vehicles and dosing — in combination with complementary therapies will be crucial for maximum therapeutic effectiveness.

Towards personalized, tumour-specific, therapeutic vaccines for cancer, Z. Hu, P. Ott, C. Wu *Nat Rev Immunol 2018*

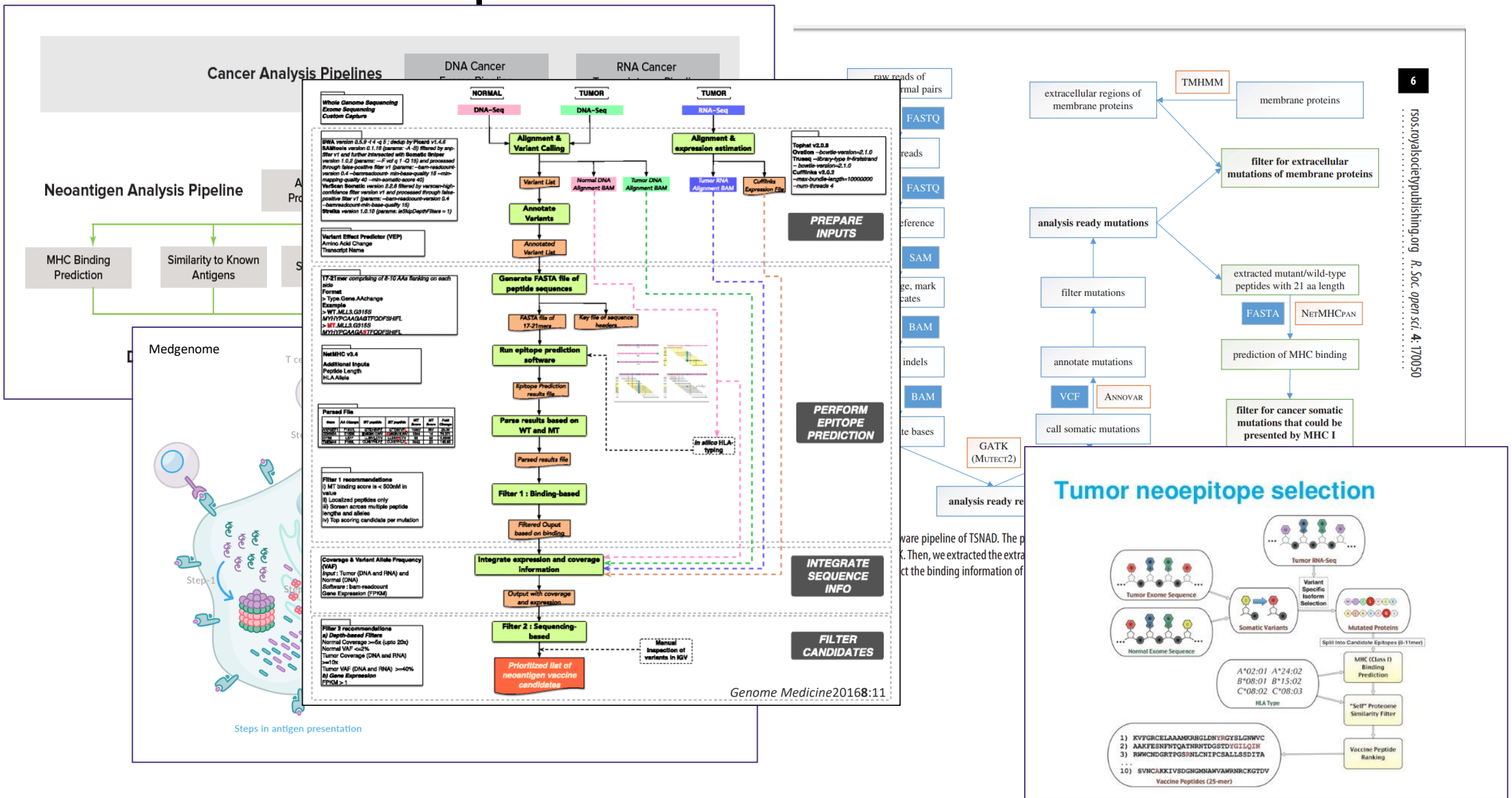


Z. Hu, P. Ott, C. Wu *Nat Rev Immunol 2018*



Z. Hu, P. Ott, C. Wu *Nat Rev Immunol 2018*

Neoepitope pipelines are becoming more common, diverse and complex



TESLA Publication

Cell

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Resource

Key Parameters of Tumor Epitope Immunogenicity Revealed Through a Consortium Approach Improve Neoantigen Prediction

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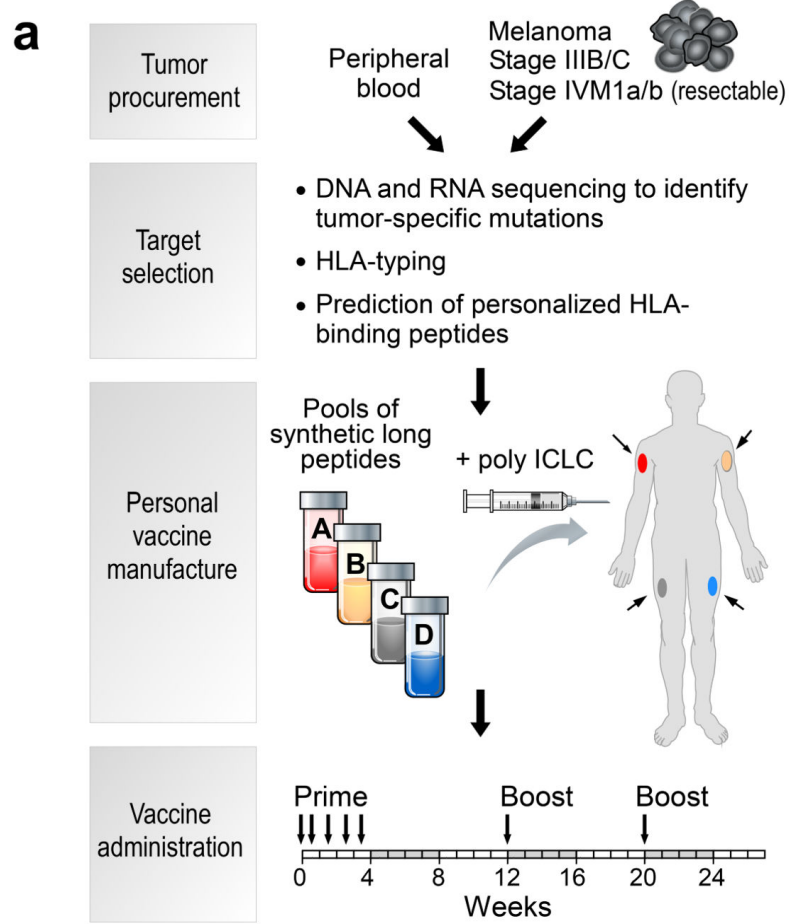
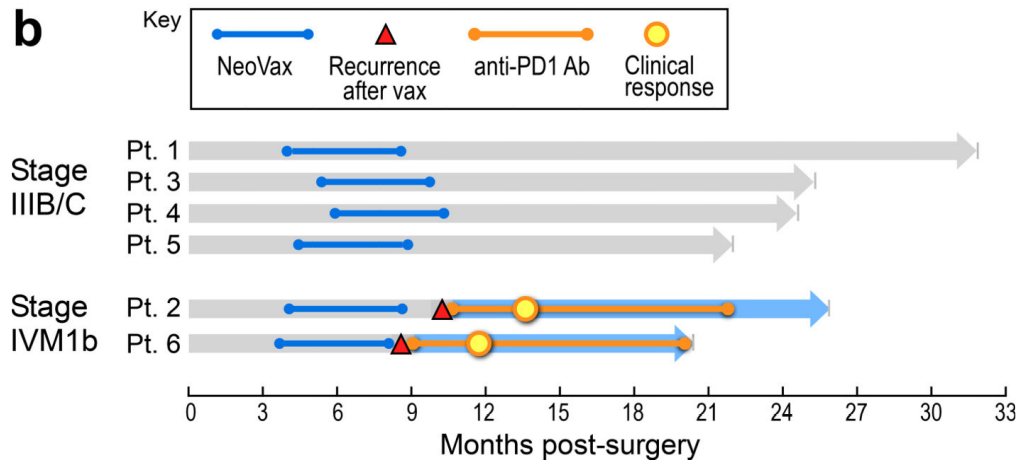
TESLA Conclusions

- Largest ever immunogenomic resource of patient tumor sequencing with matched MHC I tumor epitope validation.
  - Data resource in active use in academia and industry to improve prediction.

- 5 traits determine epitope immunogenicity in an integrated model.
  - Peptides that have strong MHC binding affinity and long half-life, are expressed highly, and have either low agretopicity or high foreignness.





Generation of a personal, multi-peptide neoantigen vaccine for patients with high-risk melanoma

A. Somatic mutations were identified by WES of melanoma and germline DNA and their expression confirmed by tumor RNA-sequencing. Immunizing peptides were selected based on HLA binding predictions. Each patient received up to 20 long peptides in 4 pools.

B. Clinical event timeline for 6 vaccinated patients from surgery until time of data cutoff (36 months from study initiation).

P.A.Ott, …C. J. Wu, An Immunogenic Personal Neoantigen Vaccine for Melanoma Patients, Nature 2017

How can we improve?

Greater success from new formulations

FixVac (BNT111)-an intravenously administered liposomal **RNA vaccine**, which targets four non-mutated, tumour-associated antigens that are prevalent in melanoma (NY-ESO-1, Tyrosinase, MAGE-A3, TPTE).

…melanoma FixVac, alone or in combination with blockade of the checkpoint inhibitor PD1, mediates durable objective responses in checkpoint-inhibitor experienced patients with unresectable melanoma (vaccine alone: 3 PR/7 SD/25; + vaccine +aPD-1: 6/17 PR)

Clinical responses are accompanied by the induction of strong $CD4^+$ and $CD8^+$ T cell immunity against the vaccine antigens.

The antigen-specific cytotoxic T-cell responses in some responders reach magnitudes typically reported for adoptive T-cell therapy and are durable. Sahin et al., Nature, 3 Sept.2020

Vaccine-induced T cell infiltration and neo-epitope-specific killing of autologous tumour cells were shown in post-vaccination resected metastases *(Sahin Nature 2017)*

Optimized RNA +	nanoparticulate	X8
4 Ag, Class I/II	liposomes	injections

Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial

- Using single-cell T cell receptor analysis, we provide evidence that ***neoantigen-specific T cells from the peripheral blood can migrate into an intracranial glioblastoma tumour***
- Neoantigen-targeting synthetic long peptide vaccines thus have the potential to favourably alter the immune milieu of glioblastoma
- GBM is a cold tumor, not highly mutated

- N=8, best responses in n=2 w/o dexamethasone

Keskin, …Wu, Reardon *Nature*, Jan 2019

The dawn of vaccines for cancer prevention

Olivera J. Finn, Ph.D., Univ. Pittsburgh

Nature Reviews Immunology **volume 18**, pages 183–194 (2018)

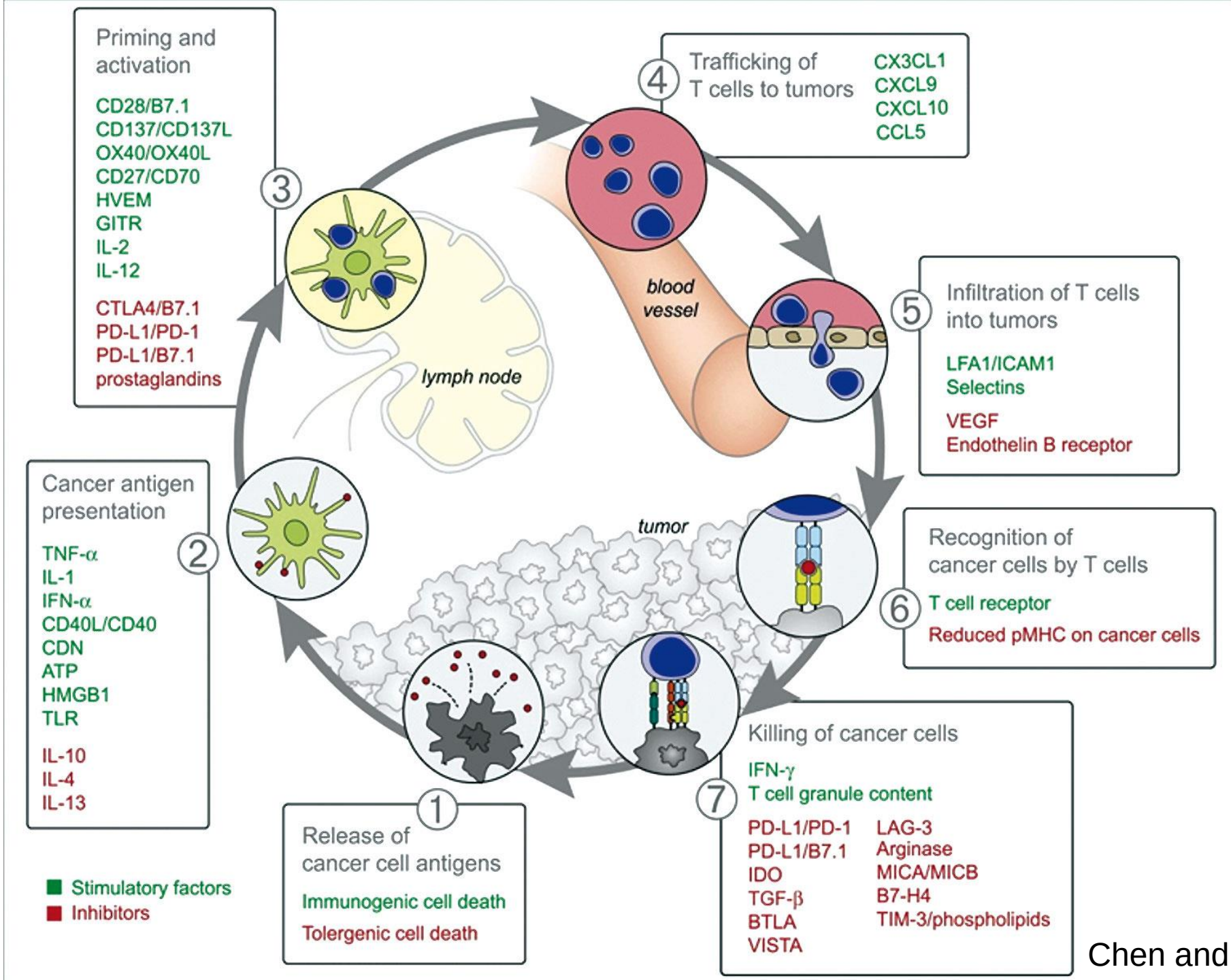
• Developments in imaging and other screening methods have made possible the detection of pre-malignant lesions.

•Therapeutic cancer vaccines based on viral antigens for the control of viral cancers have not shown effectiveness in advanced disease but have been highly effective at clearing pre-malignant lesions.

•Vaccines based on nonviral antigens might be similarly more effective against pre-malignant lesions of nonviral cancers, and the few completed or ongoing phase I and II clinical trials of preventive cancer vaccines have already shown clinical efficacy.

Can cancer vaccines work to eradicate established disease? Yes!

How can we do better than 0-10% RR?
Platform?
Antigen?
Dose?
Schedule?
Prevention?
Combination?



Chen and Mellman