# Can cancer vaccines really work? Vaccination Strategies and Identification of Neoantigens

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PARKER INSTITUTE FOR CANCER IMMUNOTHERAPY

# Disclosures:

StemImmune/Calidi Scientific and Medical Advisory Board, April 6, 2017-present
SapVax Advisory Board meetings Nov. 15, 2017; Dec. 6, 2018
NextCure, Scientific Advisory Board, 2018-present
Western Oncolytics, Scientific Advisory Board, 2018-present
Torque Therapeutics, Scientific Advisory Board, 2018-2020
Khloris, Scientific Advisory Board, 2019-present
Pyxis, Scientific Advisory Board, 2019-present
Cytomix, Scientific Advisory Board, 2019-present
Vir, Scientific Advisory Board meeting, Feb. 2020
DCprime, Scientific Advisory Board meeting, Nov. 2020
RAPT, Scientific Advisory Board, 2020-present

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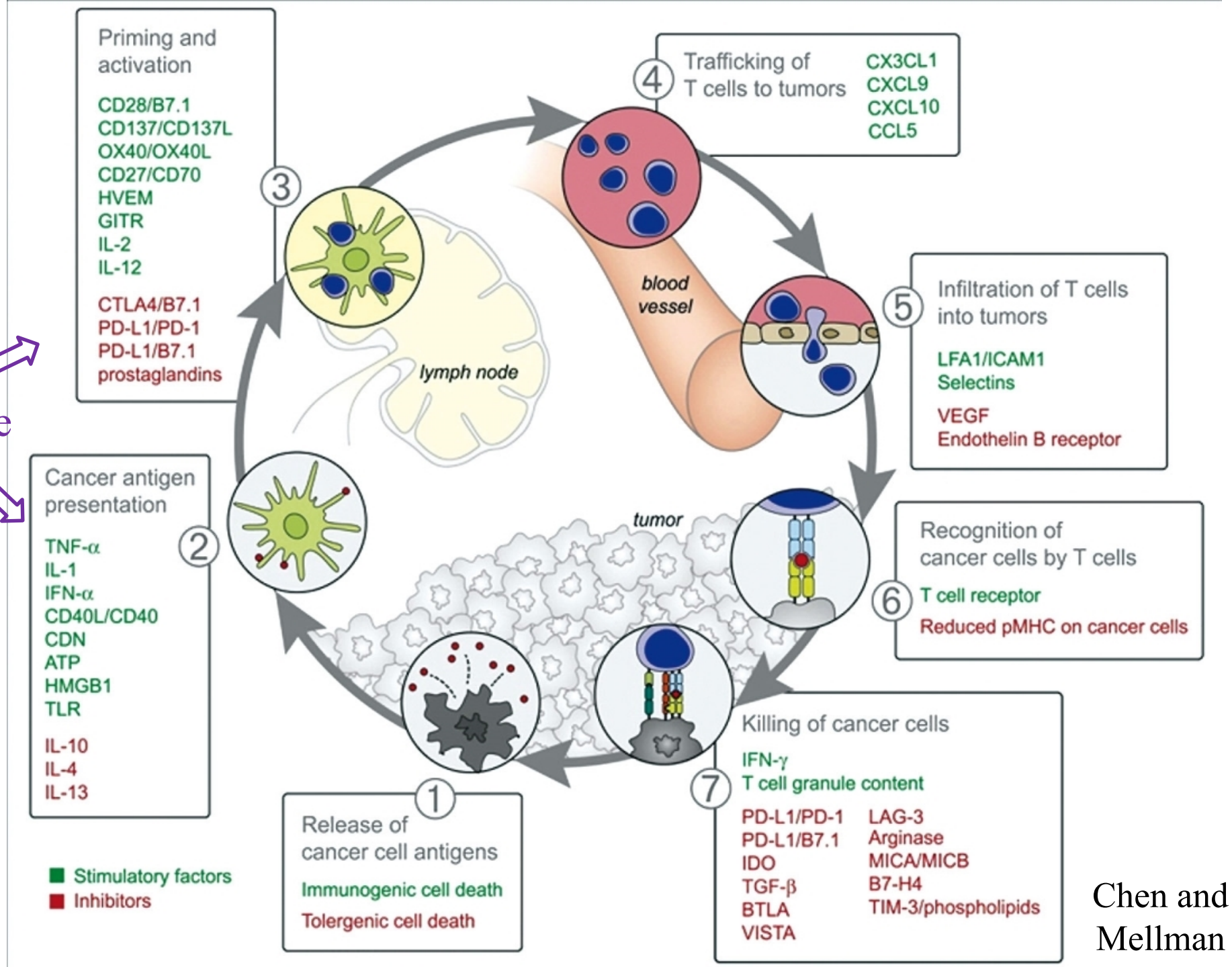



Chen and Mellman

# 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*

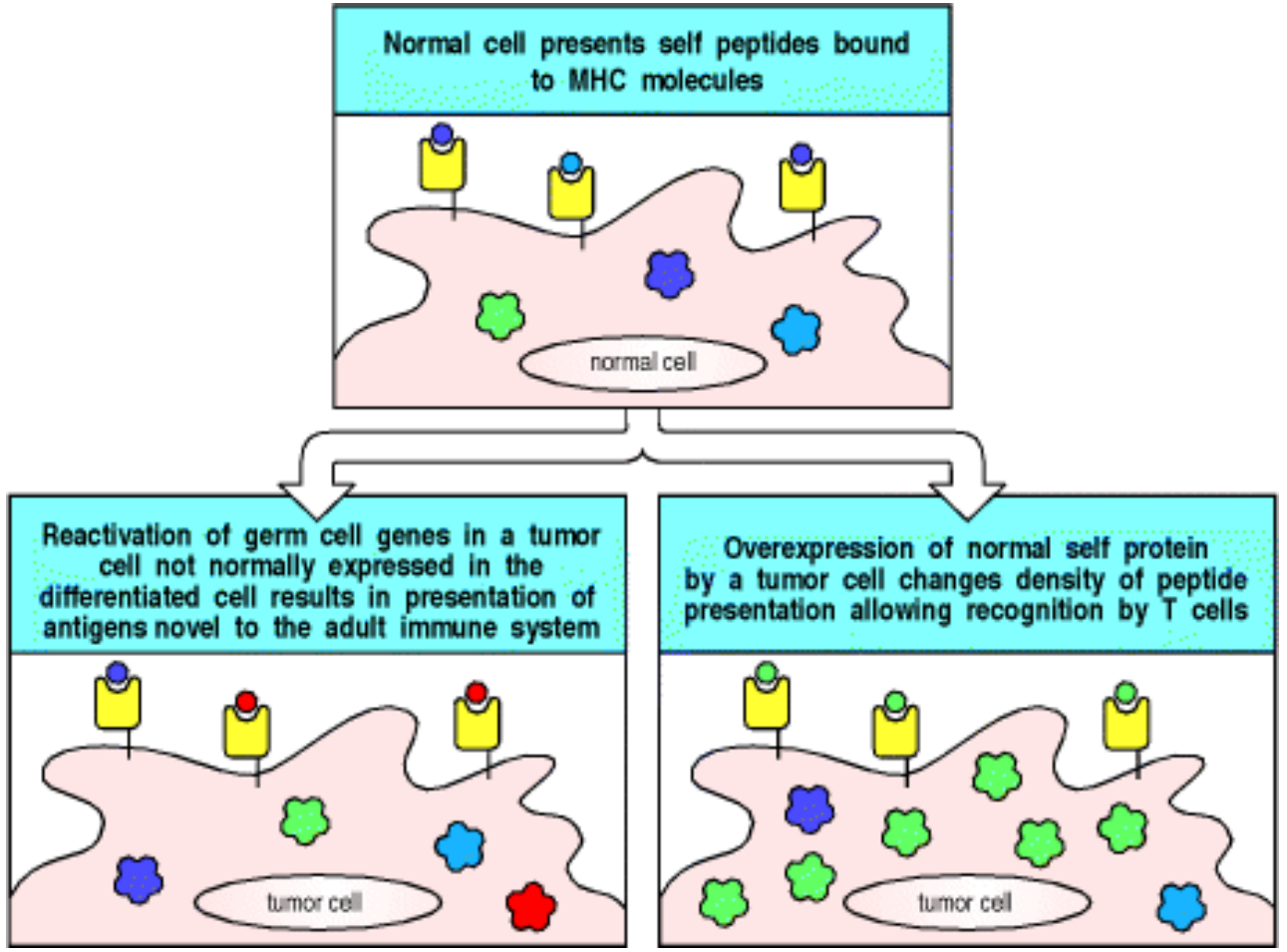
4) proteins derived from oncoviruses, like Human Papilloma Virus (HPV) proteins E6 and E7, HBV, HCV, MCPV

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

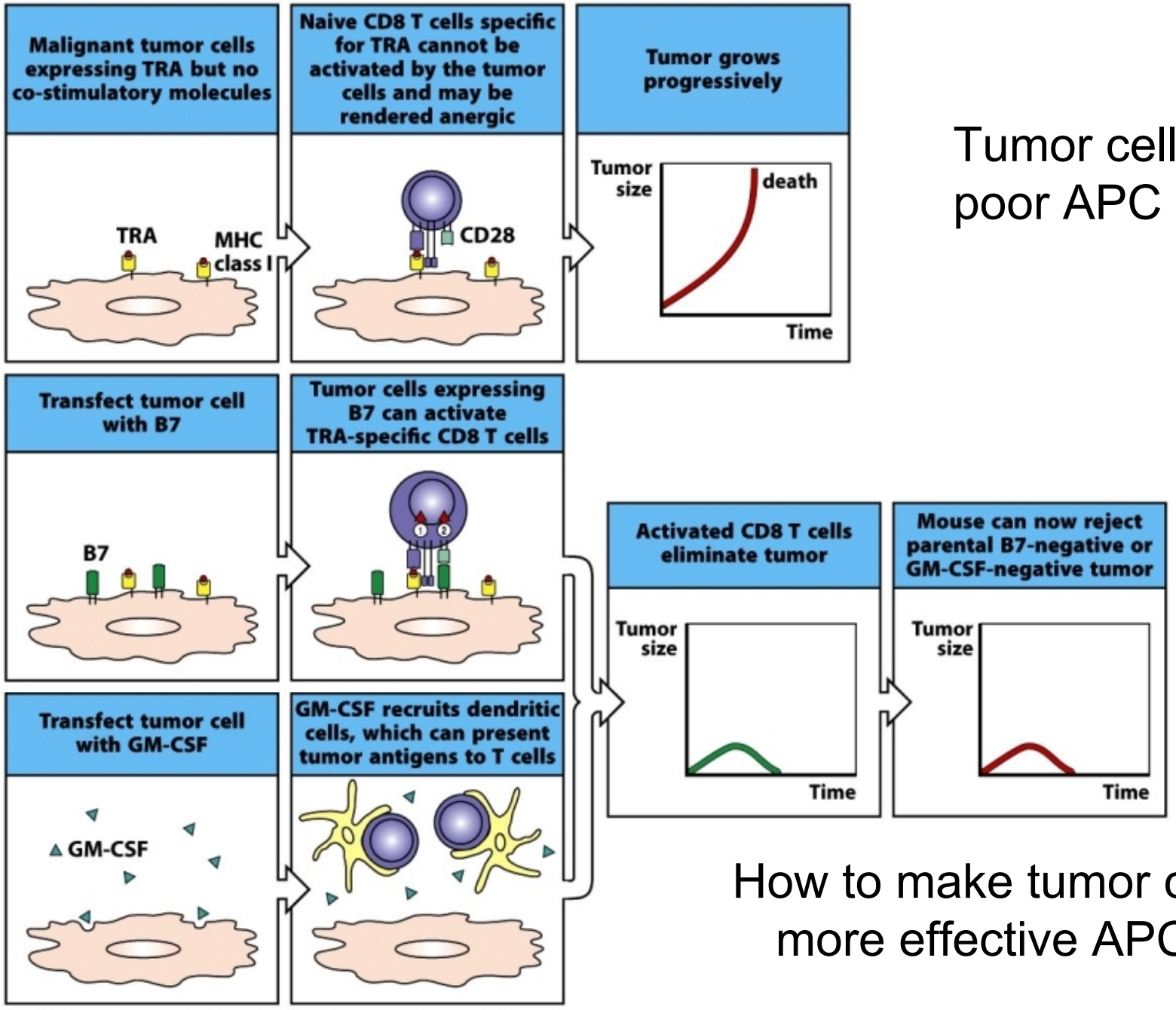
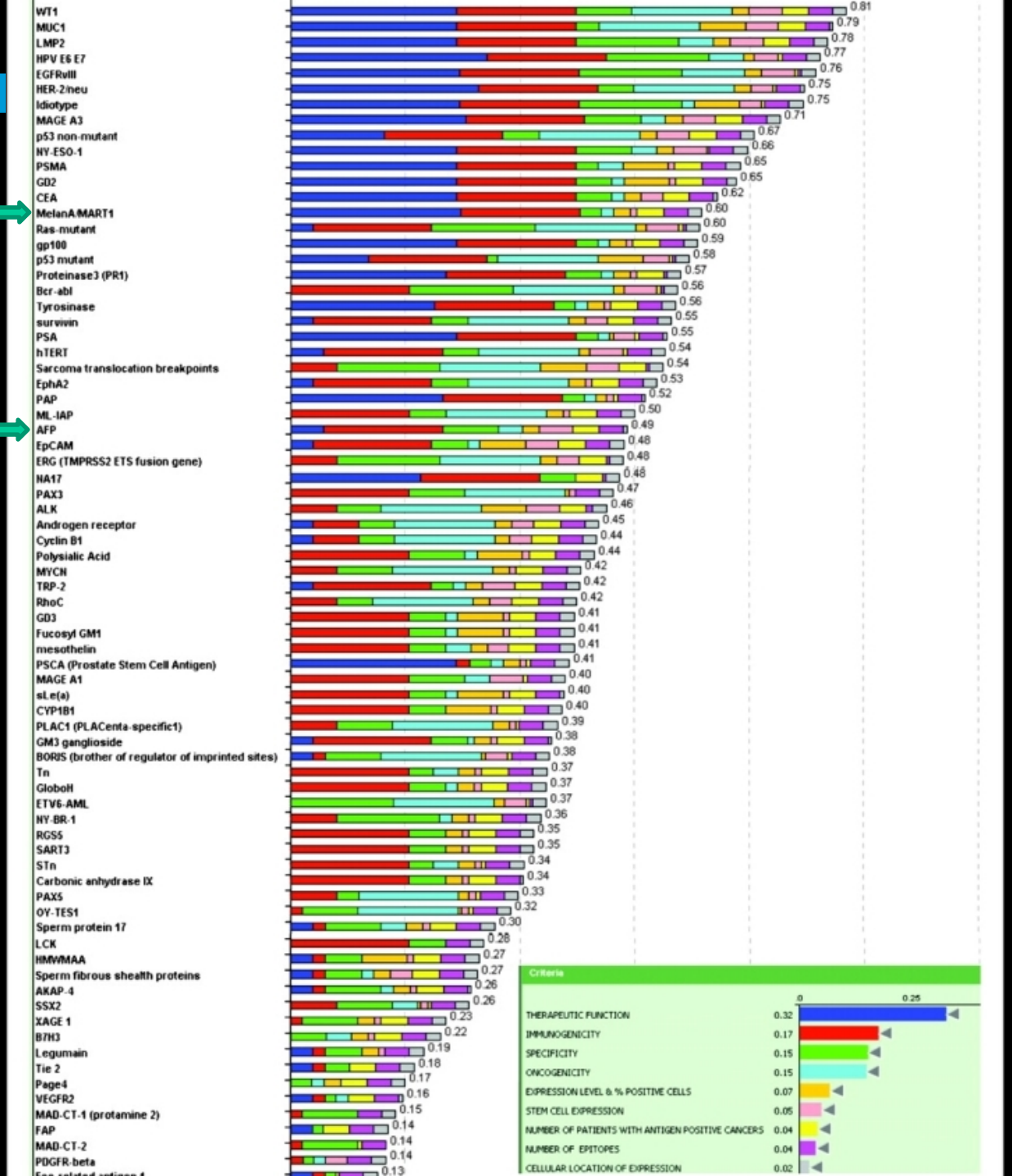



Figure 15-24 Immunobiology, 7ed. (© Garland Science 2008)

Tumor cells are poor APC

How to make tumor cells more effective APC?

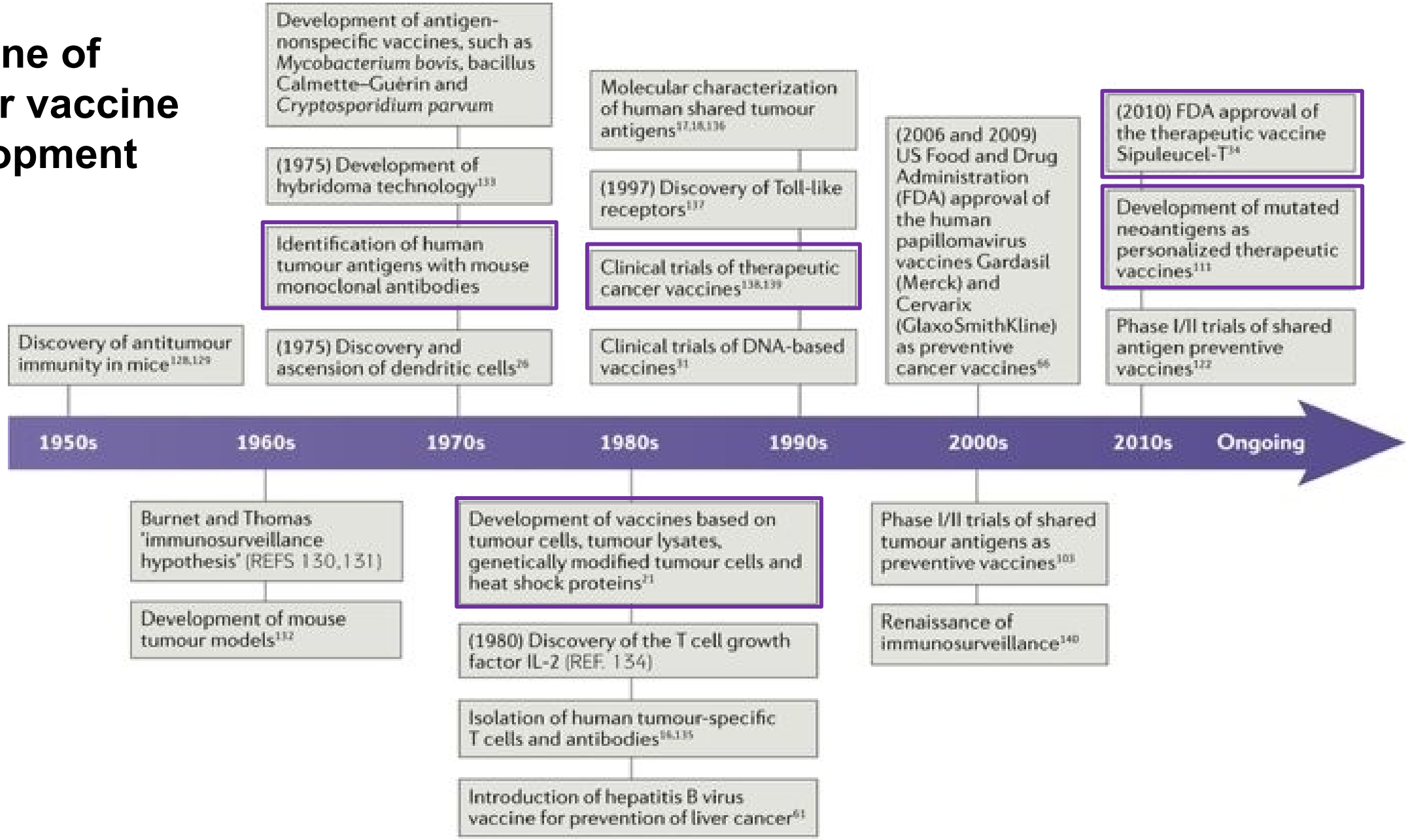



# The Prioritization of Cancer Antigens:

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

Cheever, CCR 2009

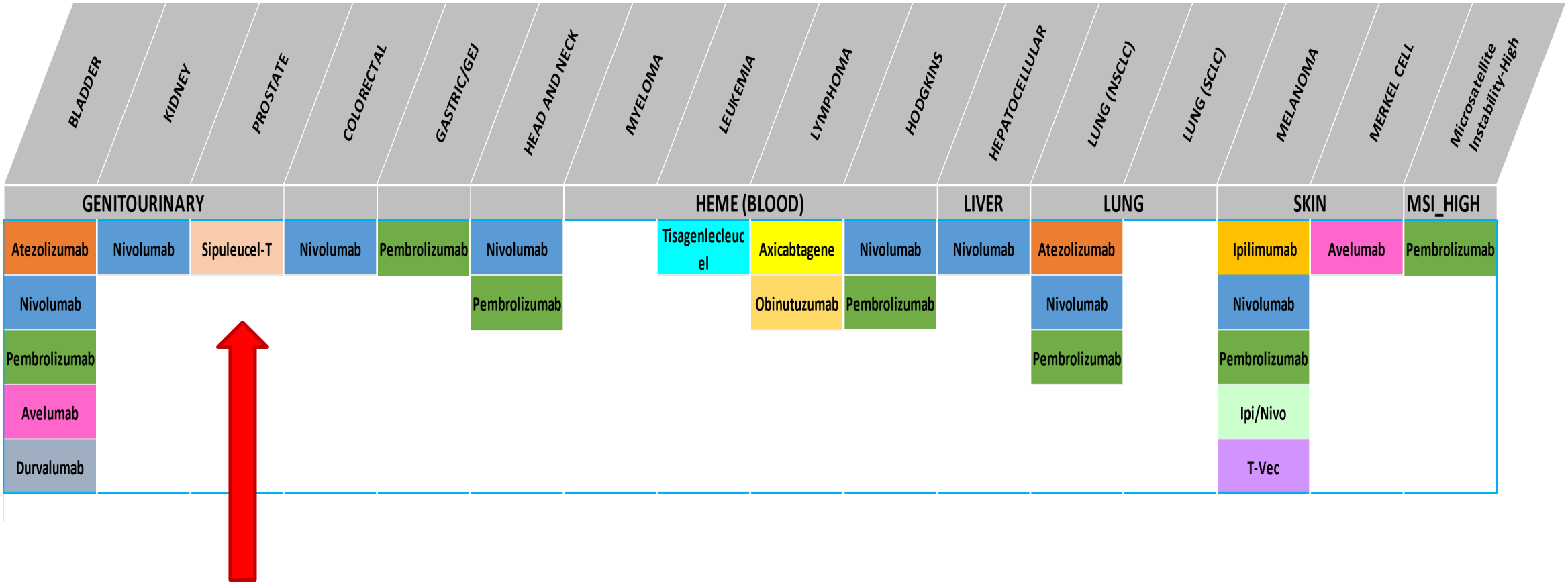
# Timeline of cancer vaccine development




O. Finn

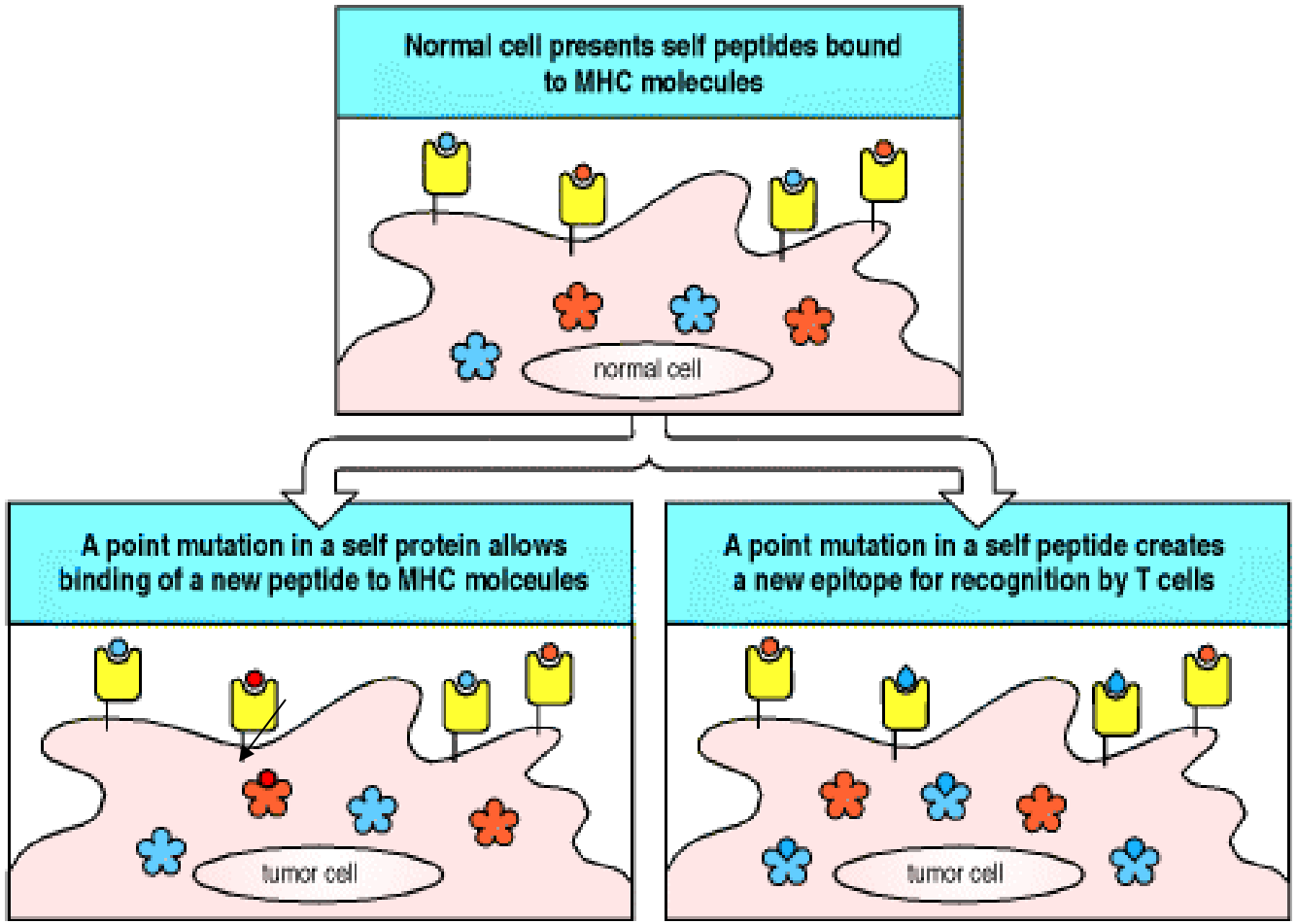
Nature Reviews | Immunology

# US Immunotherapy Approvals by tumor



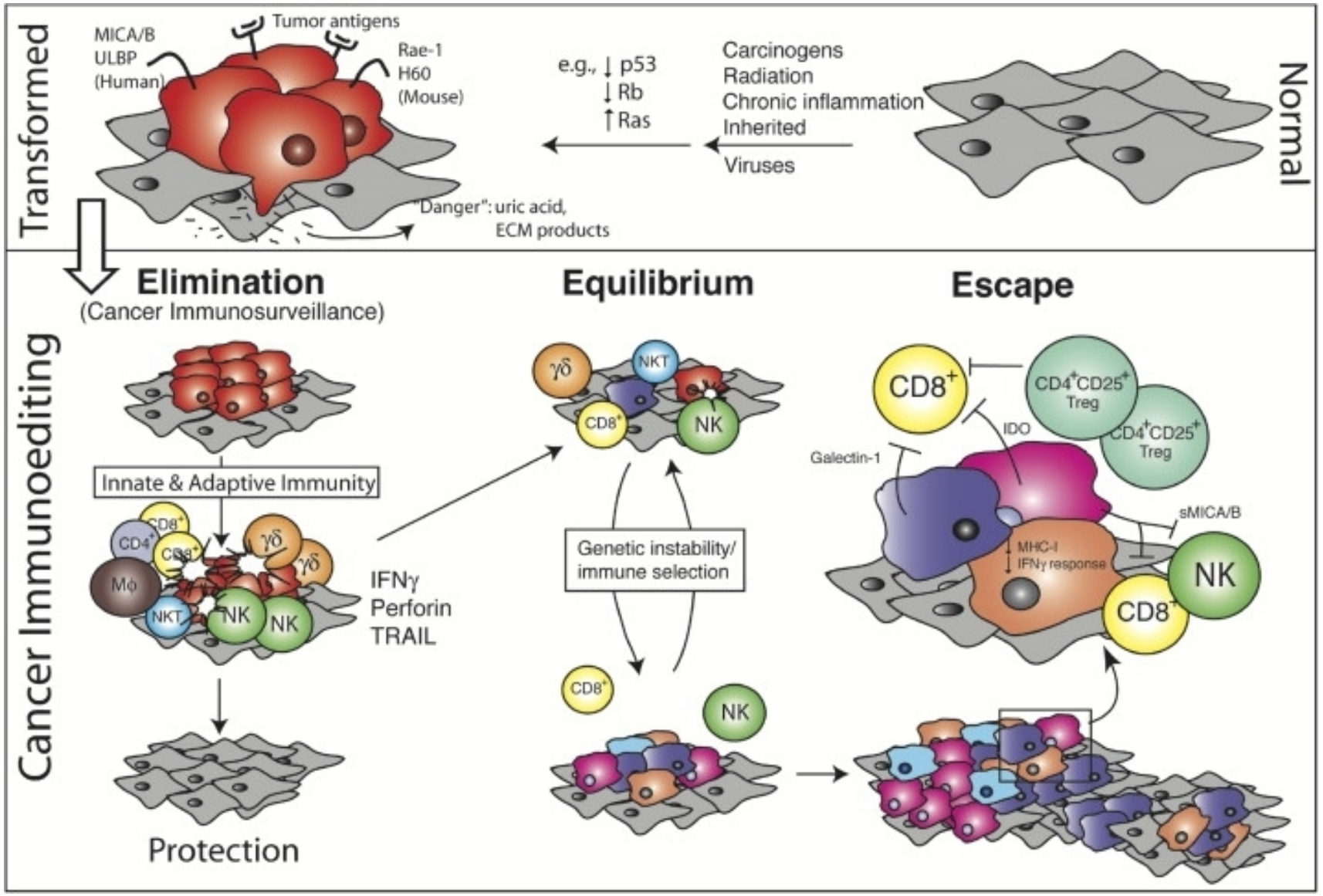

Cancer vaccine
2010

# Tumor Antigens
## “private” or patient-specific




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

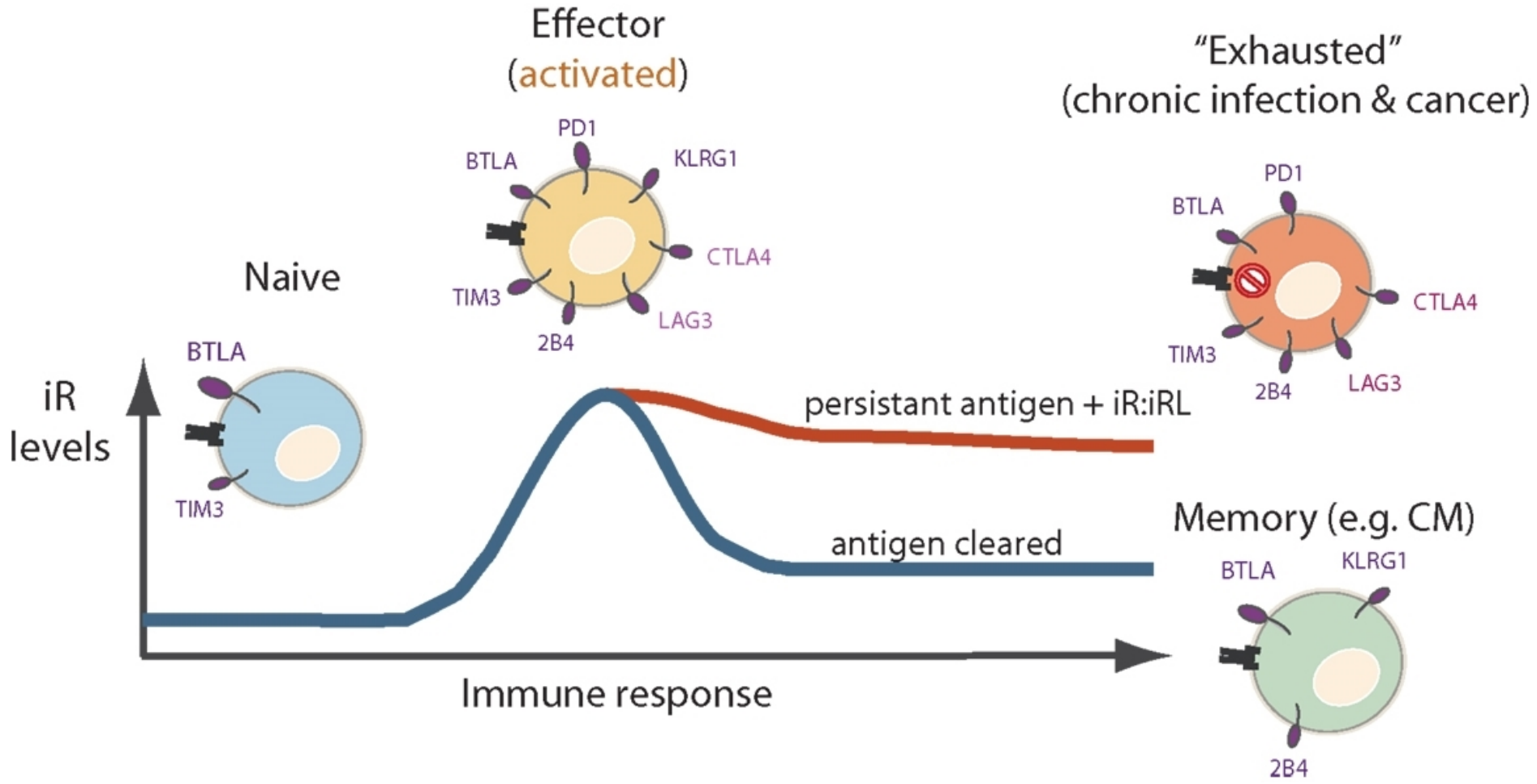
# Three Phases of the Cancer Immuno-editing




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

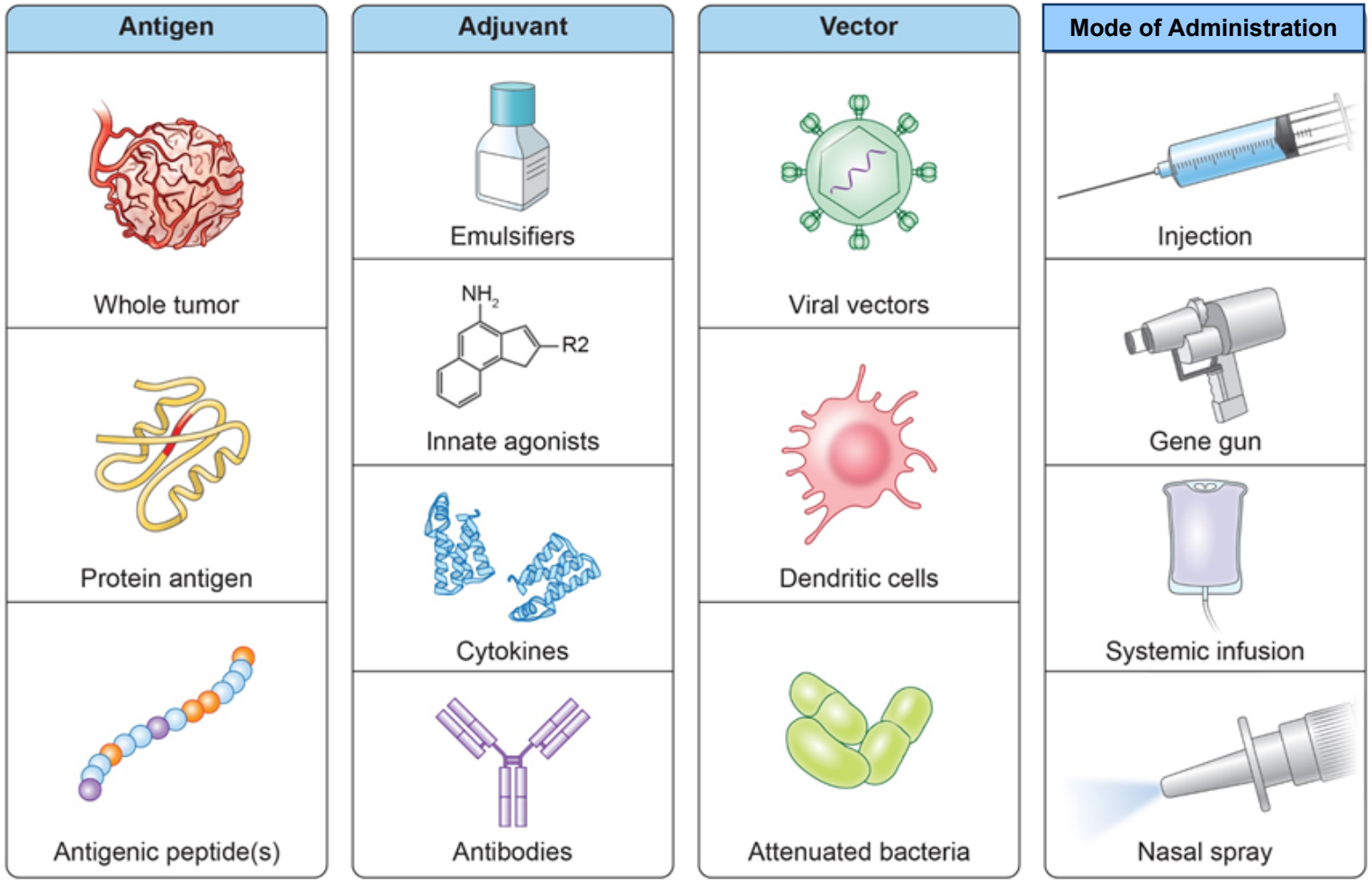



**T Cell Exhaustion**. Naïve cells express mainly BTLA and low levels of TIM3. Effector cells express a wider variety of **inhibitory receptors**. The levels of certain inhibitory receptors such as PD1, CTLA-4, LAG3, and TIM3 may peak at the effector phase. Thereafter, expression differs in chronically 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

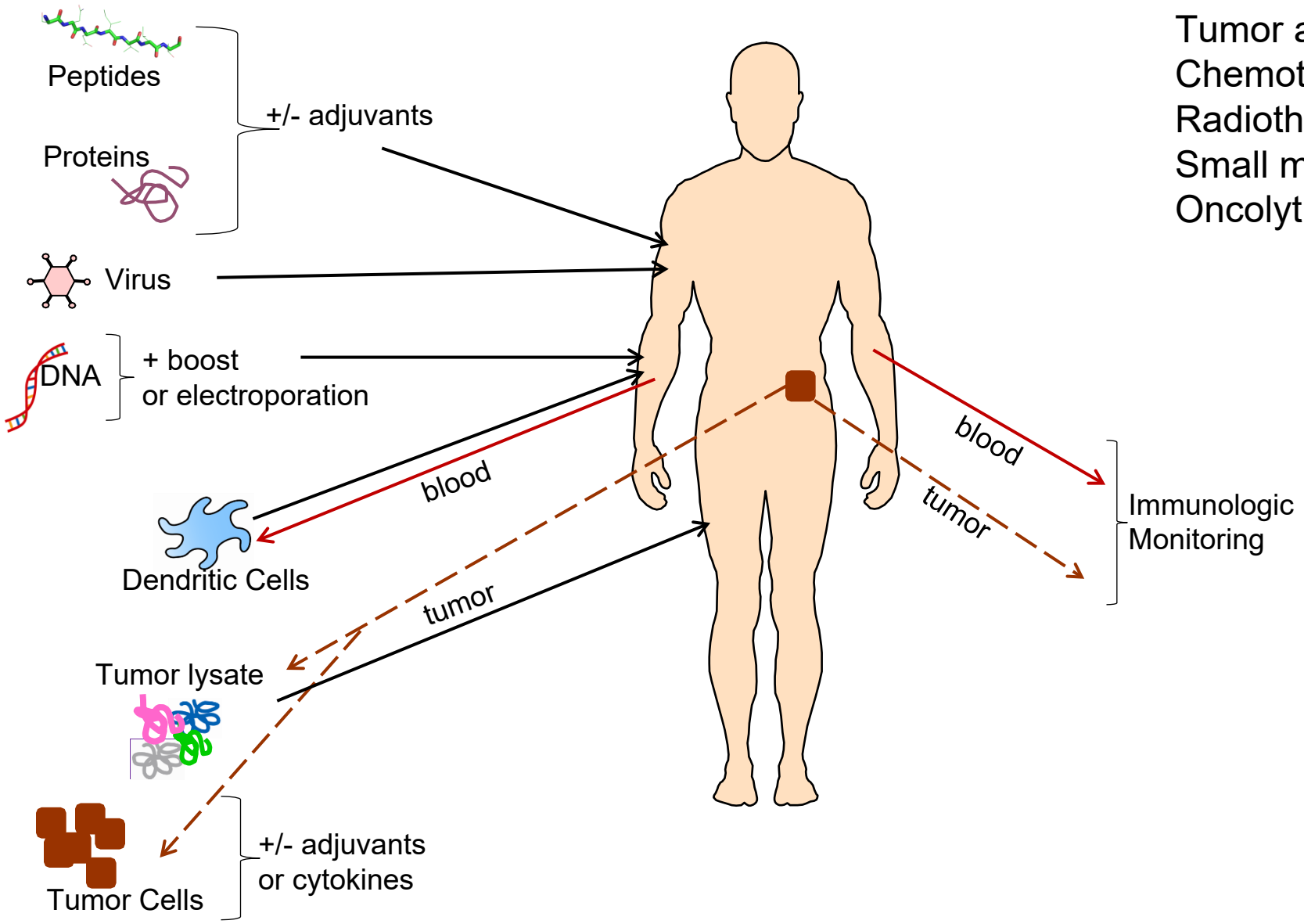
ADVANCES IN Cancer IMMUNOTHERAPY™

# Components of a cancer vaccine




And RNA/DNA

## Vaccine platforms

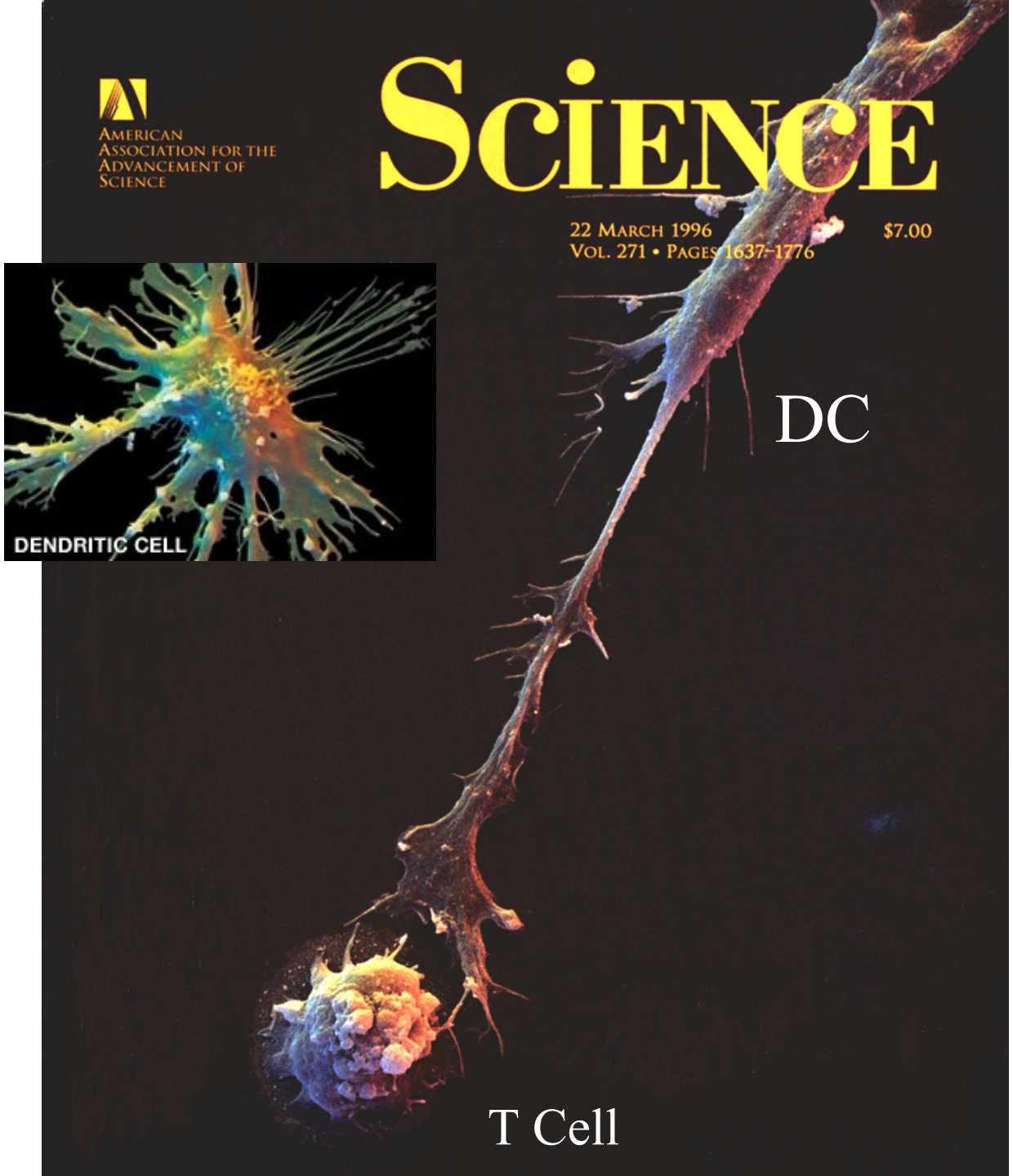



## Vaccine Effects

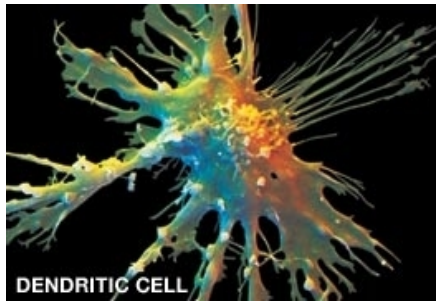
- Tumor ablation
- Chemotherapy
- Radiotherapy
- Small molecules
- Oncolytic virus

# 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.

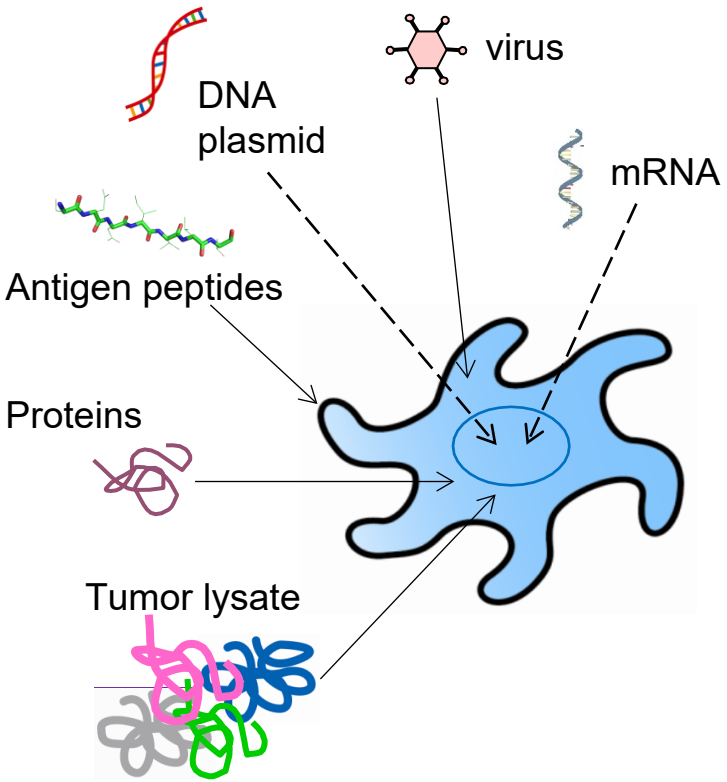
Recent DC vaccine studies (combinations, *author conclusions*):

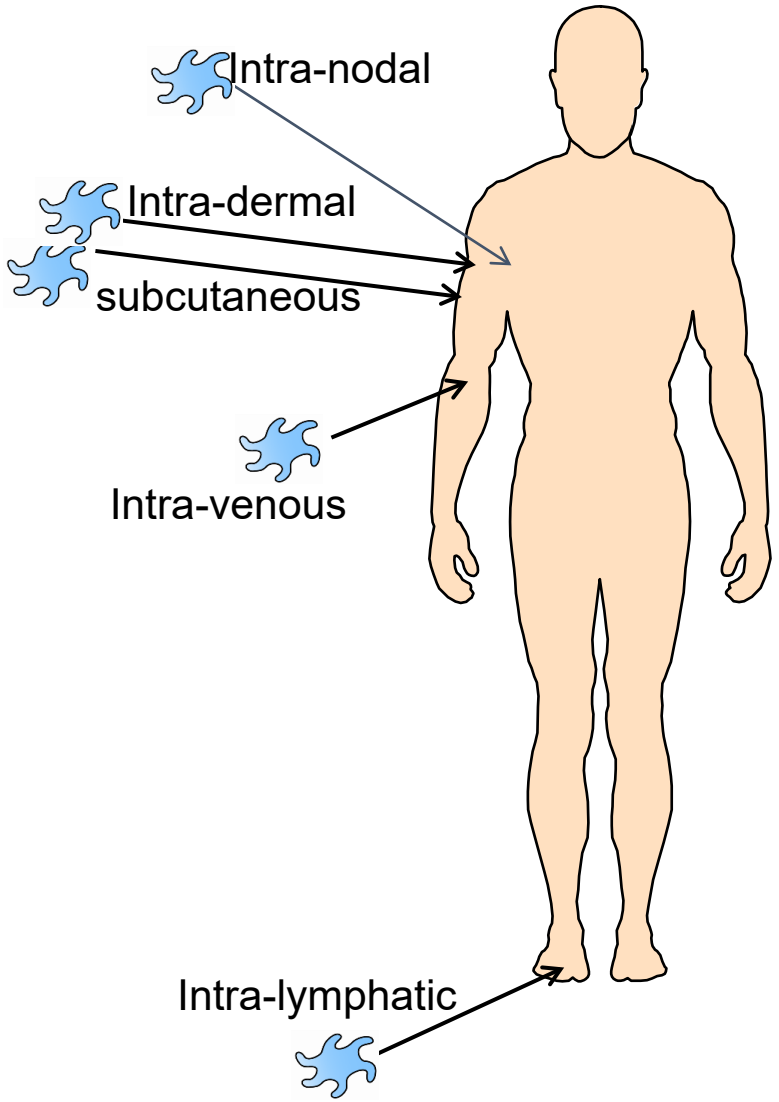
1. Kongstad, Svane: **Cytotherapy 2017**: DC + chemo in 43 prostate cancer pt. (*safe and immunogenic*)
2. Schreibelt, De Vries: **CaRes 2016**: 14 stg. IV melanoma pt., CD1c+ isolated blood DC, 16 hour culture, + gp100 and tyrosinase. *4/14 pt. PFS 12-35 mo*.
3. Wilgenhof, Neyns: **JCO 2016**: 39 “adv. 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.*

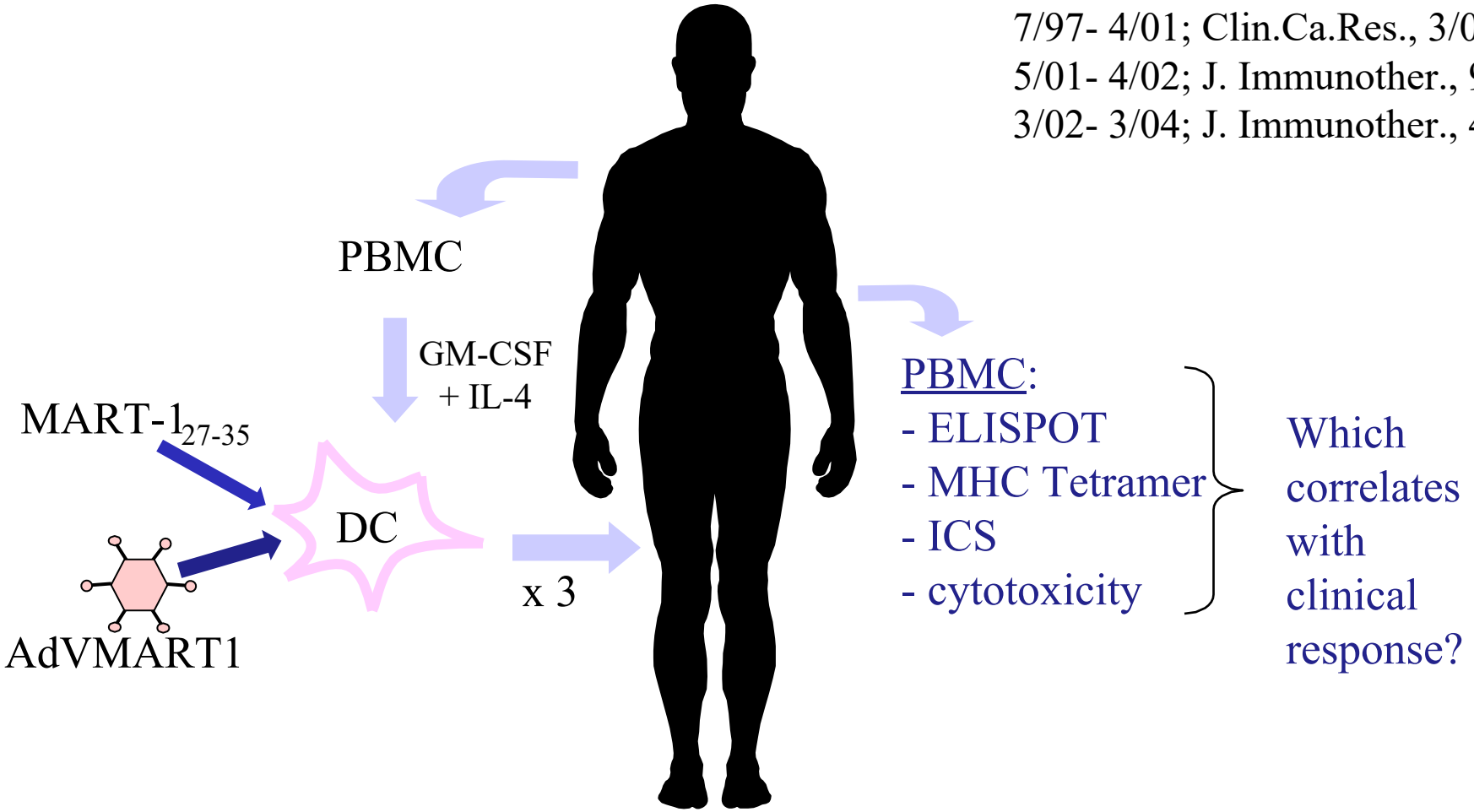
# Antigen delivery to DC



# 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




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

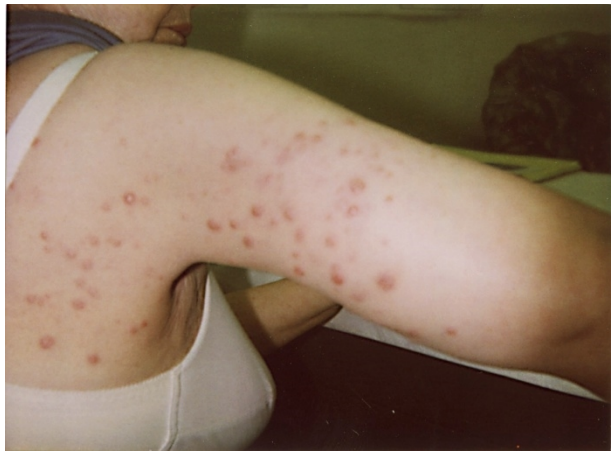
Pep. Phase II: $10^7$ DC/injection, i.d. (10 pt.)

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

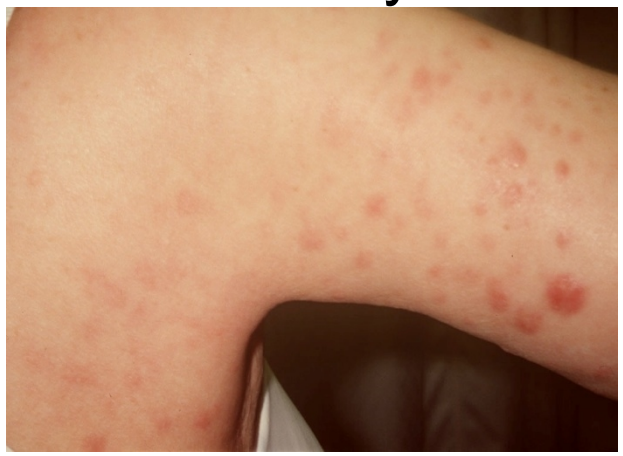
PI: J.S. Economou

# Patient E1 ($10^7$ DC, i.d.) post: 6 surgeries, 32 doses radiation, 6 infusions IFN$\alpha$. >10 yrs NED

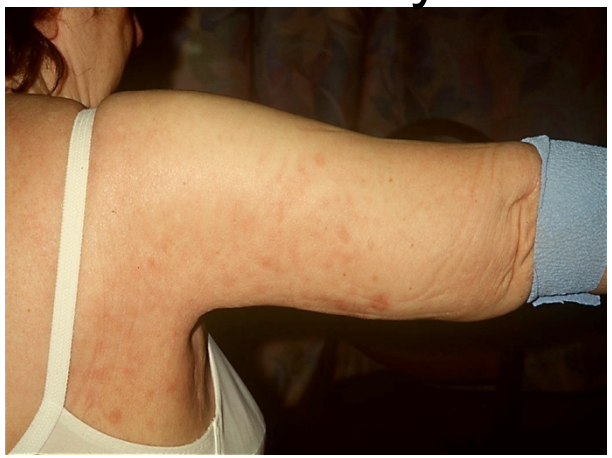
Pretreatment

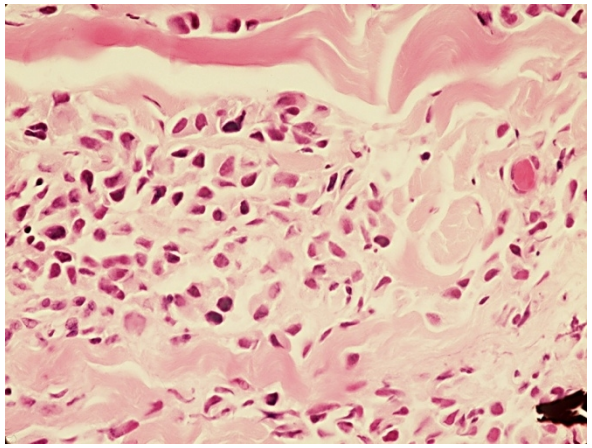


+56 days

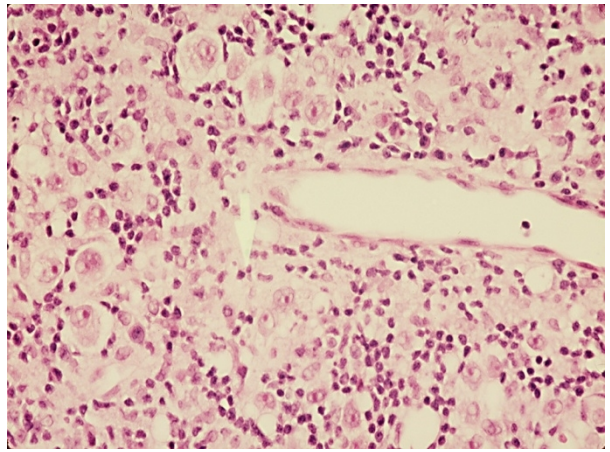


+130 days

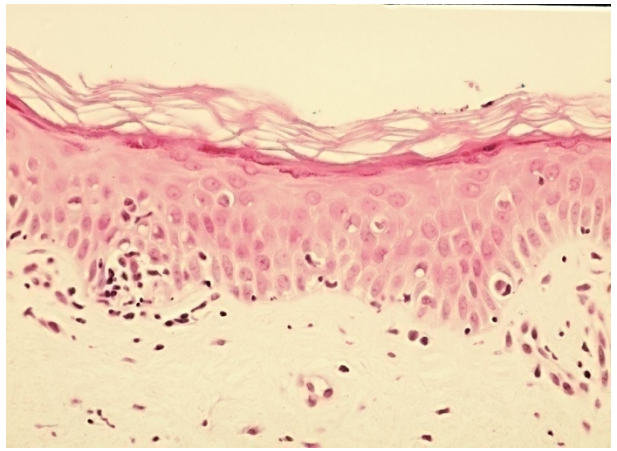




Melanoma Tumor



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:

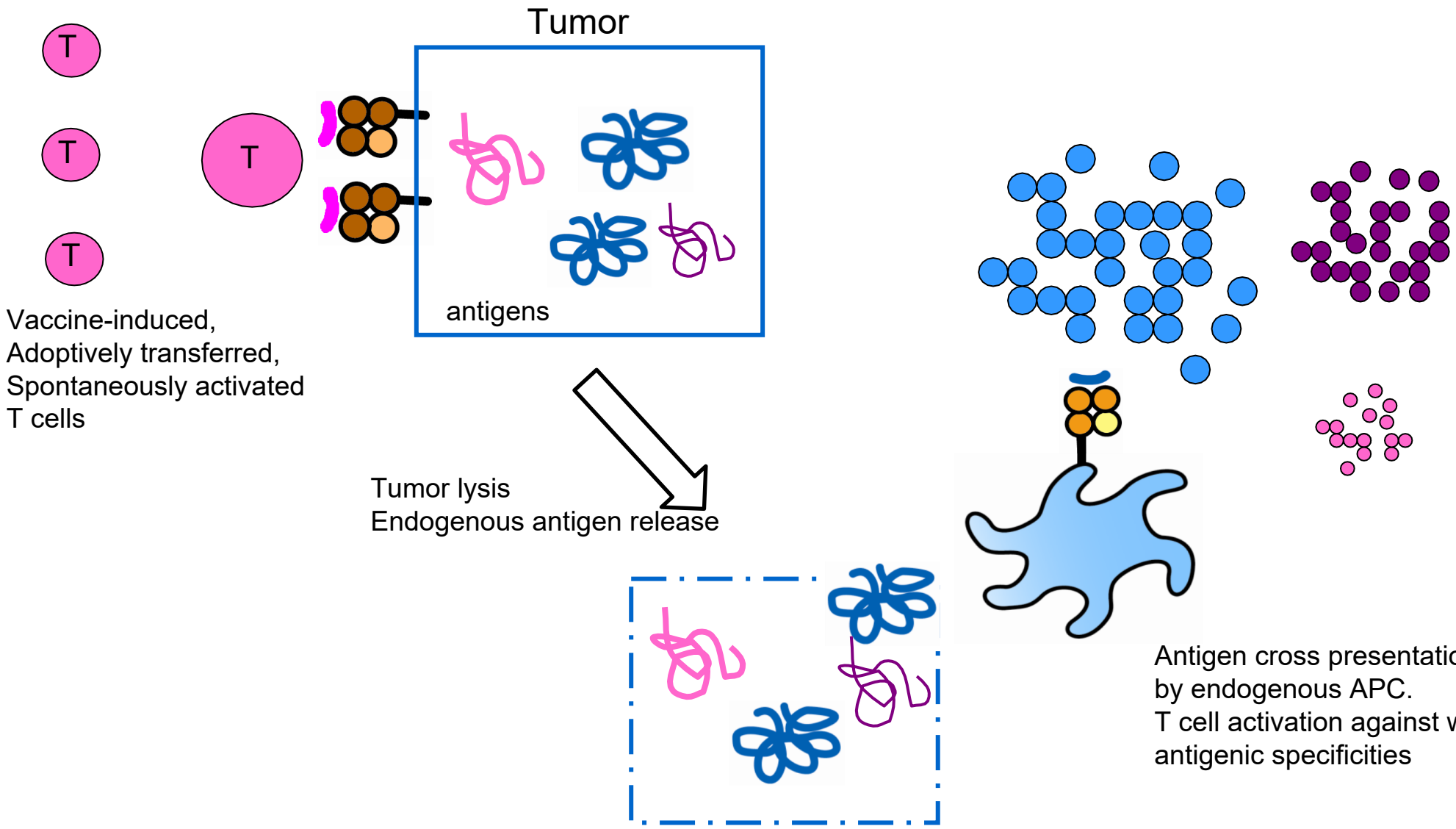
3/02-3/04 (23 enrolled); 14 received all 3 vaccines (all metastatic)

12/13 MART-1 immune responses by IFNγ ELISPOT; 9/14 MHC Tetramer+

1 “unevaluable” (54+ mo.),

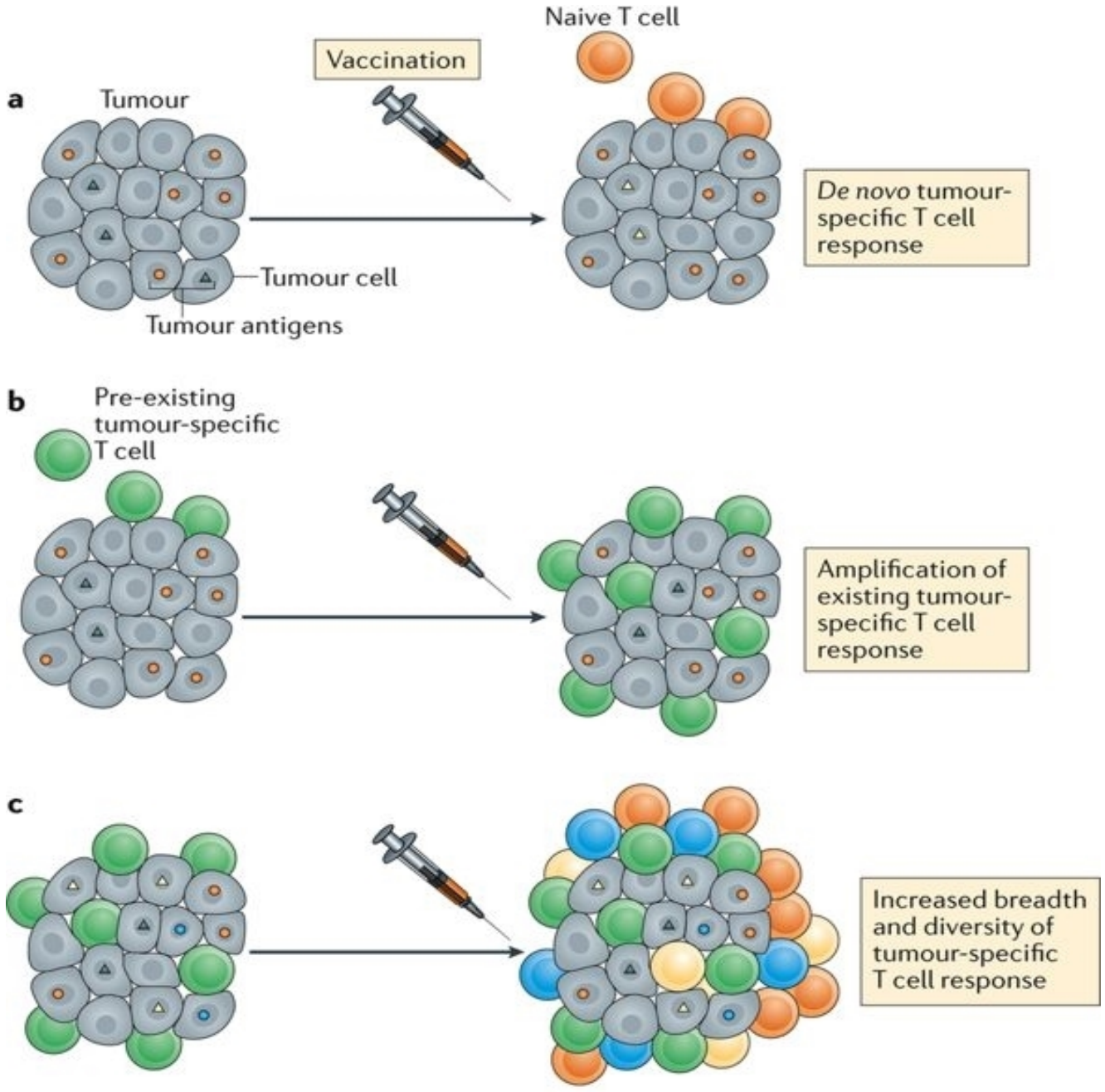
4 SD (27, 33, 36, 42 mo.), 1 became resectable/NED (56+ mo.)

# Determinant/Epitope/Antigen Spreading



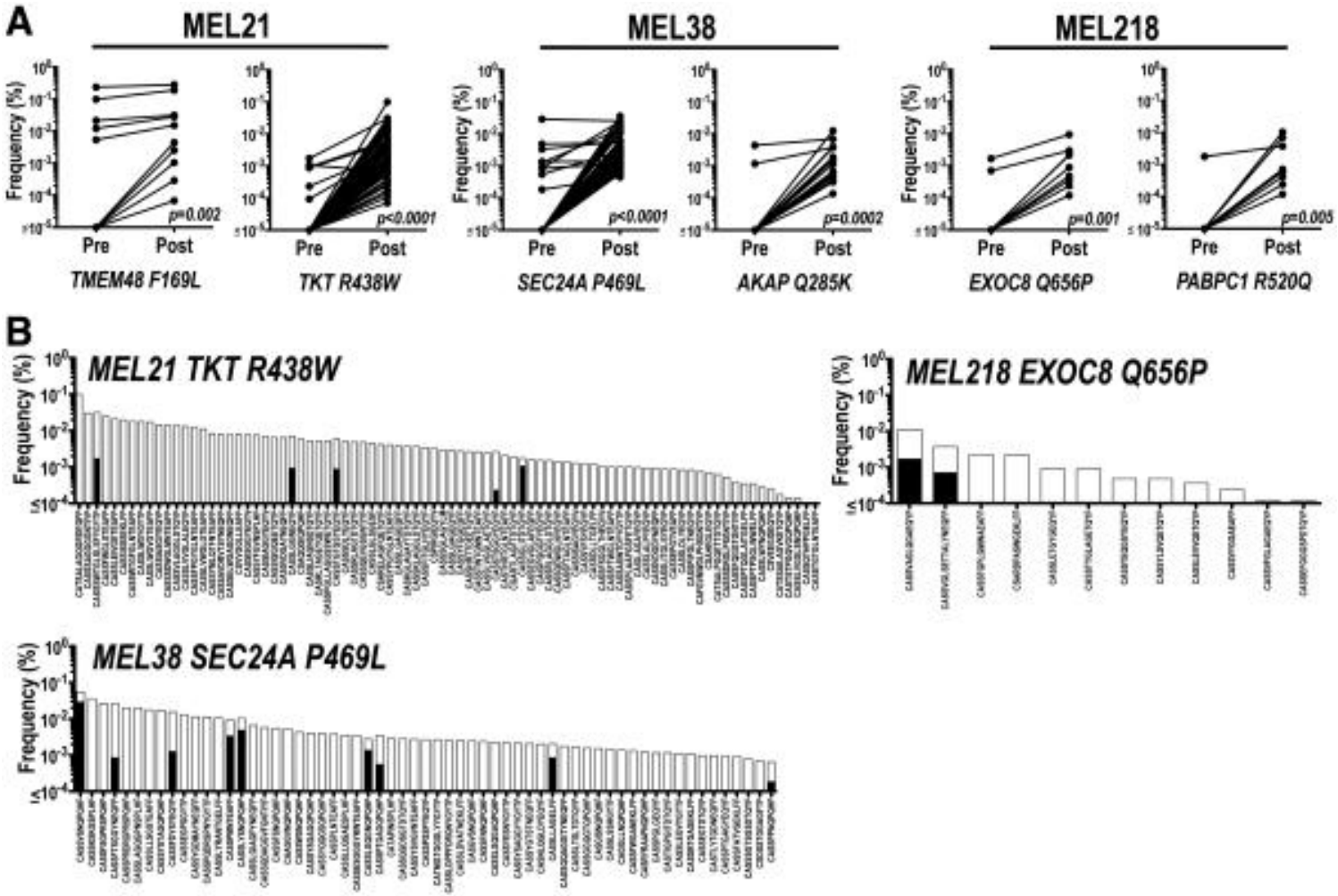

Ranieri ‘00; Disis ’02; Butterfield ‘03; Ribas ‘04; Wierecky ’06, Butterfield ’08

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**

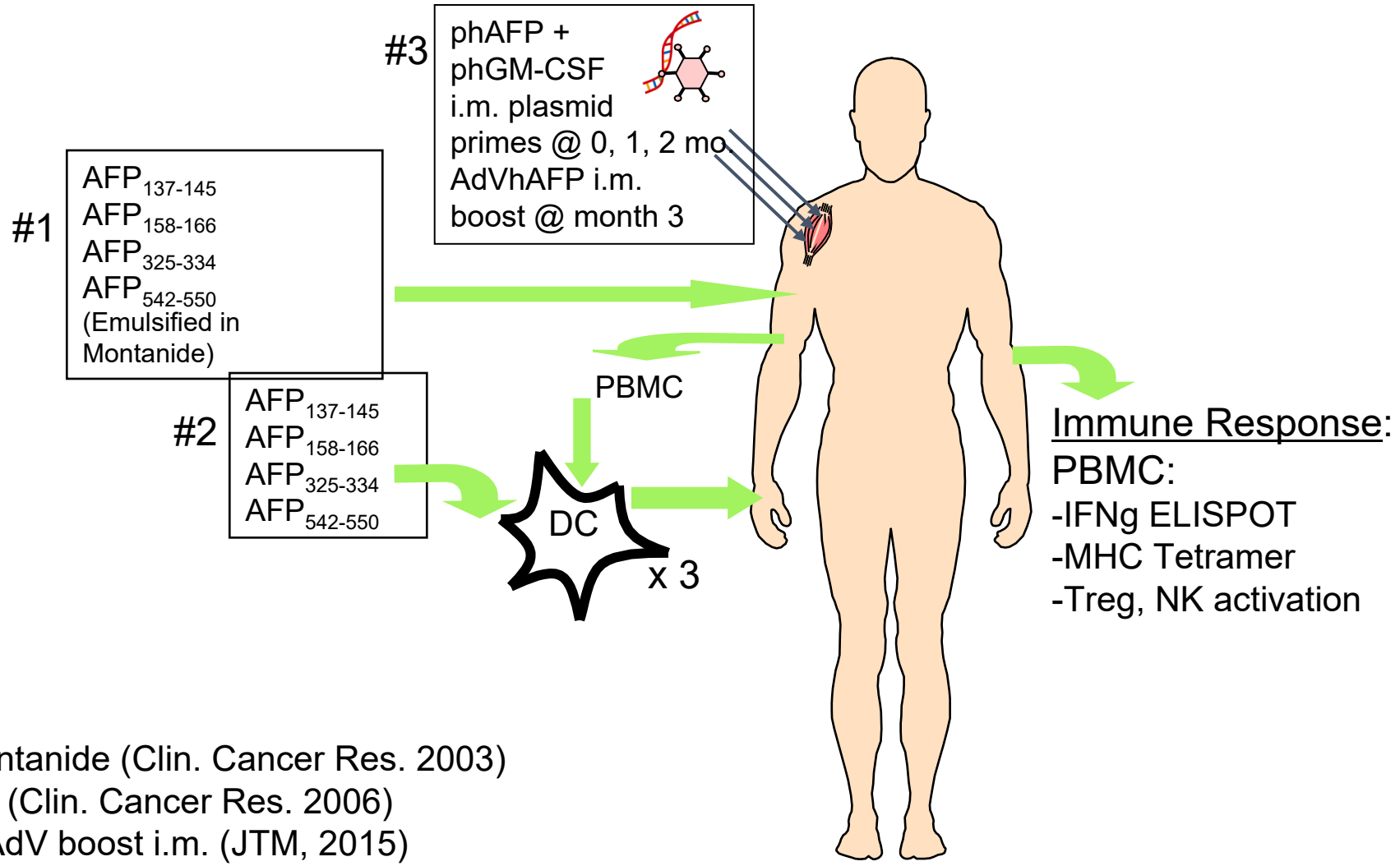
Science. 2015 May 15 Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells.
Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, Ly A, Lie WR, Hildebrand WH, Mardis ER, Linette GP

# 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:

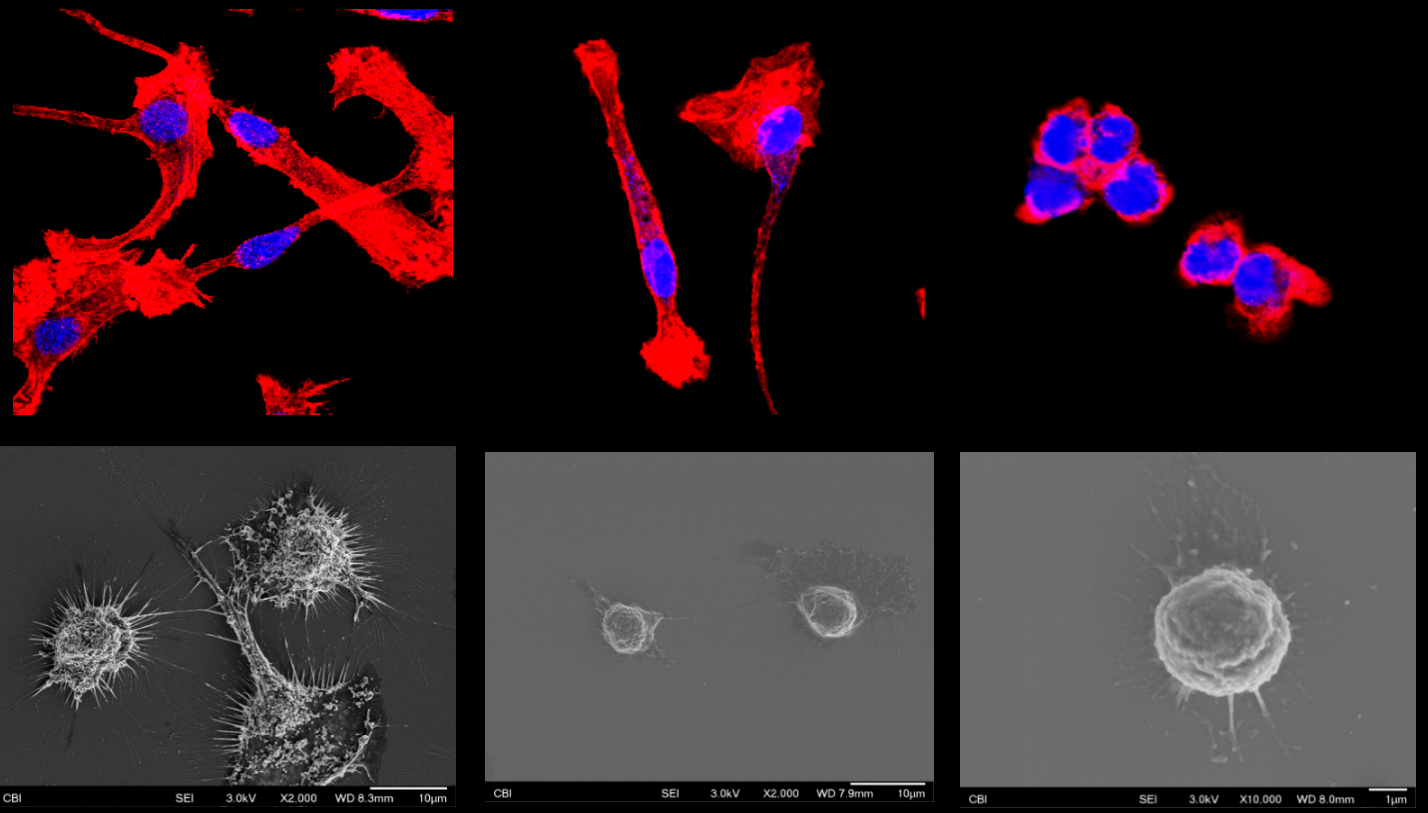
10 patients, stage III-IVb
Four AFP peptides pulsed onto autologous GM-CSF/IL-4 DC
3 injections, intradermal, no toxicities
8/10 immune responses by MHC tetramer and/or IFN γ ELISPOT
No objective clinical responses, 2 serum AFP decreases, OS = 2-35 months

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



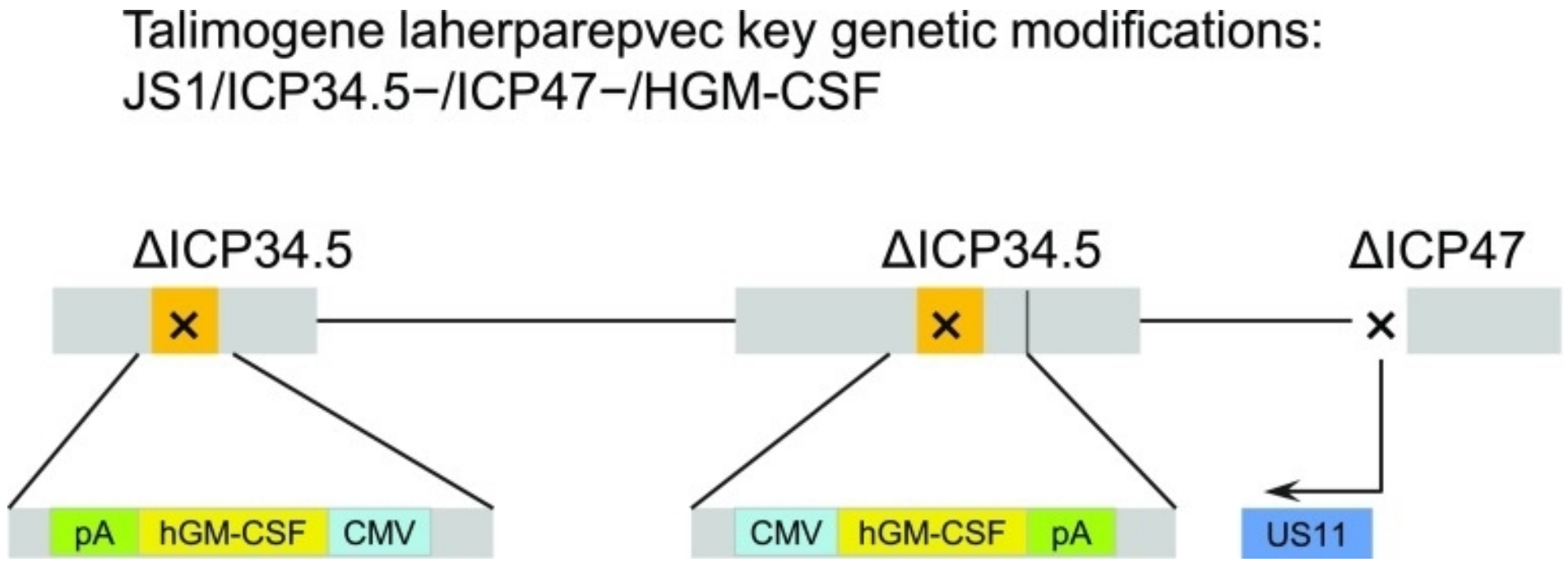

AFP alters DC phenotype to an immature phenotype that cannot be reversed by maturation, AFP inhibits DC metabolic function and T cell stimulatory capability (Pardee 2014, Santos 2019)

# Other effective platforms: Synthetic and Viral Vaccines

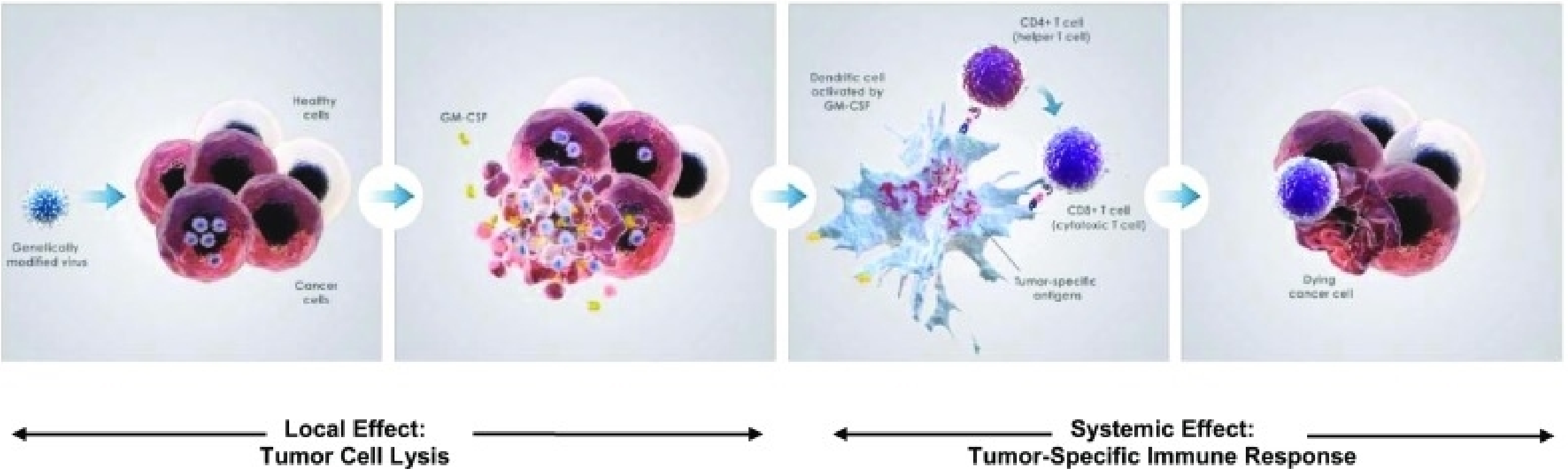
1. TVEC (Amgen) ***FDA approved 2015**
   - Oncolytic virus: HSV-1 + GM-CSF transgene
   - Metastatic melanoma, 26% response rate (vs. 6% in control arm)
2. ISA101 (Immune System Activation)
   - HPV16 Synthetic long peptide (SLP, 24-32mer) in Montanide
   - Cervical cancer
   - Appears to synergize 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*

# T-VEC:




Genetic modifications of talimogene laherparepvec. The viral gene ICP34.5 was deleted and replaced with a human granulocyte-macrophage colony-stimulating factor (hGM-CSF) expression cassette comprising the cytomegalovirus (CMV) promoter, hGM-CSF, and a bovine growth hormone polyadenylation (pA) signal. Expression of the viral gene US11 is driven by the ICP47 promoter




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

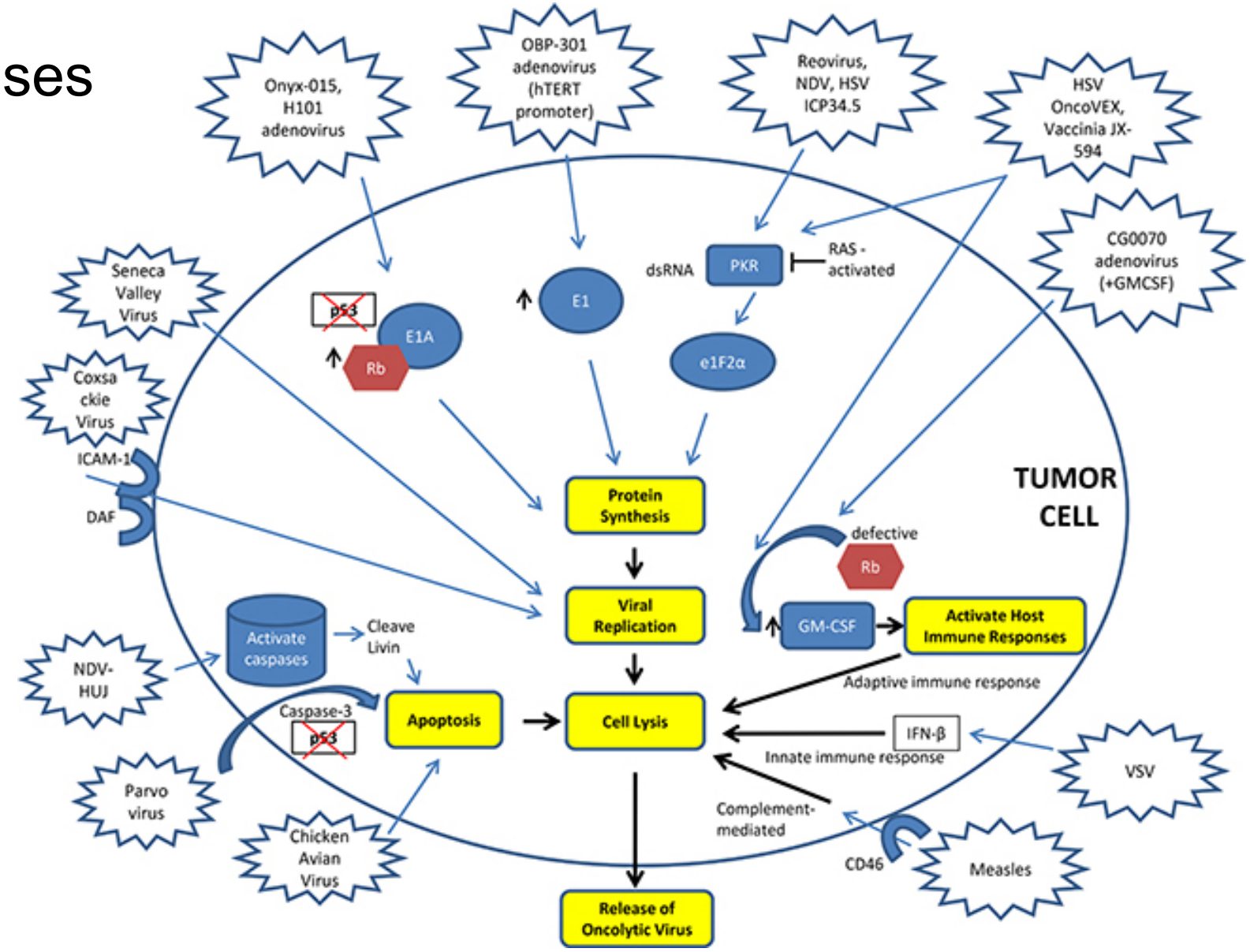



**Figure 1: Mechanisms of action of oncolytic viruses.** DAF – Decay Accelerating Factor, GM-CSF – Granulocyte Macrophage-Colony Stimulating Factor, HSV – Herpes Simplex Virus, hTERT – Human Telomerase, ICAM-1 – Intercellular Adhesion Molecule-1, ICP – Infectious Cell Protein, INF-β – Interferon beta,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

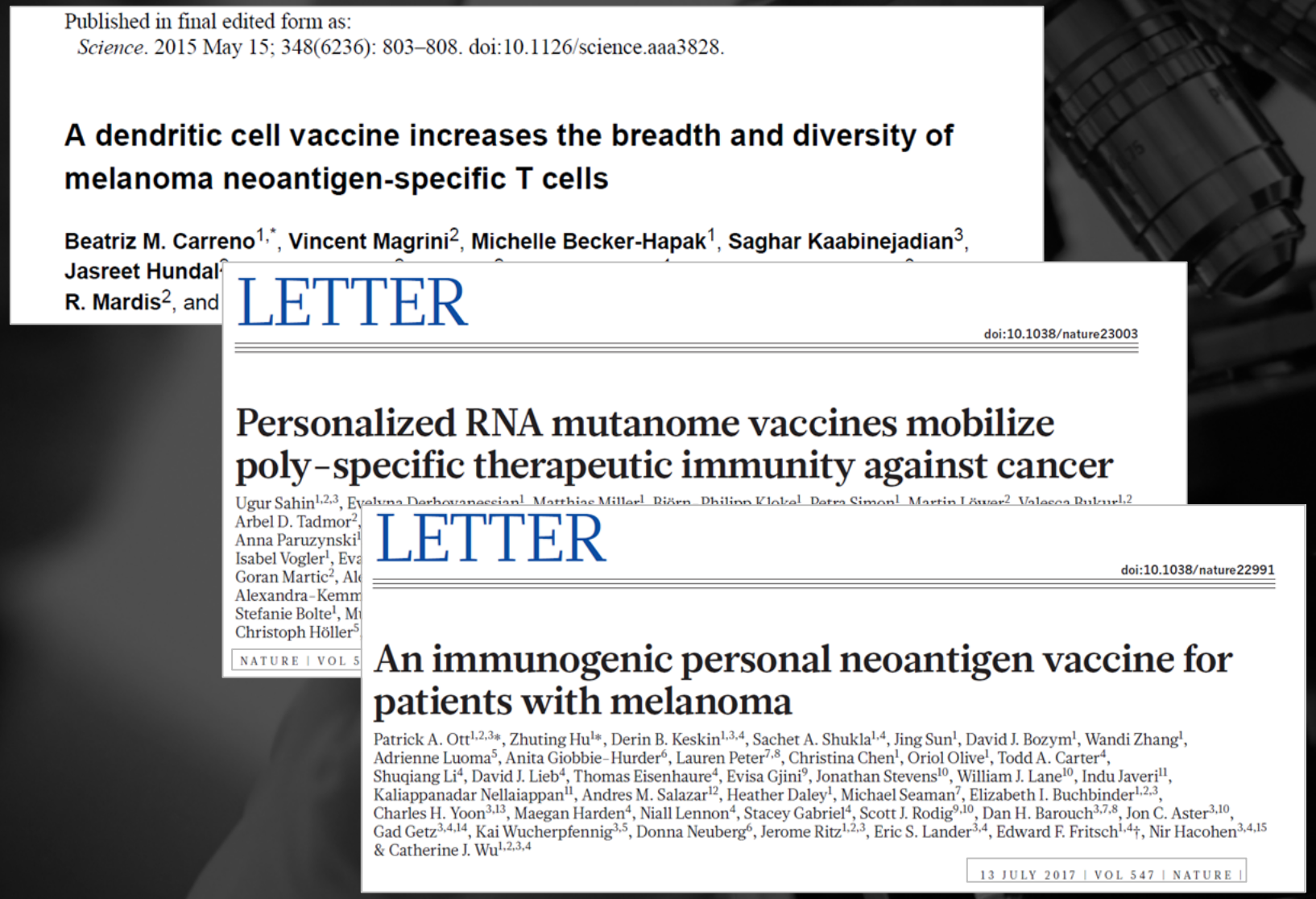
# Tumor Mutations

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**

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LETTER

doi:10.1038/nature23003

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

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LETTER

doi:10.1038/nature22991

**An immunogenic personal neoantigen vaccine for patients with melanoma**

Patrick A. Ott[1,2,3*], Zhuting Hu[1*], Derin B. Keskin[1,3,4], Sachet A. Shukla[1,4], Jing Sun[1], David J. Bozym[1], Wandi Zhang[1], Adrienne Luoma[5], Anita Giobbie-Hurder[6], Lauren Peter[7,8], Christina Chen[1], Oriol Olive[1], Todd A. Carter[4], Shuqiang Li[4], David J. Lieb[4], Thomas Eisenhaure[4], Evisa Gjini[9], Jonathan Stevens[10], William J. Lane[10], Indu Javeri[11], Kaliappanadar Nellaiappan[11], Andres M. Salazar[12], Heather Daley[1], Michael Seaman[7], Elizabeth I. Buchbinder[1,2,3], Charles H. Yoon[3,13], Maegan Harden[4], Niall Lennon[4], Stacey Gabriel[4], Scott J. Rodig[9,10], Dan H. Barouch[3,7,8], Jon C. Aster[3,10], Gad Getz[3,4,14], Kai Wucherpfennig[3,5], Donna Neuberg[6], Jerome Ritz[1,2,3], Eric S. Lander[3,4], Edward F. Fritsch[1,4†], Nir Hacohen[3,4,15] & Catherine J. Wu[1,2,3,4]

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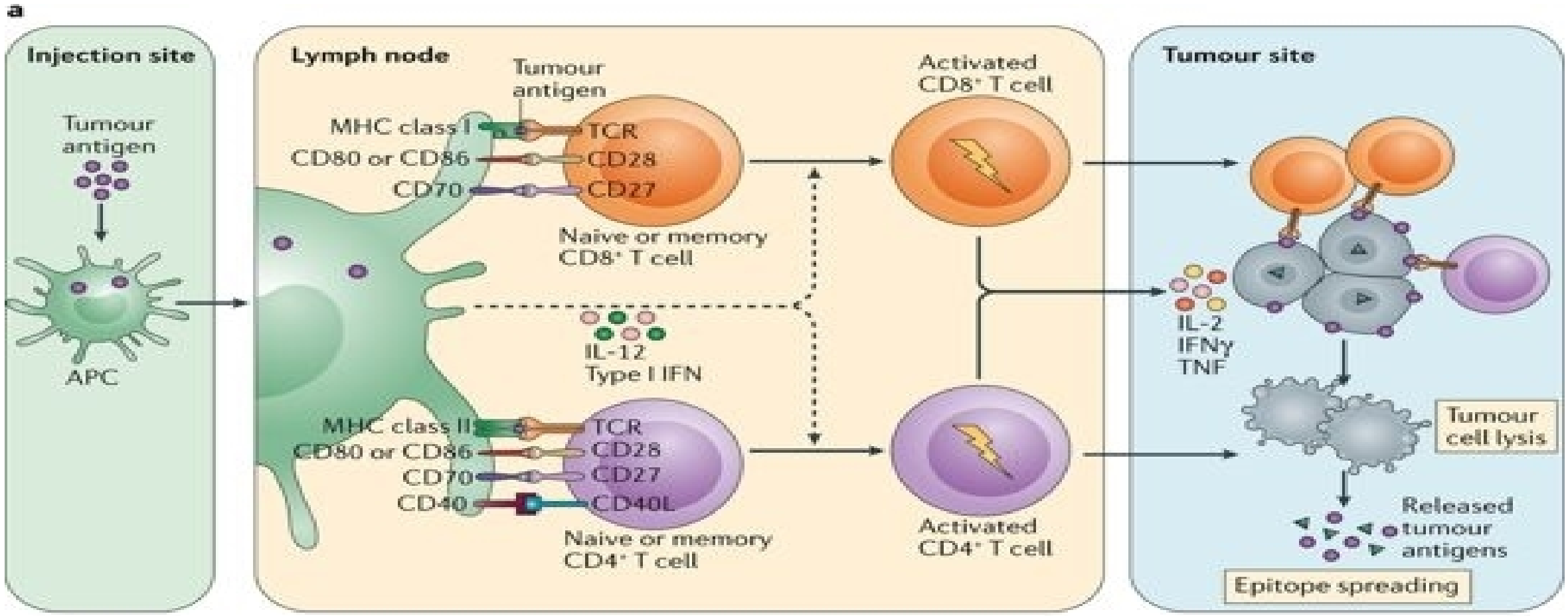
•Neoantigens have emerged as targets of effective tumour-directed T cell responses. Increased neoantigen load is associated with improved patient outcomes.

•**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.

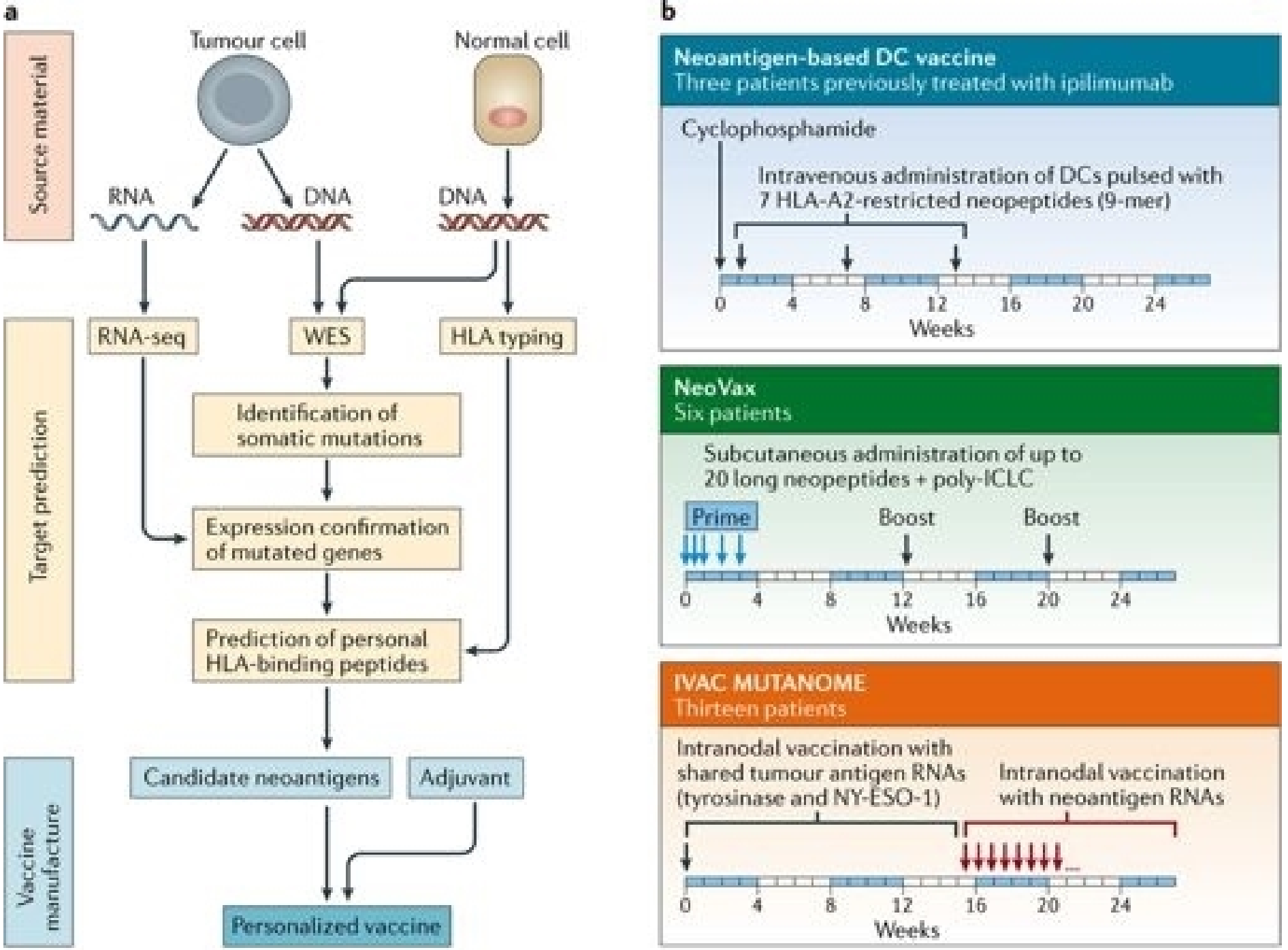
•A crucial aspect of a vaccine targeting neoantigens is the selection of epitopes that can be presented *in vivo* by tumour or antigen-presenting cells. HLA-binding prediction, high-resolution mass spectrometry and understanding of antigen processing are important research areas for further discovery.

•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*

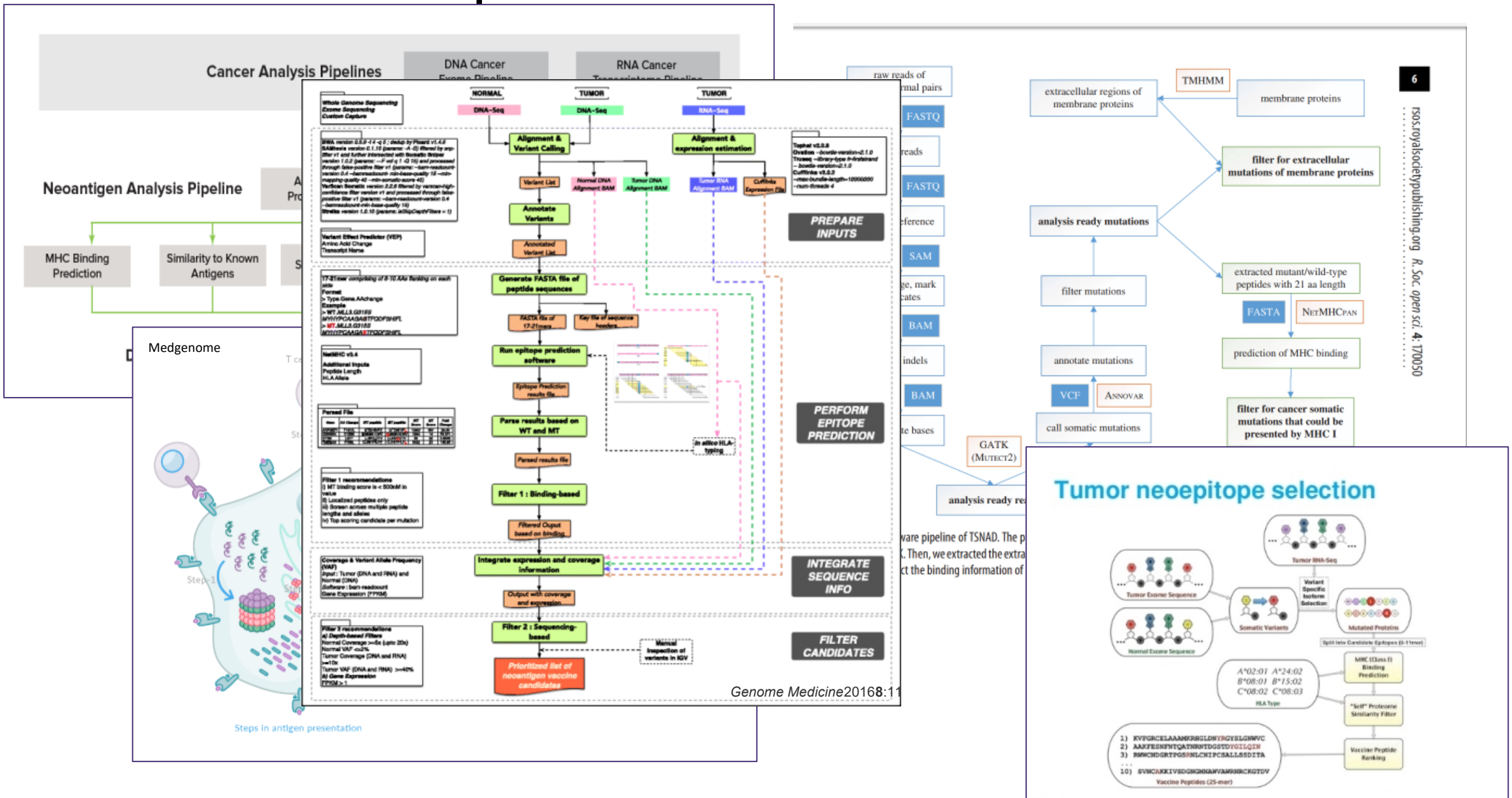



Z. Hu, P. Ott, C. Wu*Nat Rev Immunol 2018*




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

# Neoepitope pipelines are becoming more common, diverse and complex



# TESLA Publication

Cell

CellPress

Resource

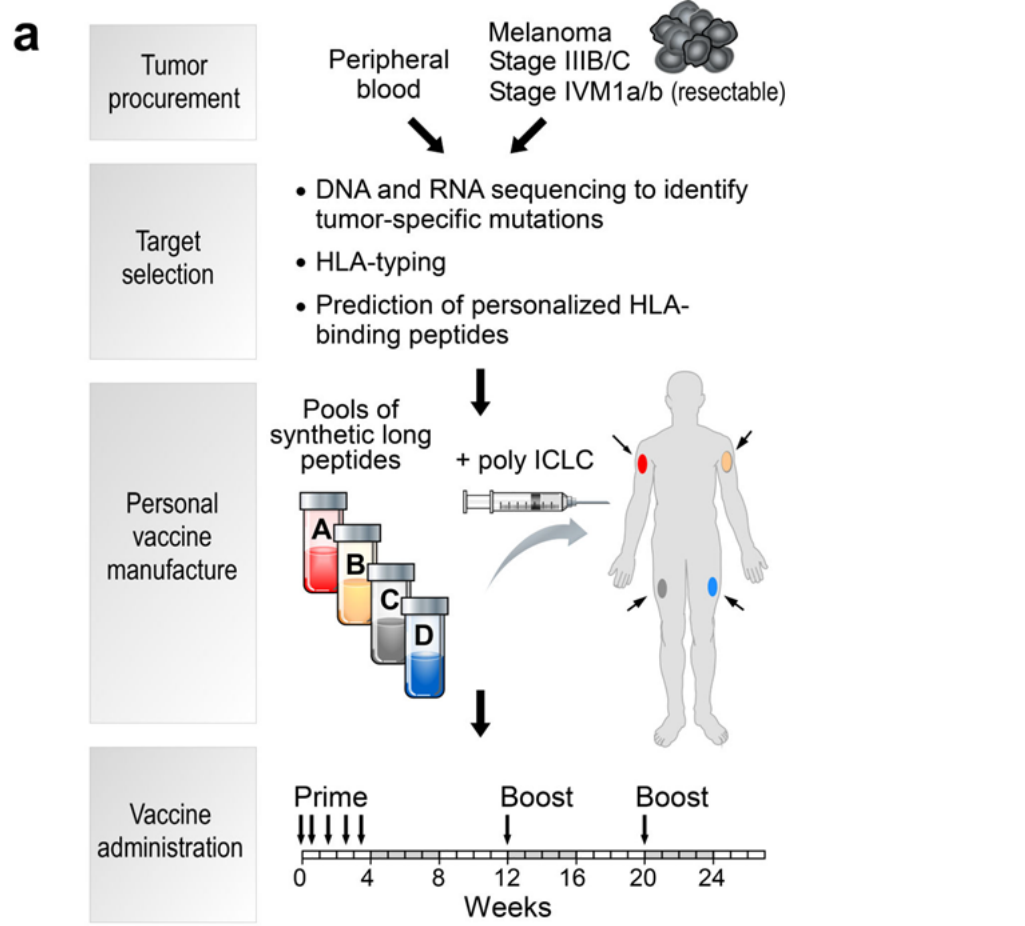
# Key Parameters of Tumor Epitope Immunogenicity Revealed Through a Consortium Approach Improve Neoantigen Prediction

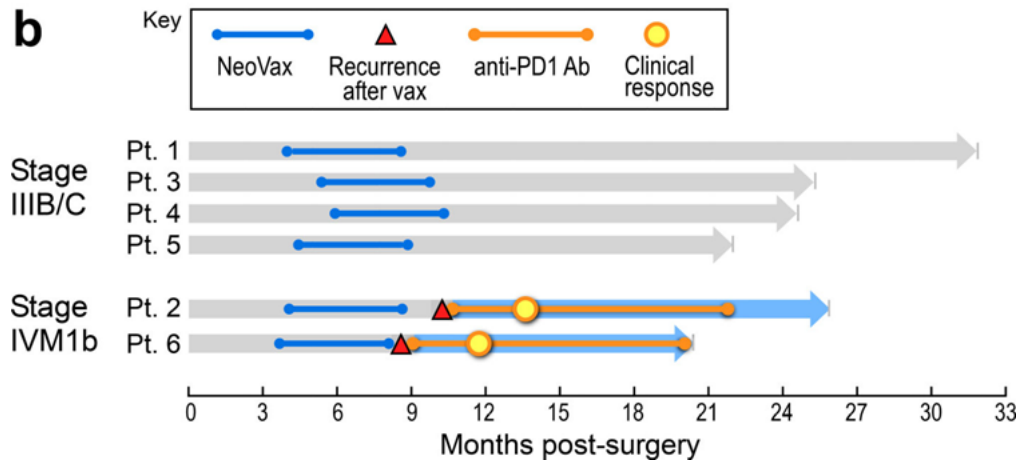
Daniel K. Wells,[1,24,26,*] Marit M. van Buuren,[2,3,24] Kristen K. Dang,[4,24] Vanessa M. Hubbard-Lucey,[5] Kathleen C.F. Sheehan,[6,7] Katie M. Campbell,[8] Andrew Lamb,[4] Jeffrey P. Ward,[9] John Sidney,[10] Ana B. Blazquez,[11] Andrew J. Rech,[1,12] Jesse M. Zaretsky,[8] Begonya Comin-Anduix,[1,13] Alphonsus H.C. Ng,[14] William Chour,[15] Thomas V. Yu,[4] Hira Rizvi,[16] Jia M. Chen,[8] Patrice Manning,[1] Gabriela M. Steiner,[1] Xengie C. Doan,[4] The Tumor Neoantigen Selection Alliance, Taha Merghoub,[1,17,18] Justin Guinney,[4,19] Adam Kolom,[1,5] Cheryl Selinsky,[1] Antoni Ribas,[1,8,9] Matthew D. Hellmann,[1,16,17,18] Nir Hacohen,[20,21] Alessandro Sette,[11,22] James R. Heath,[1,14] Nina Bhardwaj,[1,11] Fred Ramsdell,[1] Robert D. Schreiber,[1,6,7,25] Ton N. Schumacher,[23,25] Pia Kvistborg,[2,25] and Nadine A. Defranoux[1,25,*]

# 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.



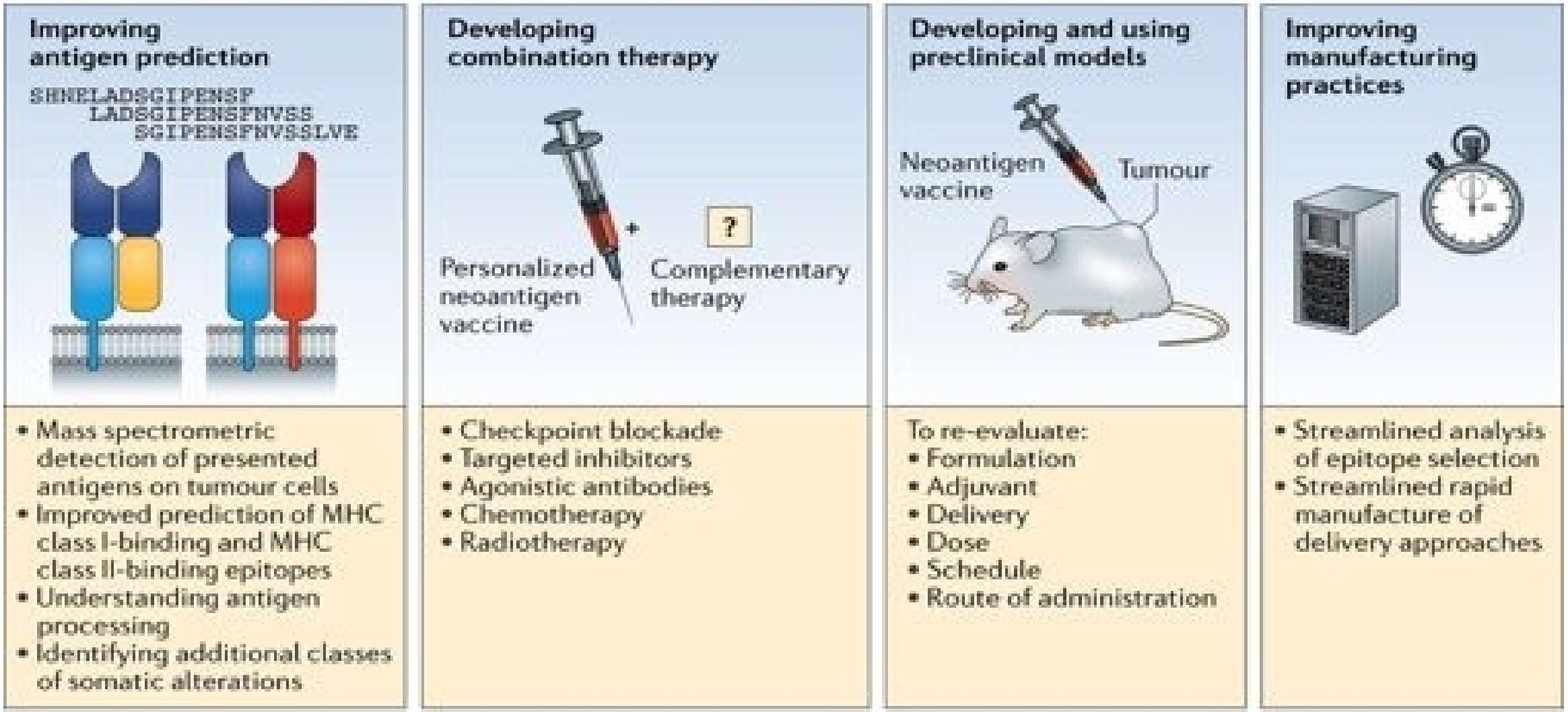




# Generation of a personal, multi-peptide neoantigen vaccine for patients with high-risk melanoma

**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*




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

# Measuring Immunity in Immunotherapy Clinical Trials:

- Was the cytokine induced (right time/place/level)?
- *Did the vaccine activate tumor-specific T cells?*
- *What is a quality/function of those T cells?*
- *Did spreading occur? To neoantigens?*
- Did the adoptively transferred effector cells survive/traffic to the tumor/kill the tumor?
- Was immune suppression reversed?
- Were the target cells/molecules activated?
- Did the target cells/molecules get to the tumor site and show activity?

- *Was the therapeutic intervention an improvement?*
- *Why or why not?*

# The dawn of vaccines for cancer prevention

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

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

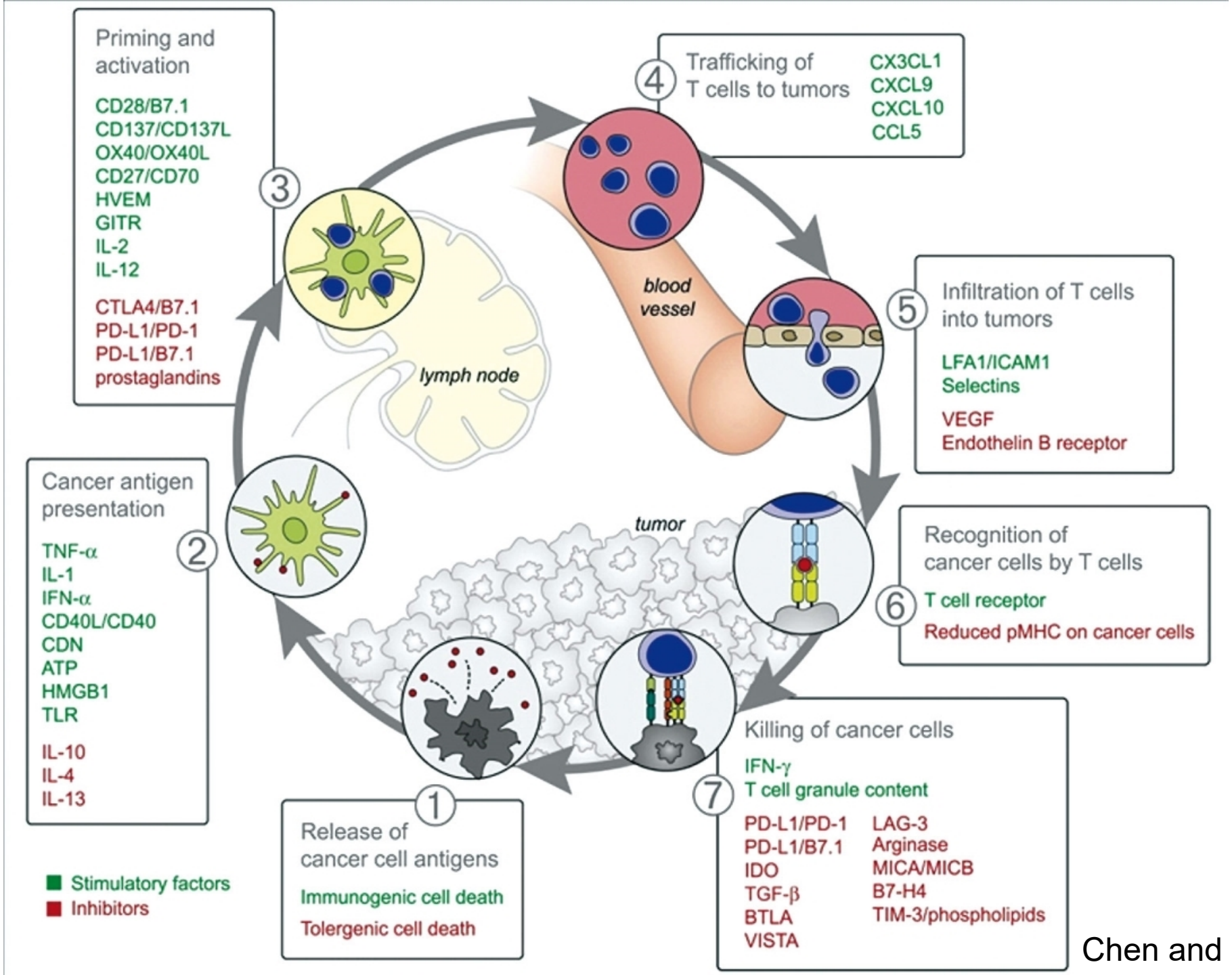
• Developments in imaging and other screening methods have made possible the detection of pre-malignant lesions.

•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