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

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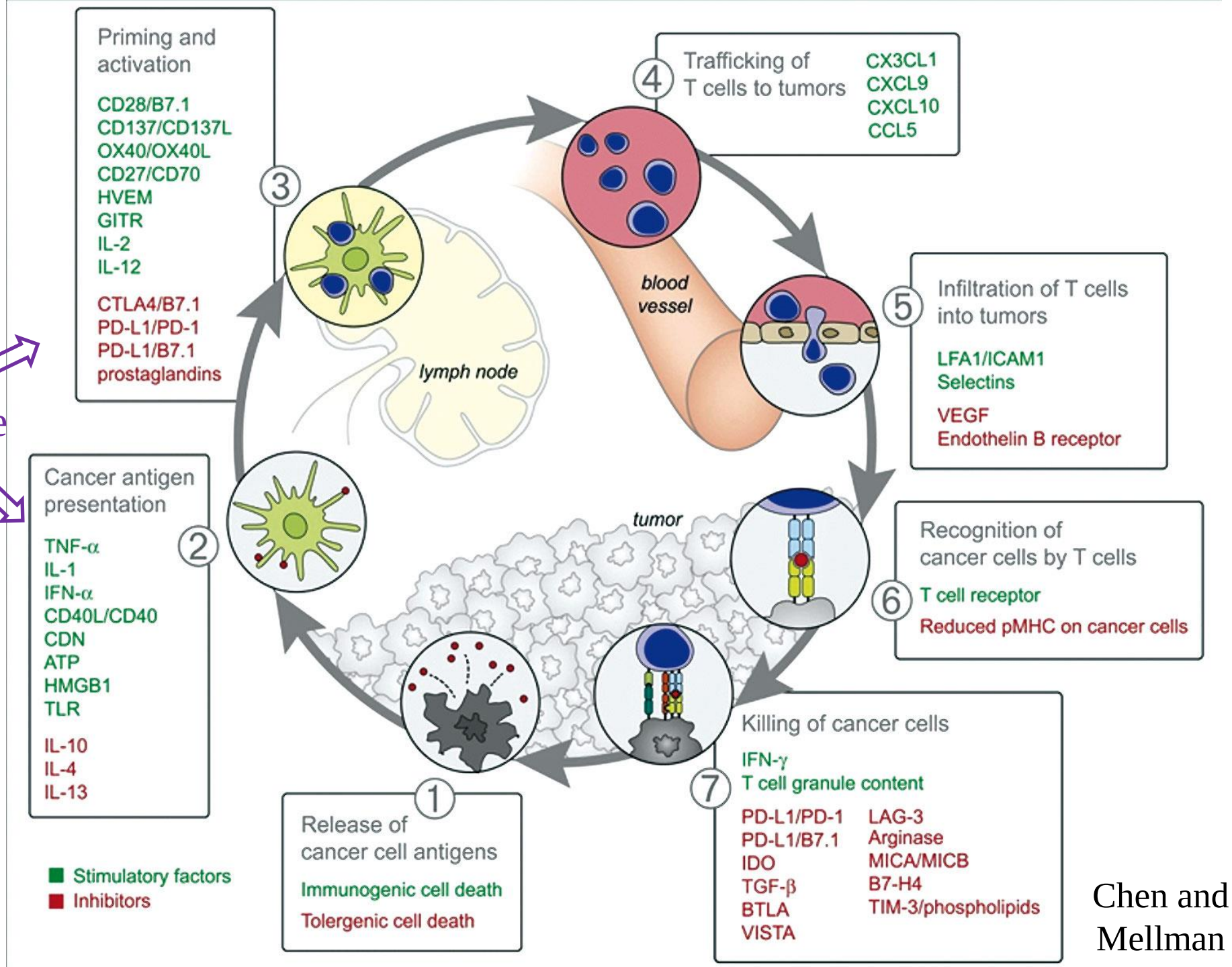
PARKER INSTITUTE FOR CANCER IMMUNOTHERAPY

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
Replimmune, Scientific Advisory Board, 2018-present
Western Oncolytics, Scientific Advisory Board, 2018-present
Torque Therapeutics, Scientific Advisory Board, 2018-present
Khloris, Scientific Advisory Board, 2019-present
Pyxis, Scientific Advisory Board, 2019-present
Cytomix, Scientific Advisory Board, 2019-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*

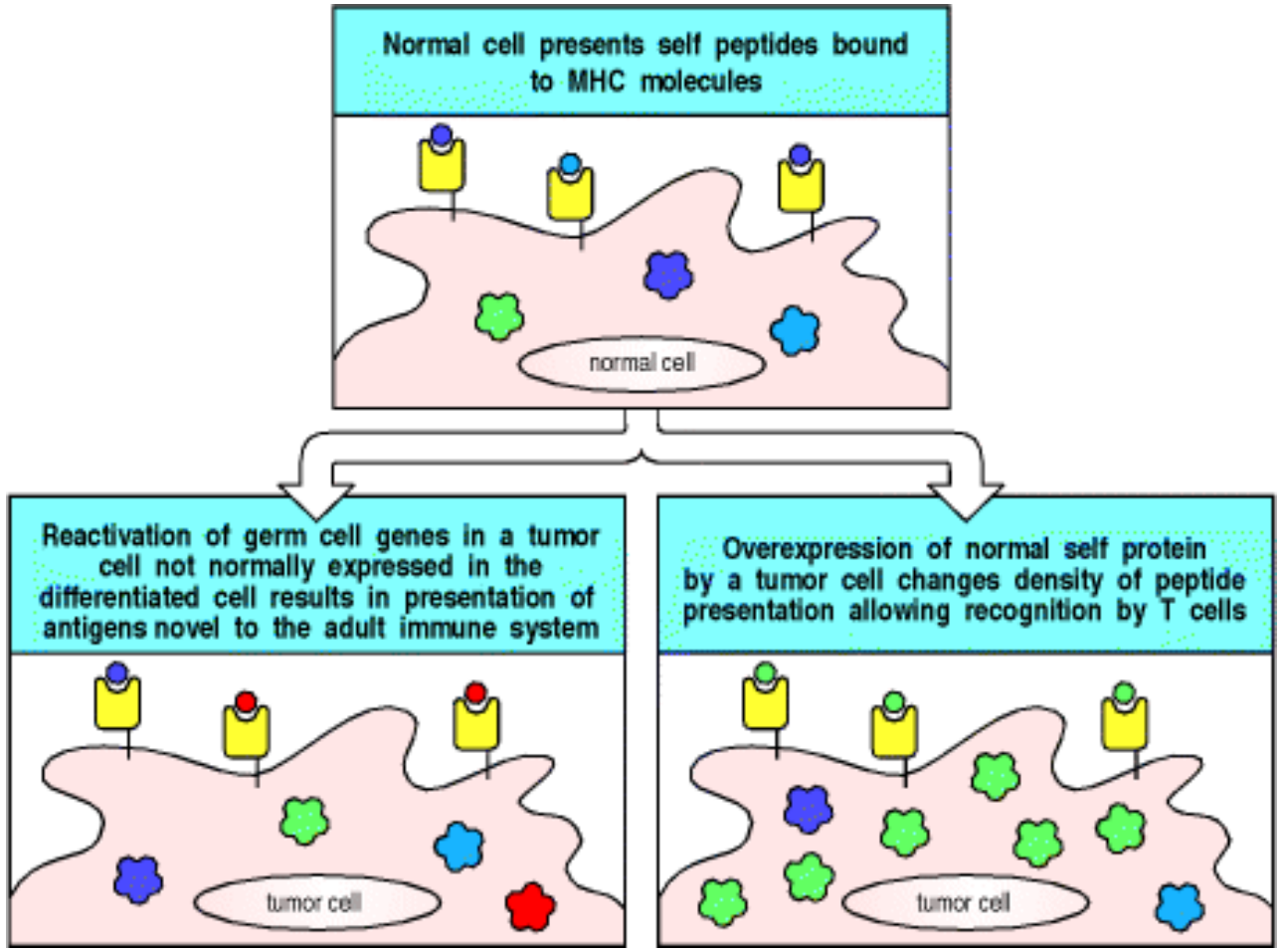
4) proteins derived from oncoviruses, like Human Papilloma Virus (HPV) proteins E6 and E7, HBV, HCV, MCPV

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

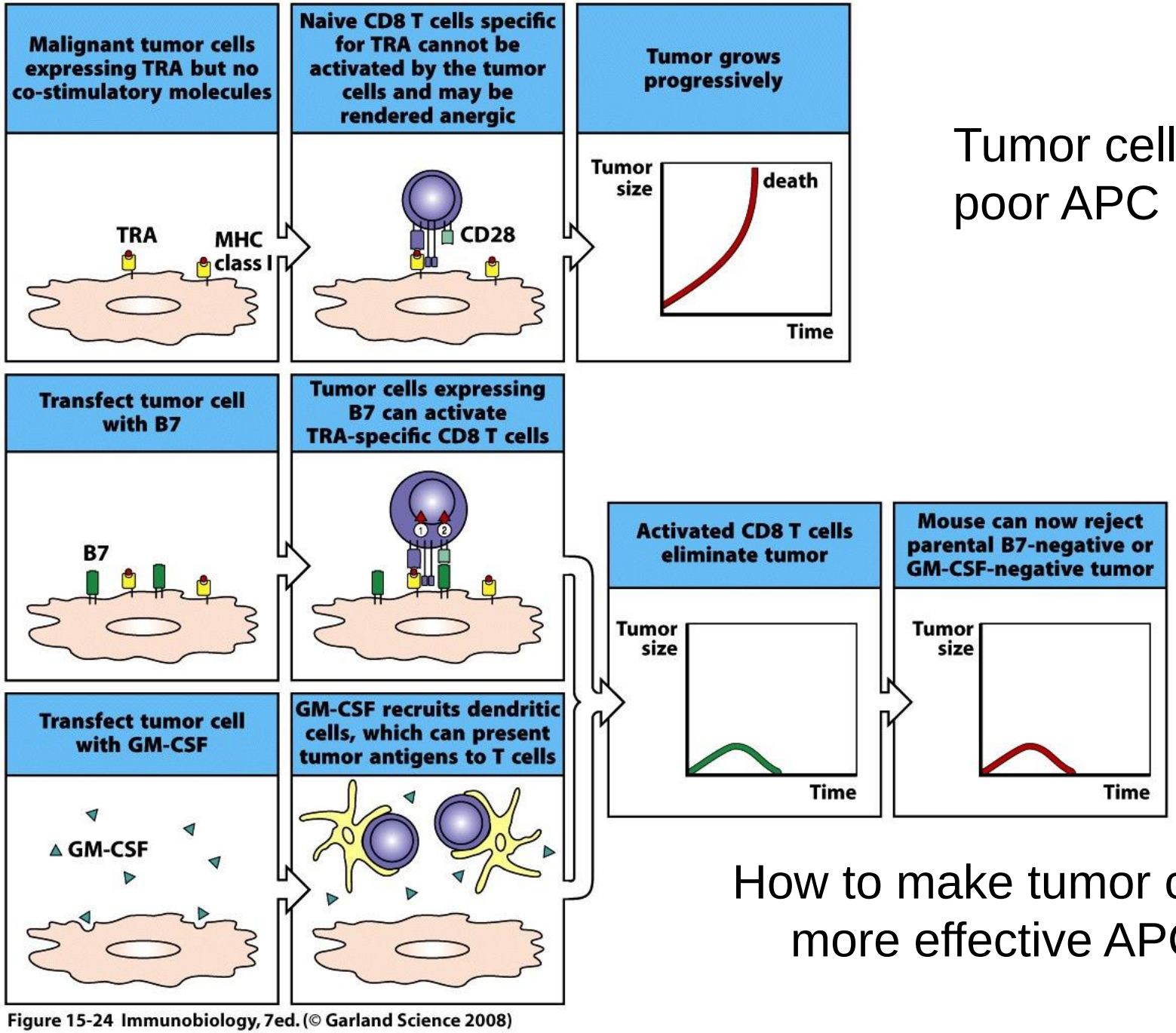
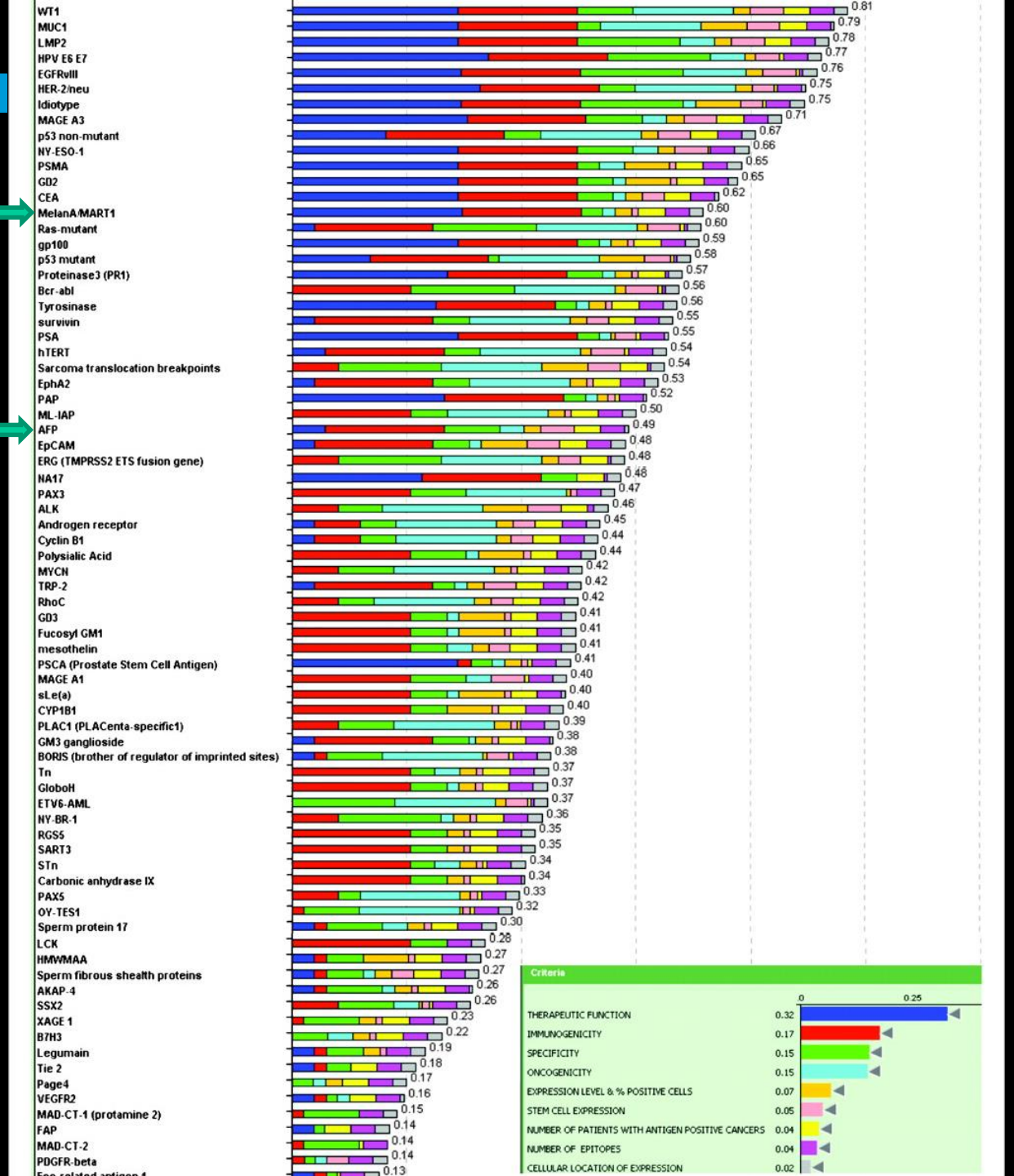


Figure 15-24 Immunobiology, 7ed. (© Garland Science 2008)

Tumor cells are poor APC

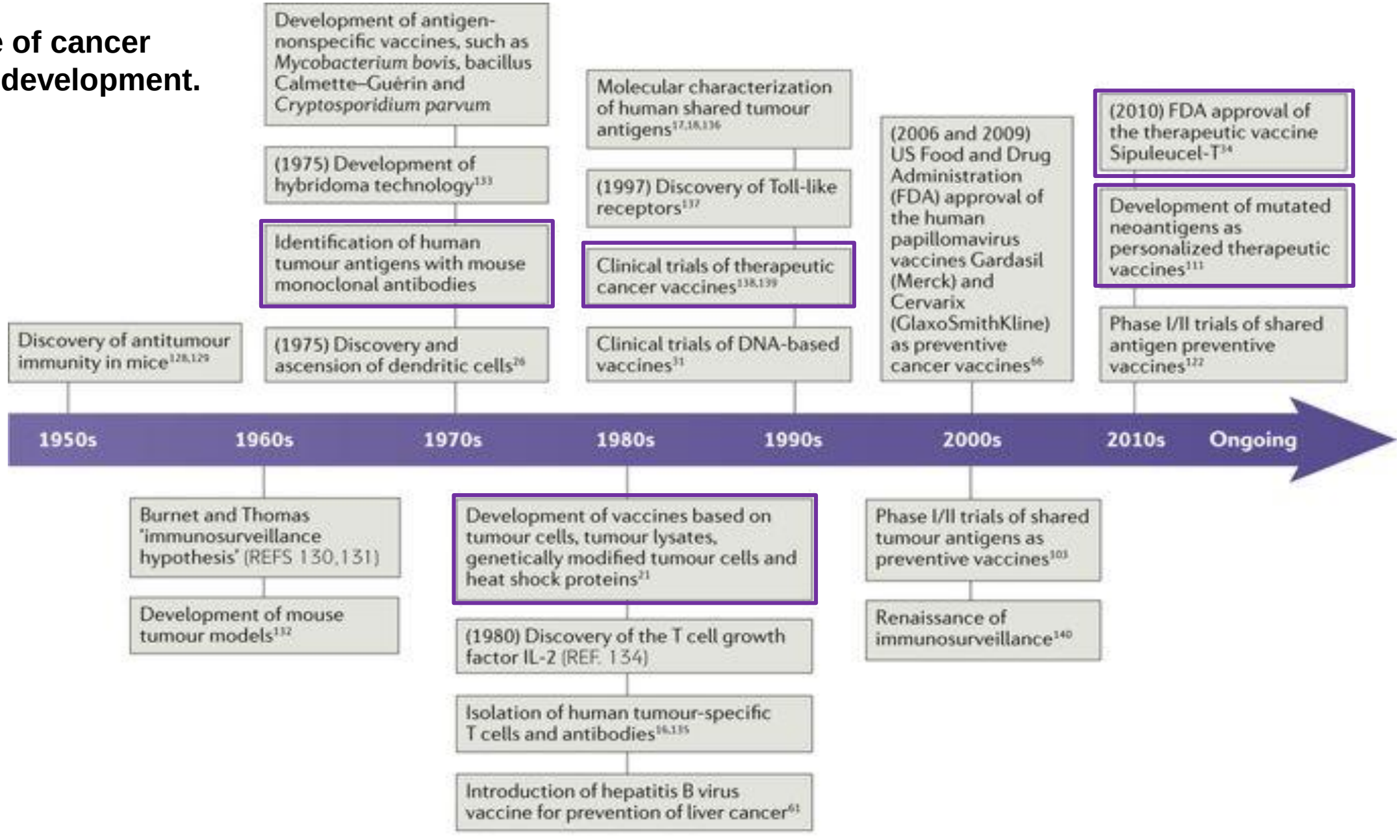
How to make tumor cells more effective APC



The Prioritization of Cancer Antigens:

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

Timeline of cancer vaccine development.



O. Finn

Nature Reviews | Immunology

Recent US immunotherapy approvals by type

TABLE of CONTENTS [Generic Drug Name (trade name): Manufacturer]

Checkpoint Inhibitors: anti PD-1 type (monoclonal antibodies)

Nivolumab (Opdivo): Bristol-Myers Squibb

Pembrolizumab (Keytruda): Merck

Checkpoint Inhibitors: anti PD-L1 type (monoclonal antibodies)

Atezolizumab (Tecentriq): Genentech

Avelumab (Bavencio): EMD Serono

Durvalumab (Imfinzi): Astrazeneza

Checkpoint Inhibitors: anti CTLA-4 type (monoclonal antibodies)

Ipilimumab (Yervoy): Bristol-Myers Squibb

Monoclonal antibody targeting CD20

Obinutuzumab (Gazyva): Genentech

Chimeric Antigen Receptor T-cells "CAR-Ts":

Axicabtagene (Yescarta): Kite Pharma

Tisagenlecleucel (Kymriah): Novartis

Oncolytic Virus:

Talimogene laherparepvec "T-VEC" (Imlygic): Amgen

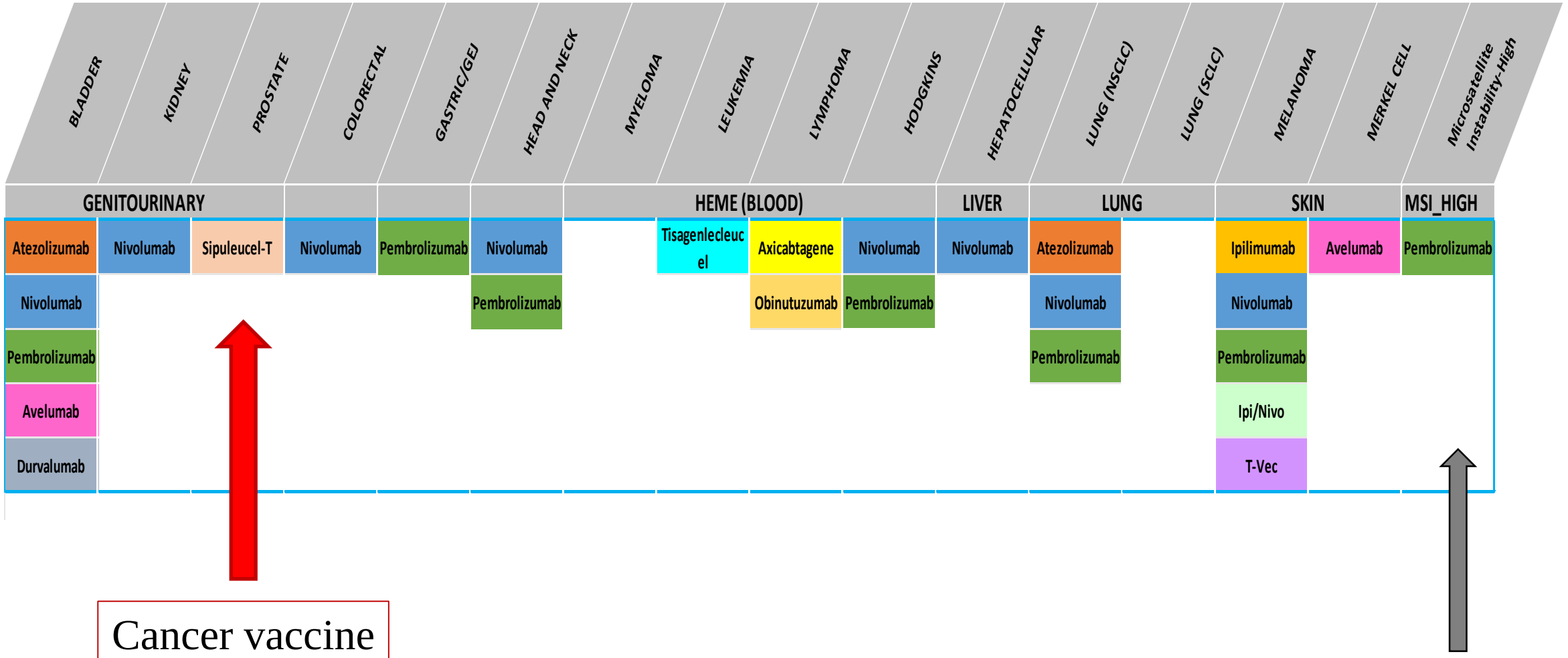
Recombinant Antigen Vaccine:

Sipuleucel T (Provenge): Dendreon

COMBINATION THERAPIES:

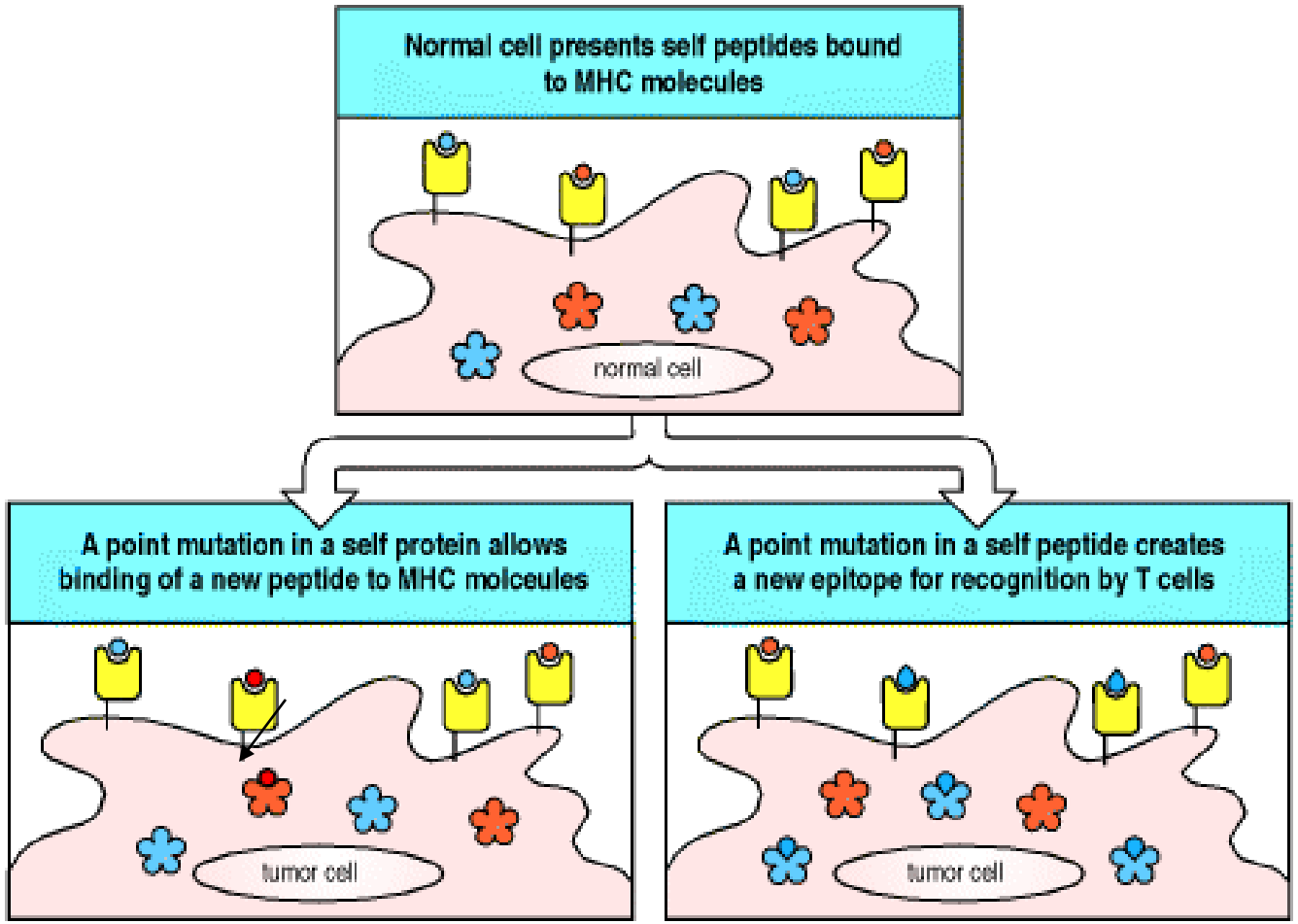
Ipilimumab + Nivolumab

US Immunotherapy Approvals by tumor



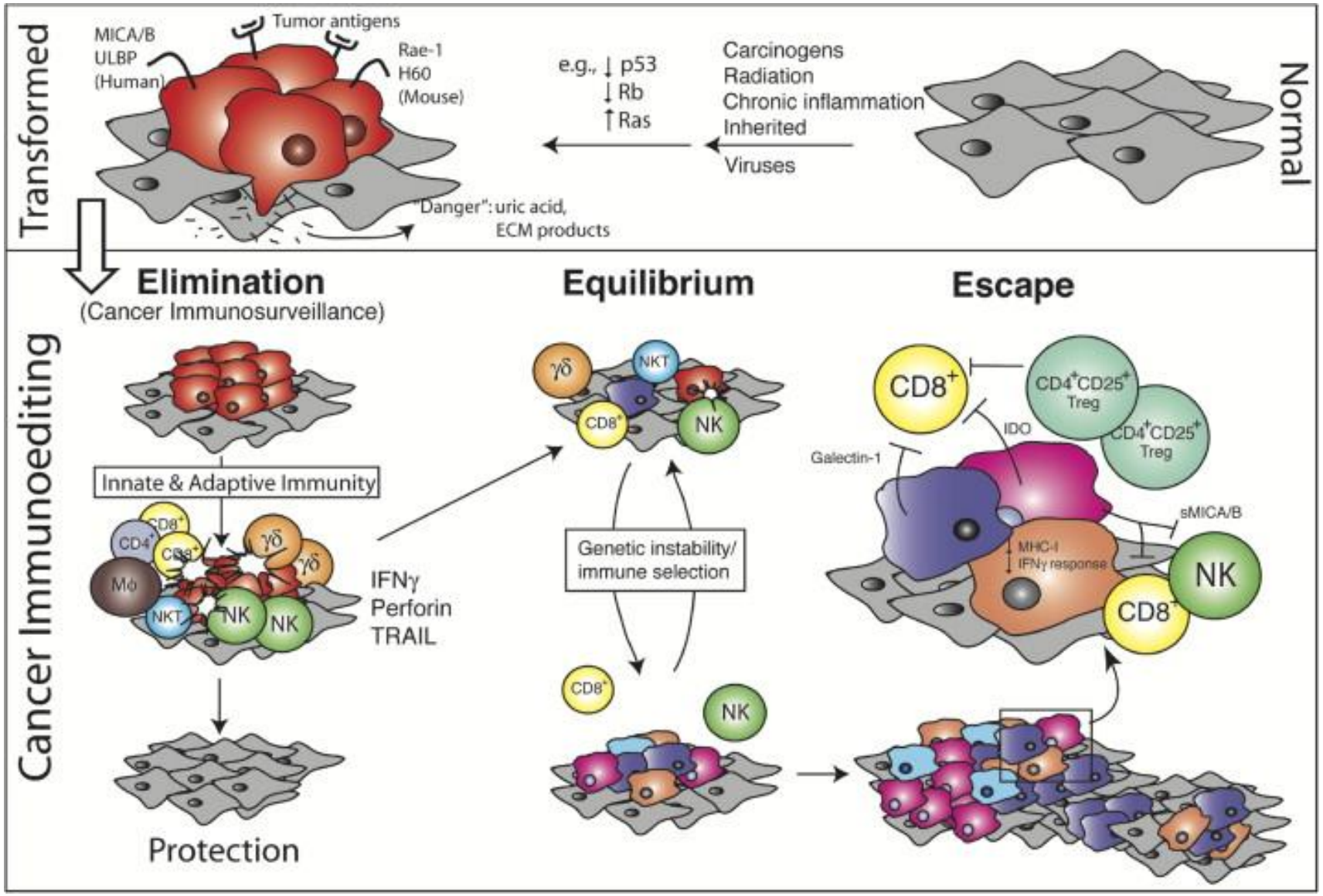
MSI-high tumors of any histology

Tumor Antigens
“private” or patient-specific



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

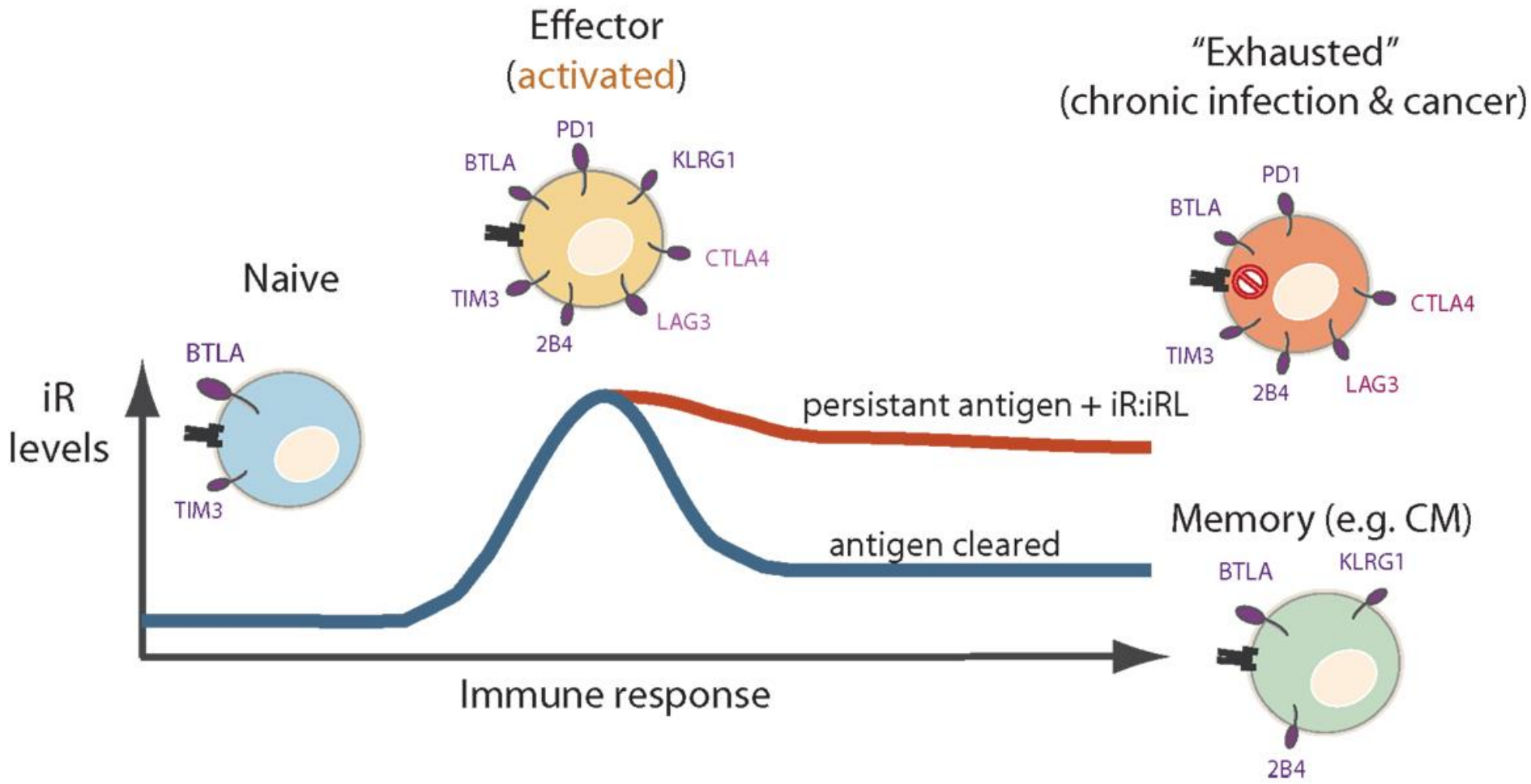
Three Phases of the Cancer Immuno-editing



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

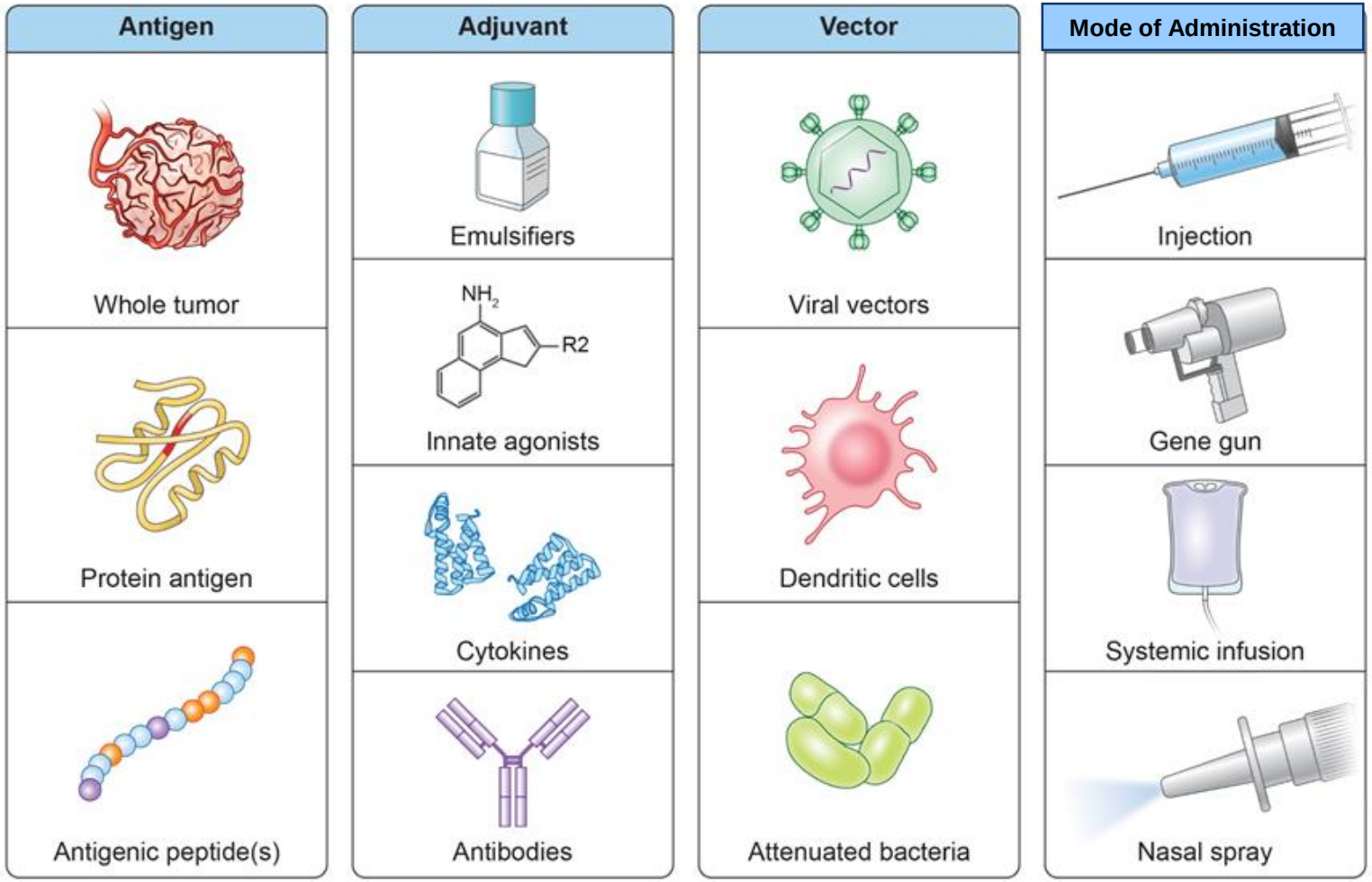
Cell Therapies for Cancer: Vaccines

Antigen Presenting Cells:

Allogeneic tumor cells (+/- cytokines like GM-CSF)
Autologous tumor cells (+/- cytokines like GM-CSF)
Transfected cell lines (MRC-5 + tumor DNA/RNA)
Activated B cells
Dendritic Cells

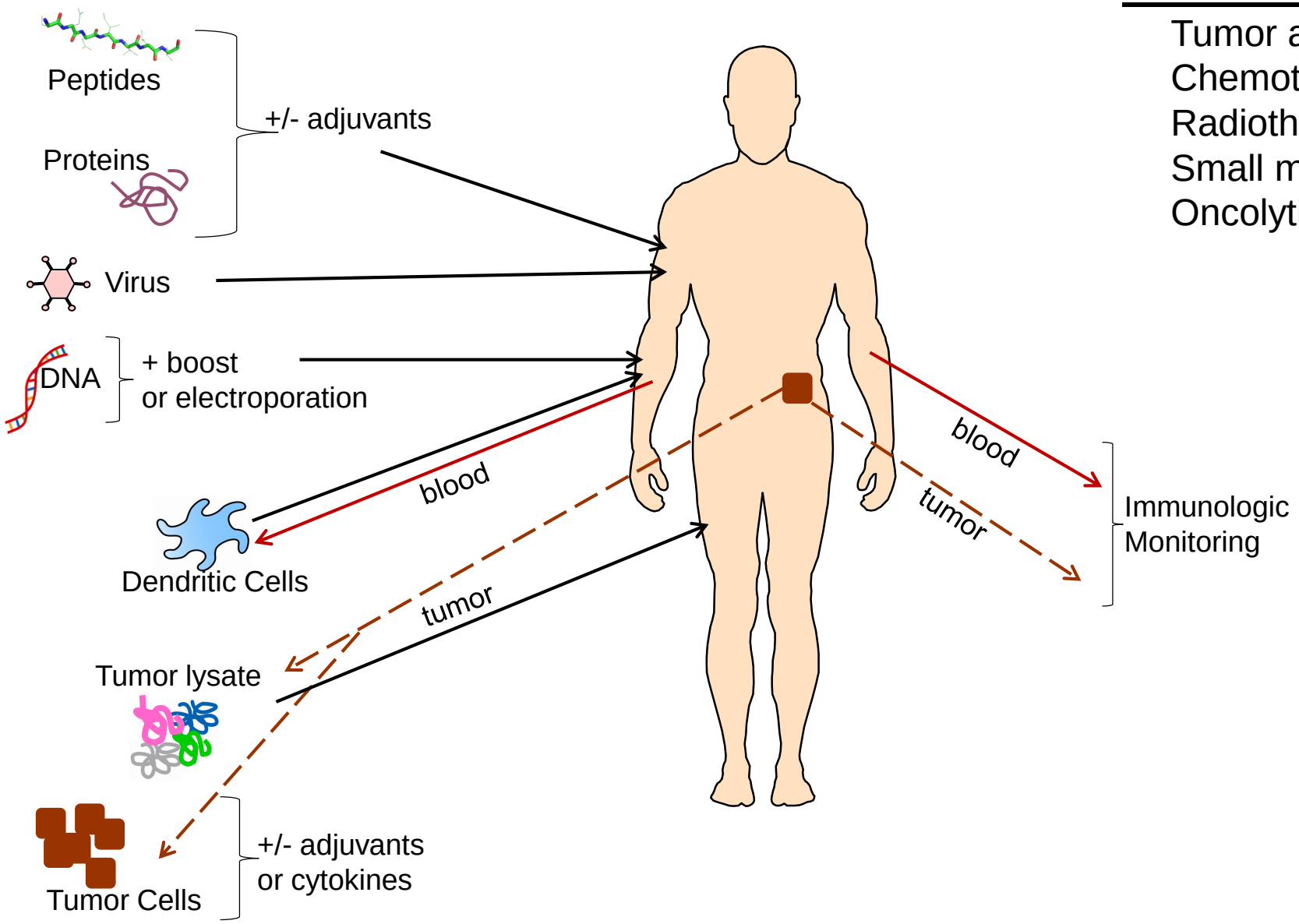
ADVANCES IN Cancer IMMUNOTHERAPY™

Components of a cancer vaccine



And RNA/DNA

Vaccine platforms

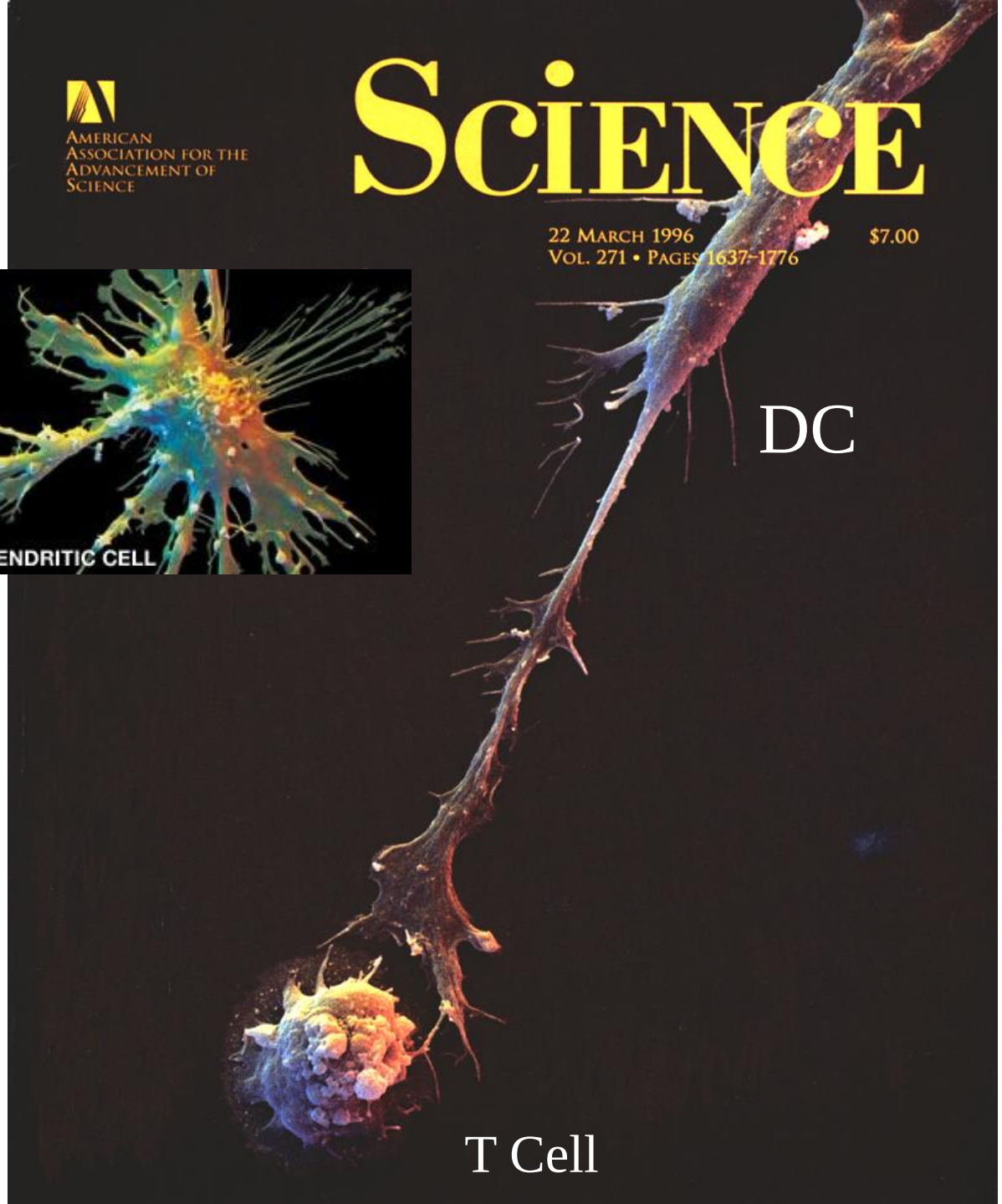


Vaccine Effects

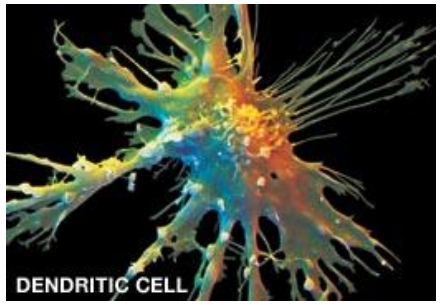
- Tumor ablation
- Chemotherapy
- Radiotherapy
- Small molecules
- Oncolytic virus

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.

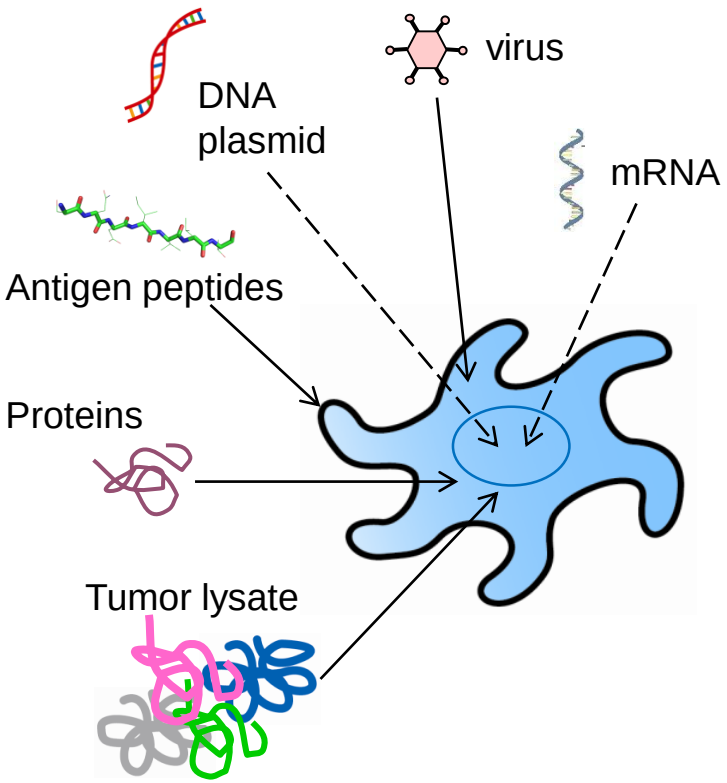
Recent DC vaccine studies (combinations, *author conclusions*):

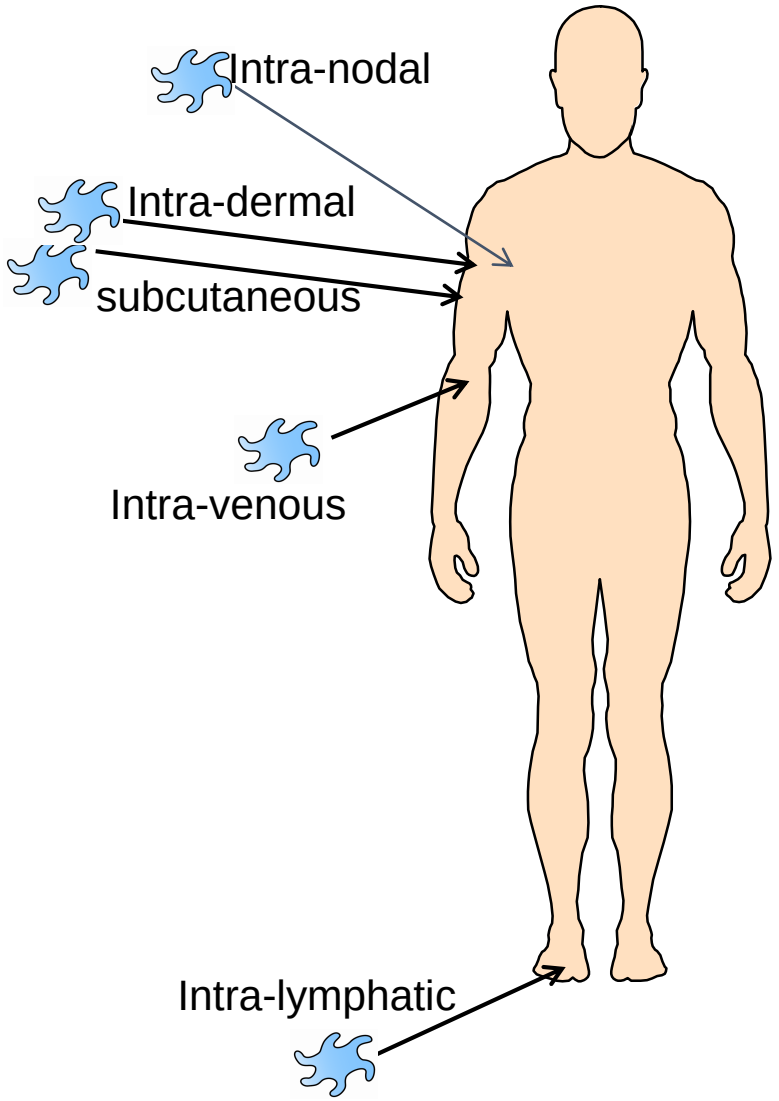
1. Kongstad, Svane: **Cytotherapy 2017**: DC + chemo in 43 prostate cancer pt. (*safe and immunogenic*)
2. Schreibelt, De Vries: **CaRes 2016**: 14 stg. IV melanoma pt., CD1c+ isolated blood DC, 16 hour culture, + gp100 and tyrosinase. *4/14 pt. PFS 12-35 mo*.
3. Wilgenhof, Neyns: **JCO 2016**: 39 “adv.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

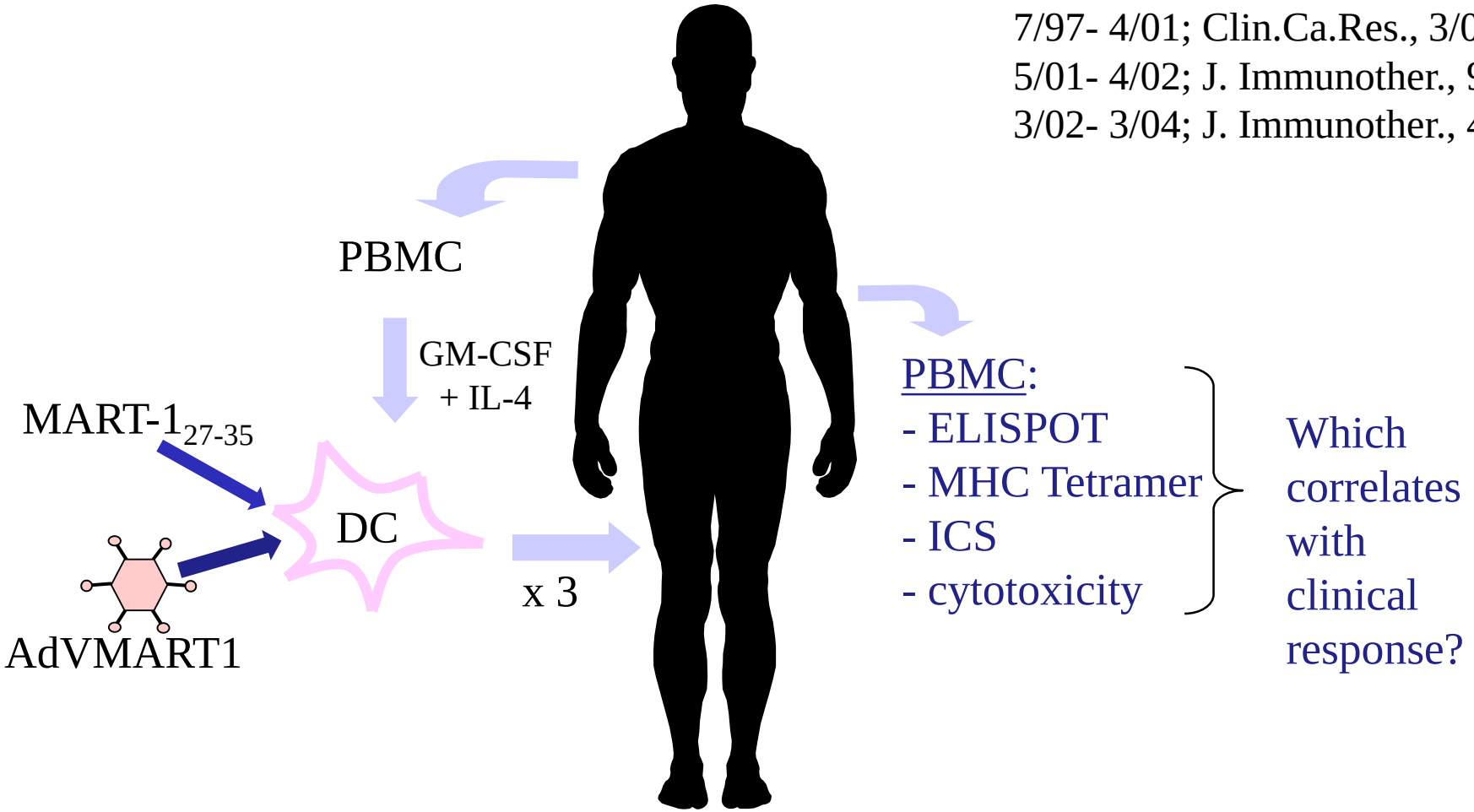
Antigen delivery to DC





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.)

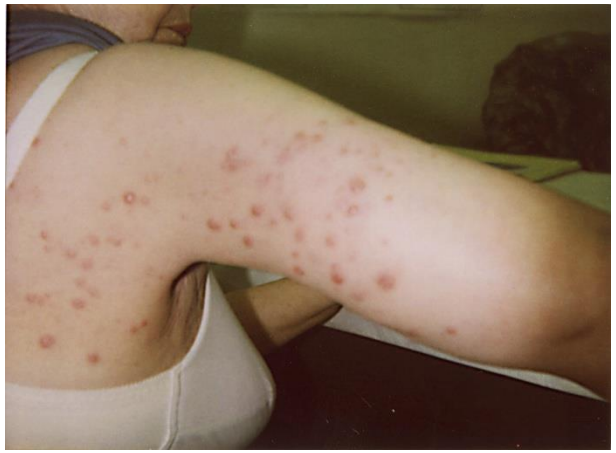
Pep. Phase II: 10^7 DC/injection, i.d. (10 pt.)

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

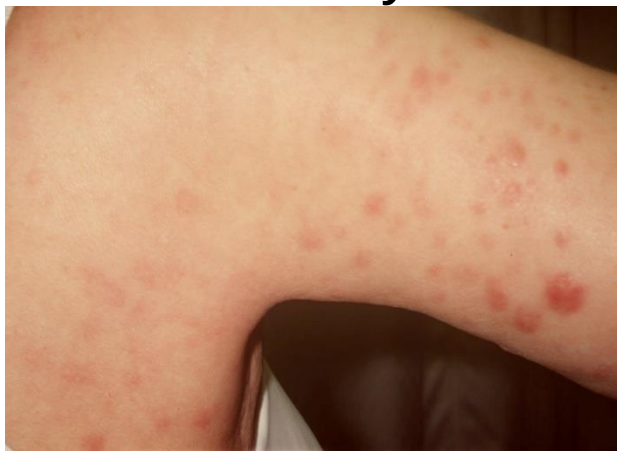
PI: J.S. Economou

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

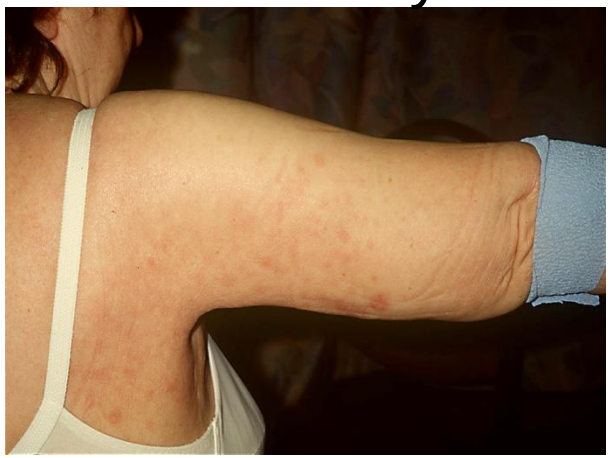
Pretreatment

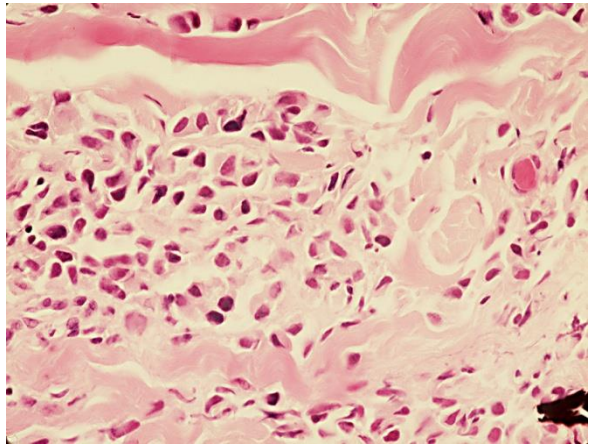


+56 days

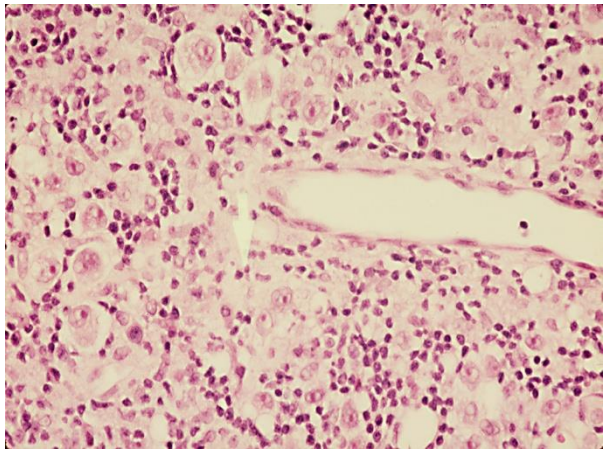


+130 days

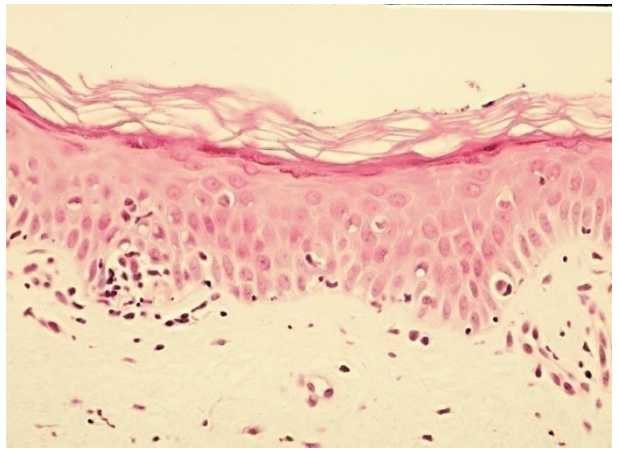




Melanoma Tumor



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:

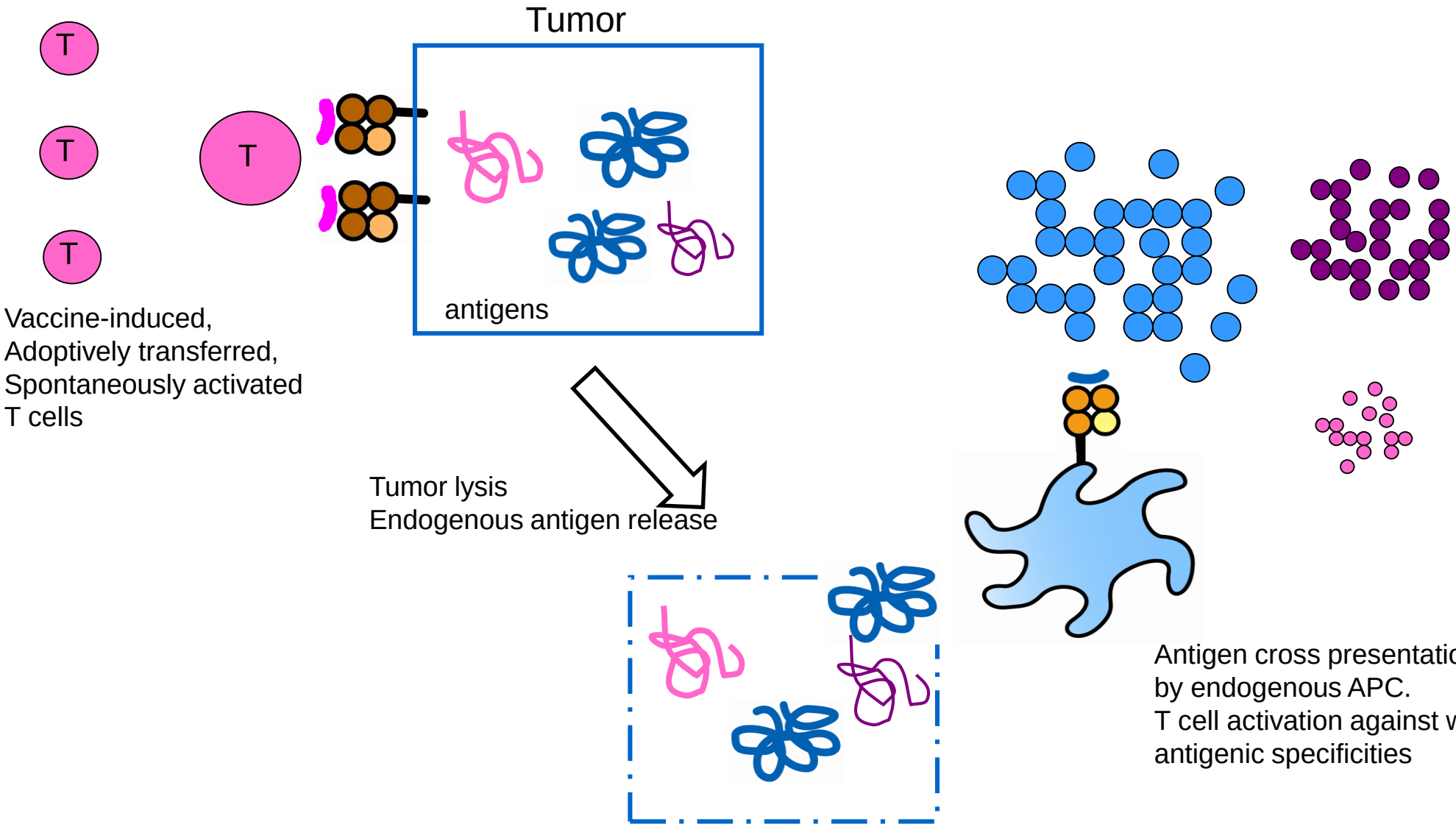
3/02-3/04 (23 enrolled); 14 received all 3 vaccines (all metastatic)

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

1 “unevaluable” (54+ mo.),

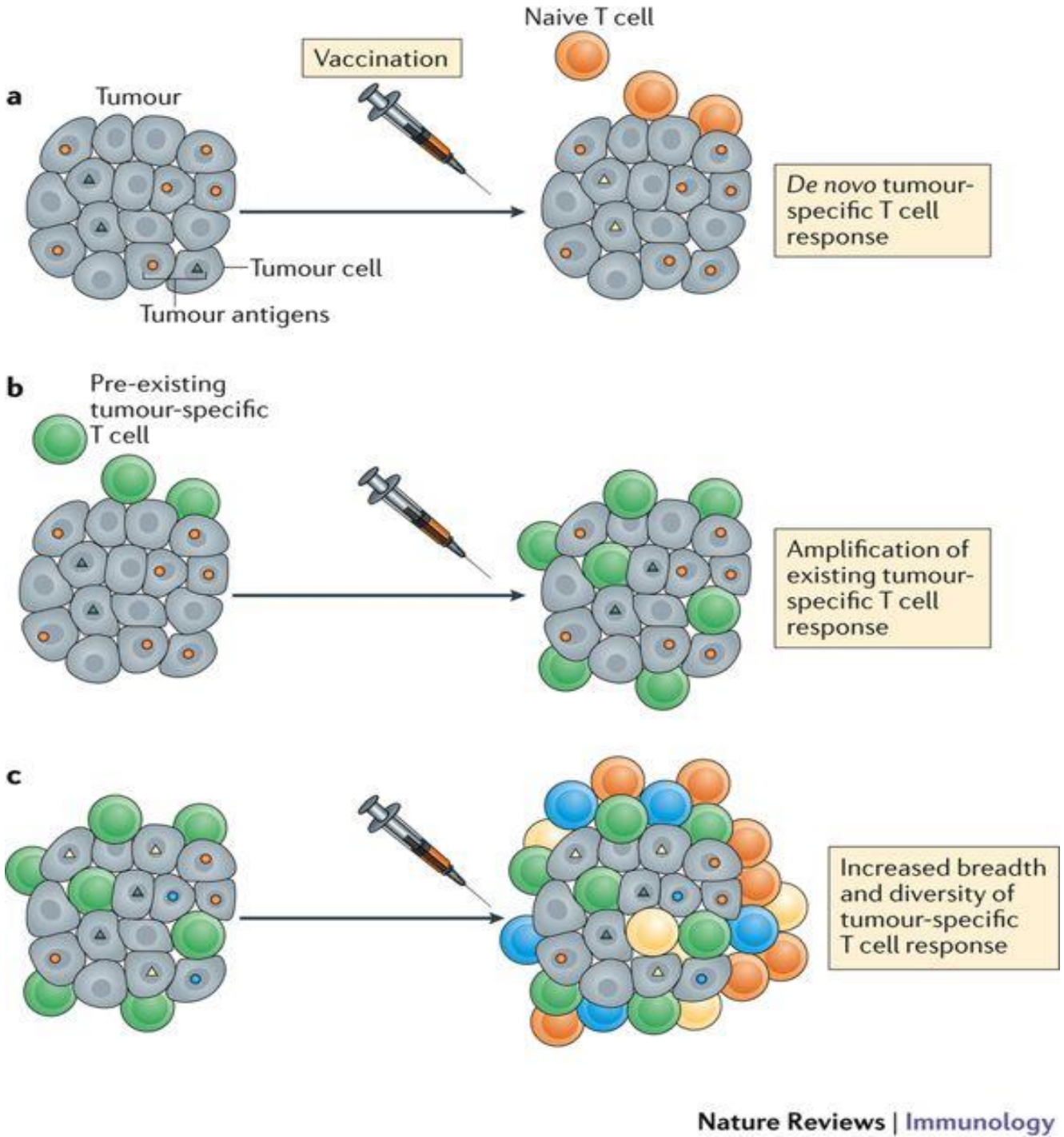
4 SD (27, 33, 36, 42 mo.), 1 became resectable/NED (56+ mo.)

Determinant/Epitope/Antigen Spreading



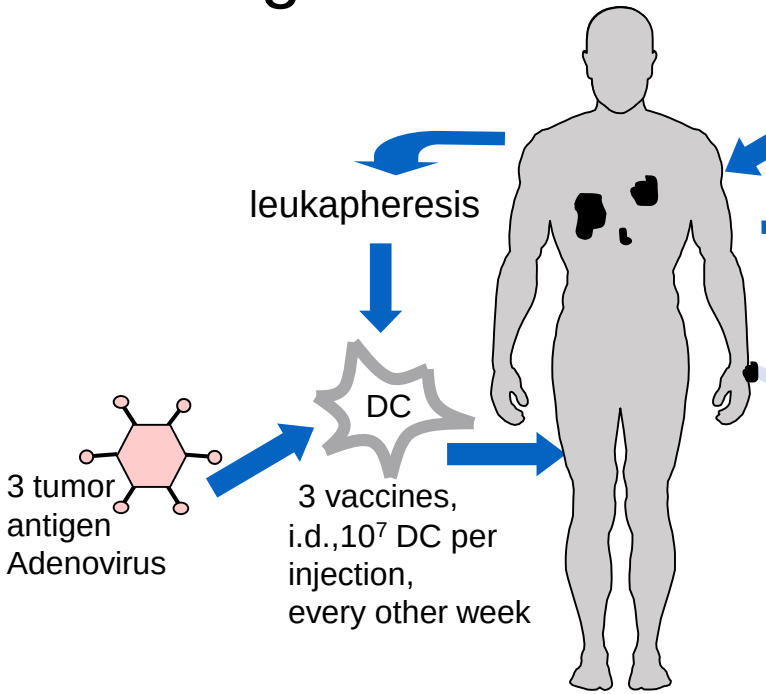
Ranieri ‘00; Disis ’02; Butterfield ‘03; Ribas ‘04; Wierecky ’06, Butterfield ’08

What have vaccines been shown to do?



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

Multi-Antigen-AdV-Transduced DC +/- IFNα Boost Trial

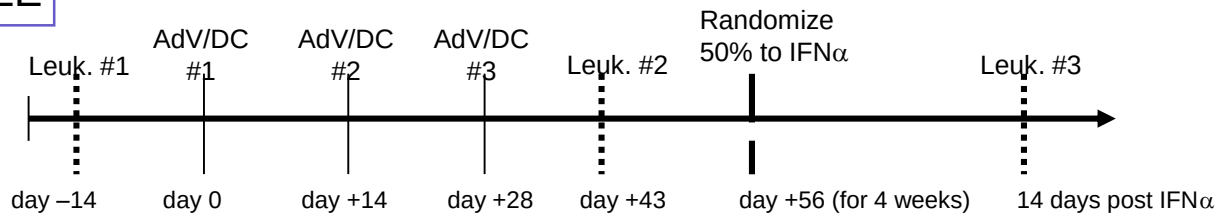


Leukapheresis/Biopsy:
CD8+/CD4+ PBMC:
-Multi-cytokine ELISPOT for immunizing antigens
-Determinant Spreading ELISPOT
-Serum Luminex
-Tetramer Assay/phenotyping
-Avidity (A2/DR4)
-NK activation
-TIL analyses
-Tumor antigen analysis

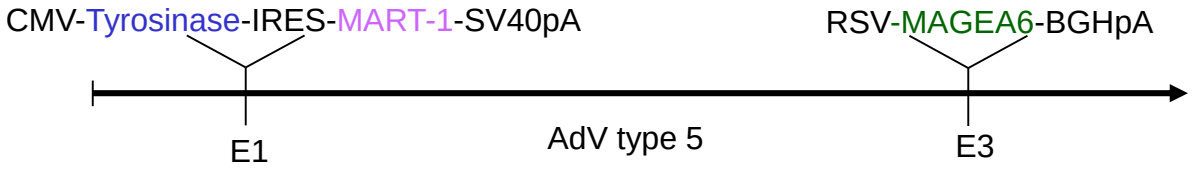
30 Patients Randomized:
1:1 to high dose i.v. IFNα

Tumor Biopsy:
Analysis of vaccine antigens
Analysis of additional antigens

SCHEDULE



VECTOR MAP

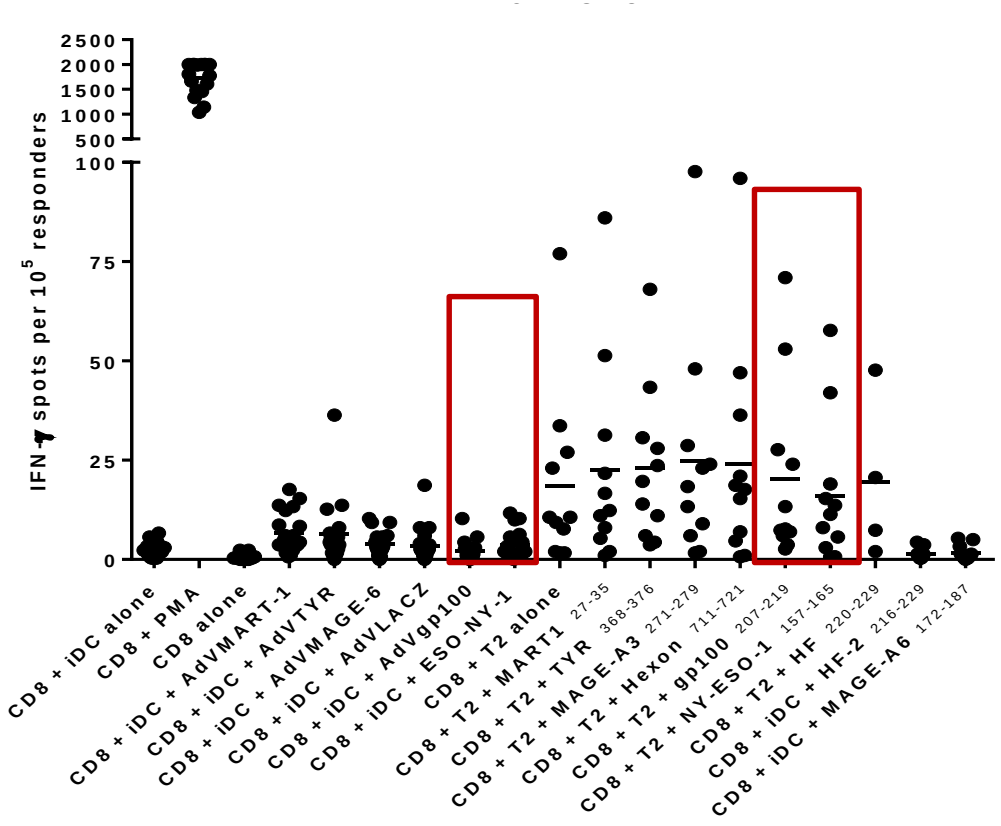


JITC, 2019

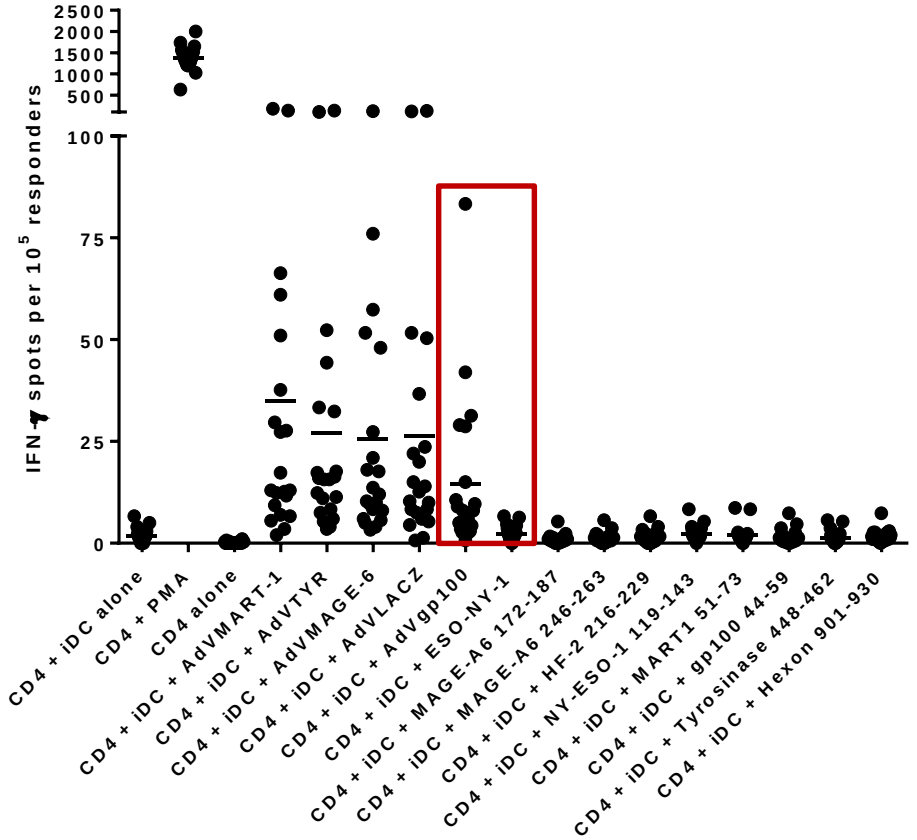
T cell subset ELISPOT analysis
Determinant spreading antigens

Day 43 post vaccines:

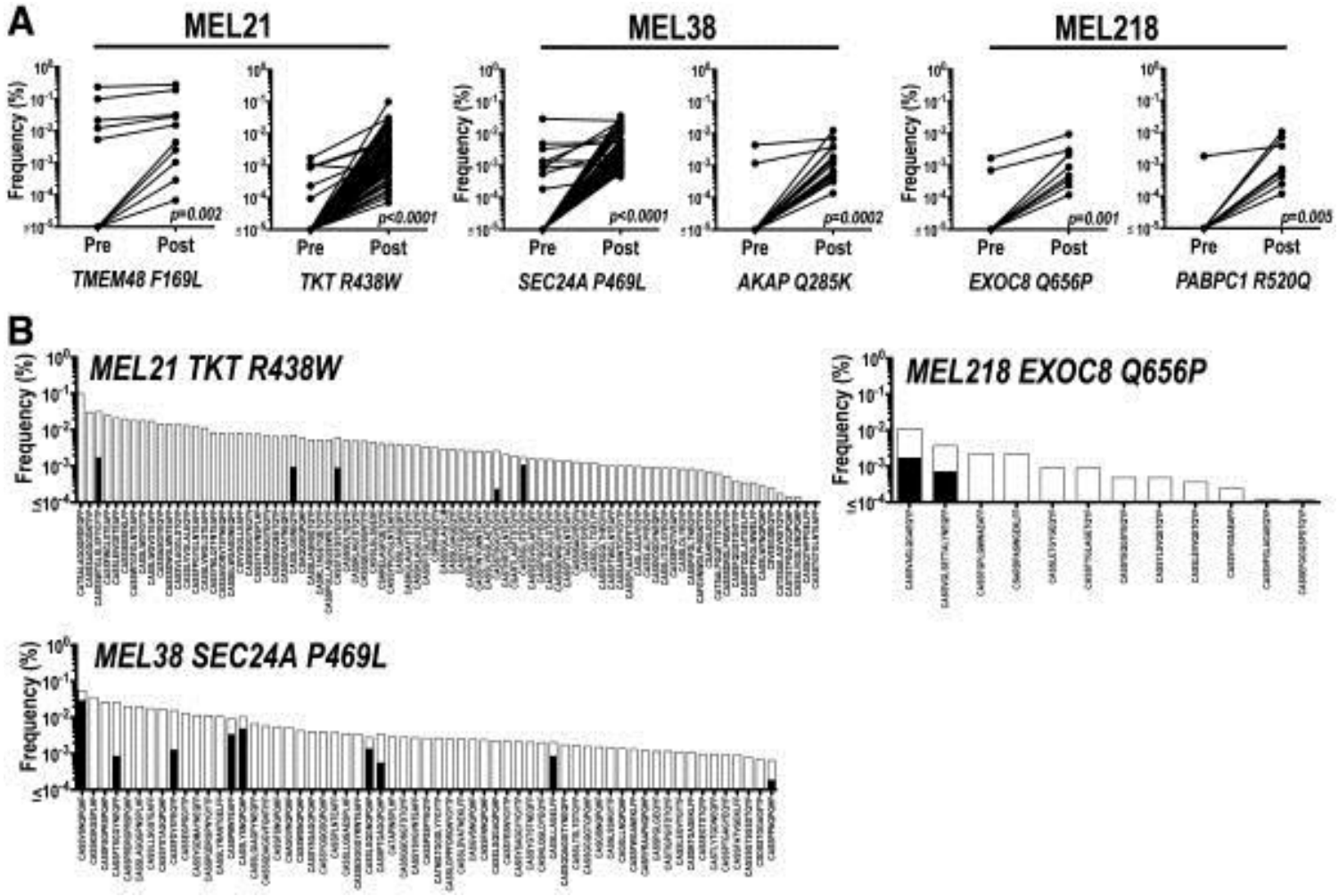
CD8+



CD4+



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

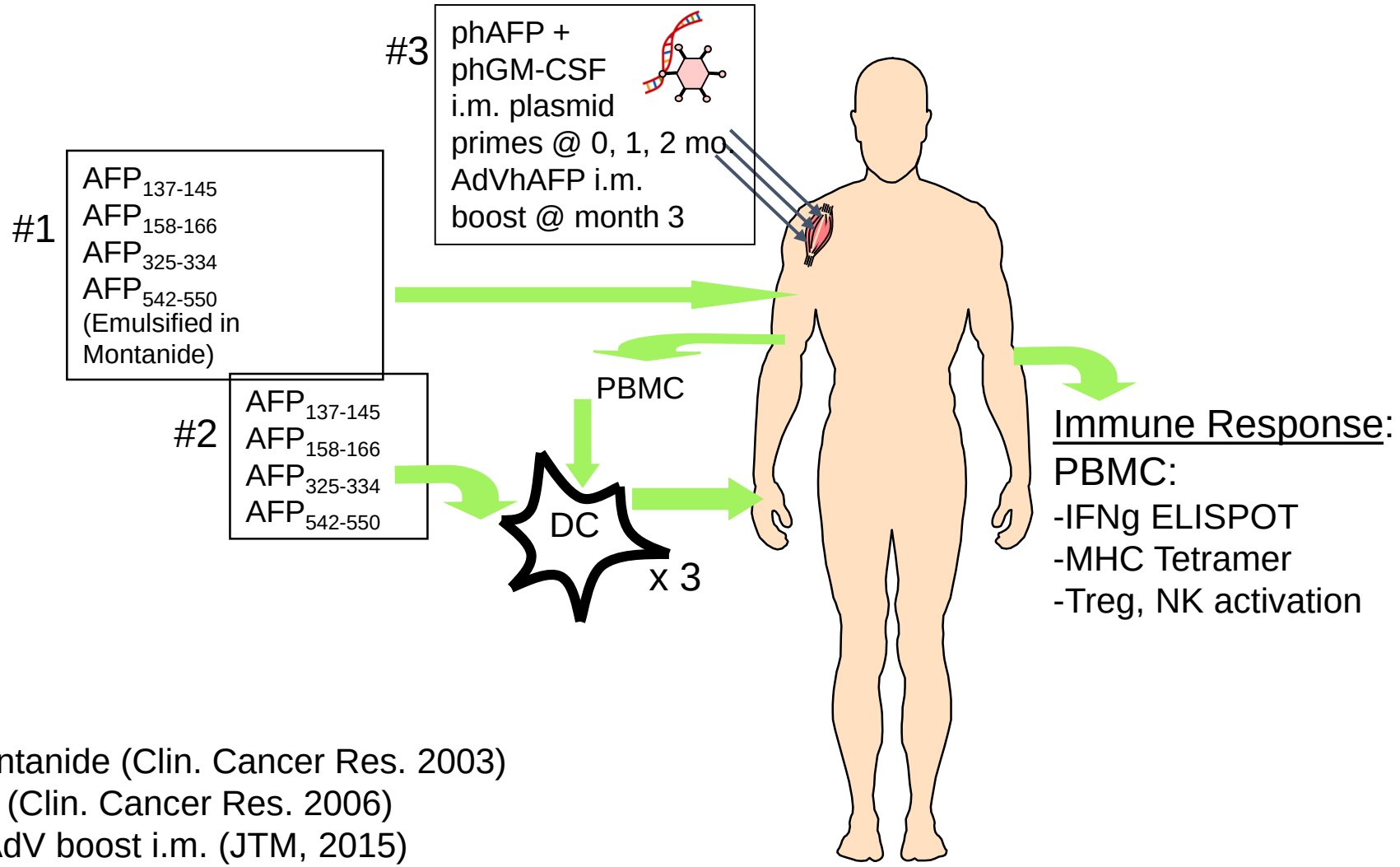
Science. 2015 May 15 Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells.
Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, Ly A, Lie WR, Hildebrand WH, Mardis ER, Linette GP

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

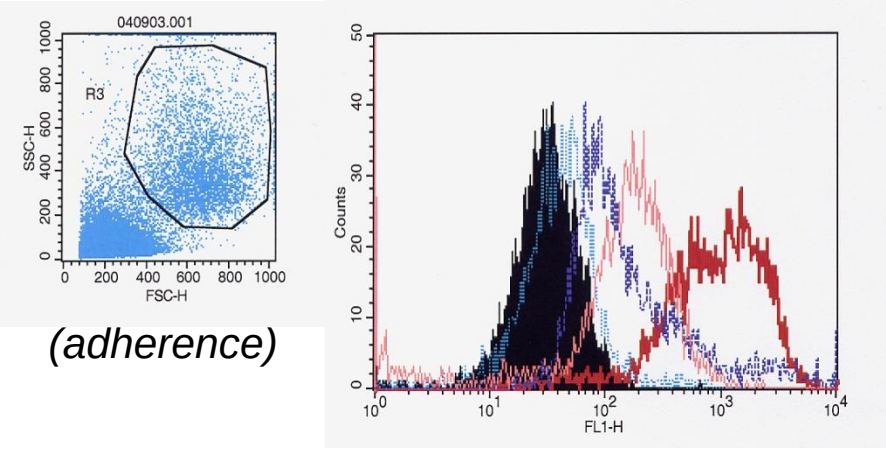
AFP peptides/DC:

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

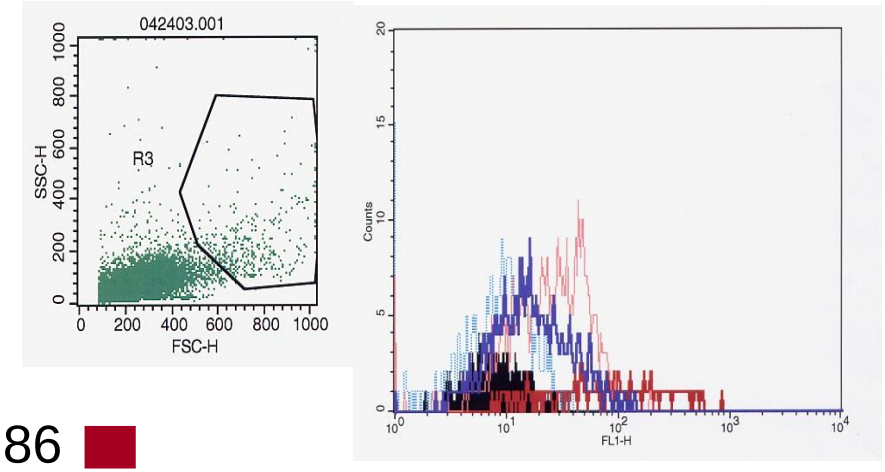
AFP DNA prime/AFPAdV boost:

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.

Patient Autologous DC Vaccine Cells



(adherence)



CD86
CD40
CD83
CCR7

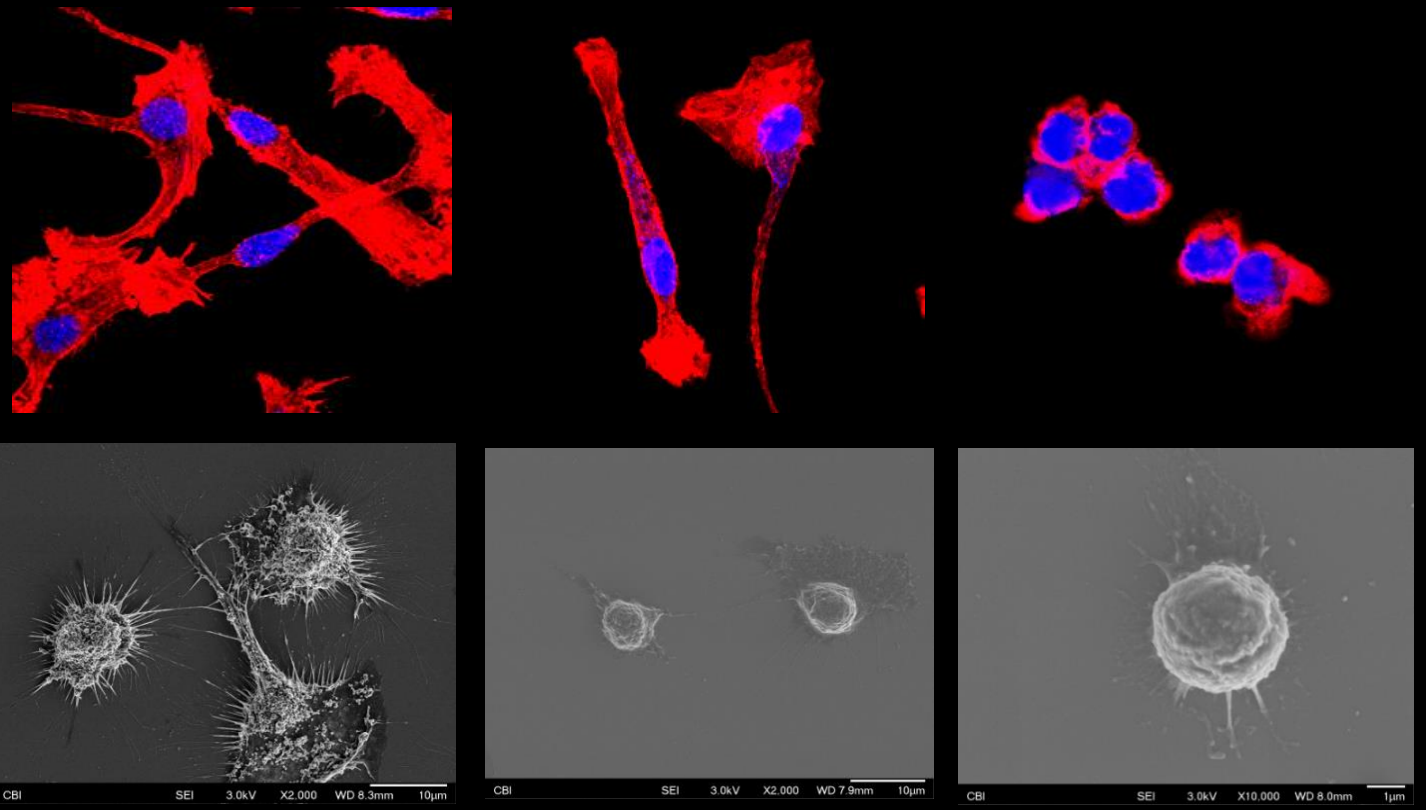
Example from an immunotherapy vaccine study. Some patients were able to expand large numbers of DC bearing cell surface markers CD40, CD83, CD86 and CCR7, but not all. **These 2 patients did not receive the same vaccine.**

Important data in dot plots and histograms often not presented in published papers

Butterfield, CCR 2006

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

OVA nAFP tAFP



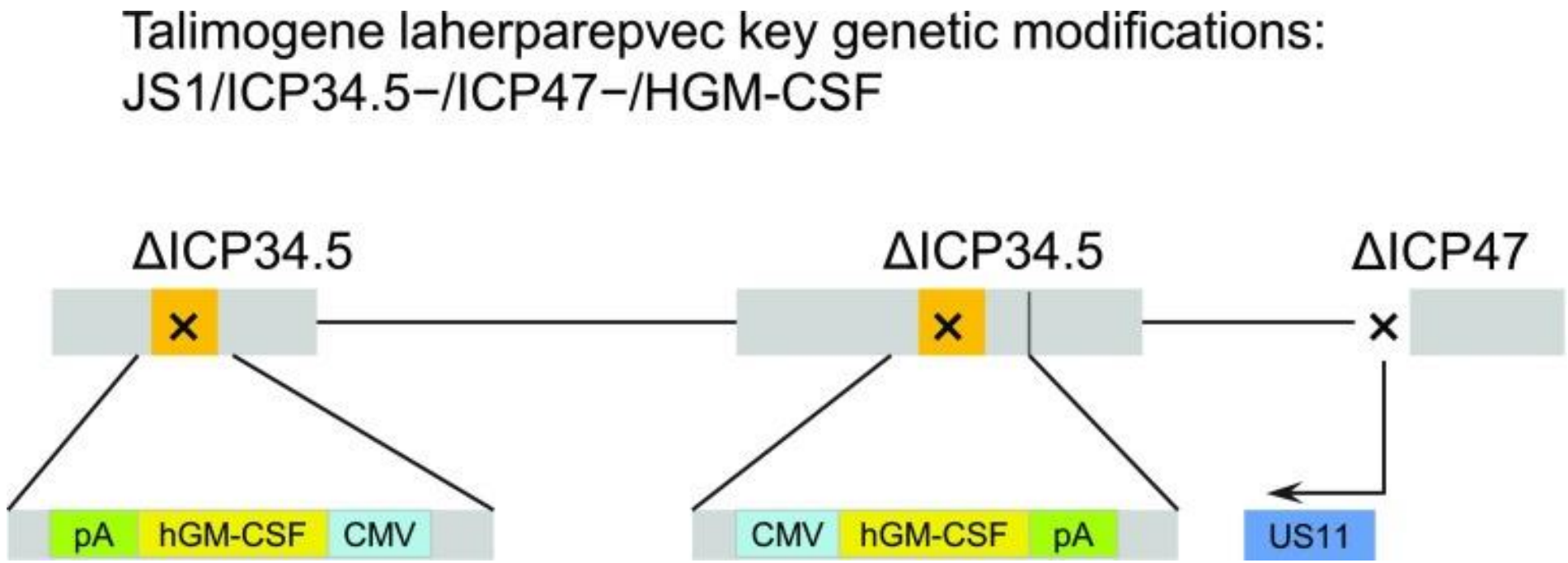
AFP alters DC phenotype to an immature phenotype that cannot be reversed by maturation, AFP inhibits DC metabolic function and T cell stimulatory capability (Pardee 2014, Santos 2019)

Other effective platforms: Synthetic and Viral Vaccines

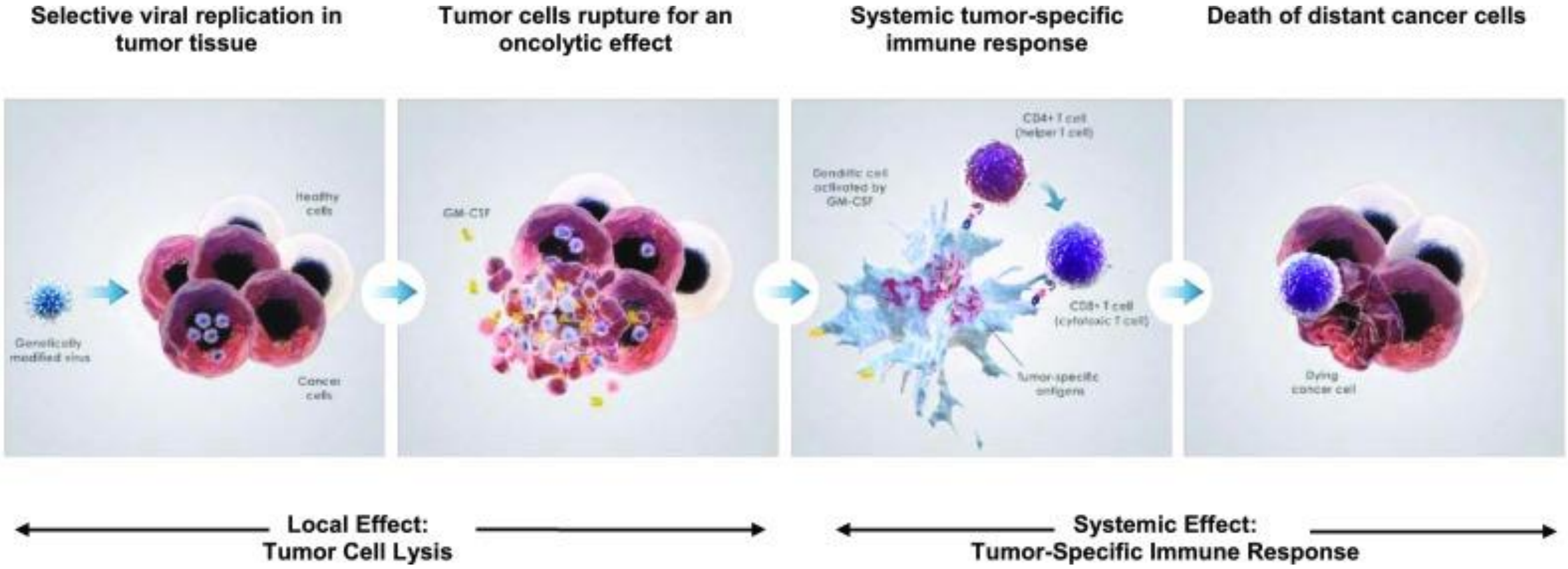
1. TVEC (Amgen) ***FDA approved 2015**
   - Oncolytic virus: HSV-1 + GM-CSF transgene
   - Metastatic melanoma, 26% response rate (vs. 6% in control arm)
2. ISA101 (Immune System Activation)
   - HPV16 Synthetic long peptide (SLP, 24-32mer) in Montanide
   - Cervical cancer
   - Appears to synergize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

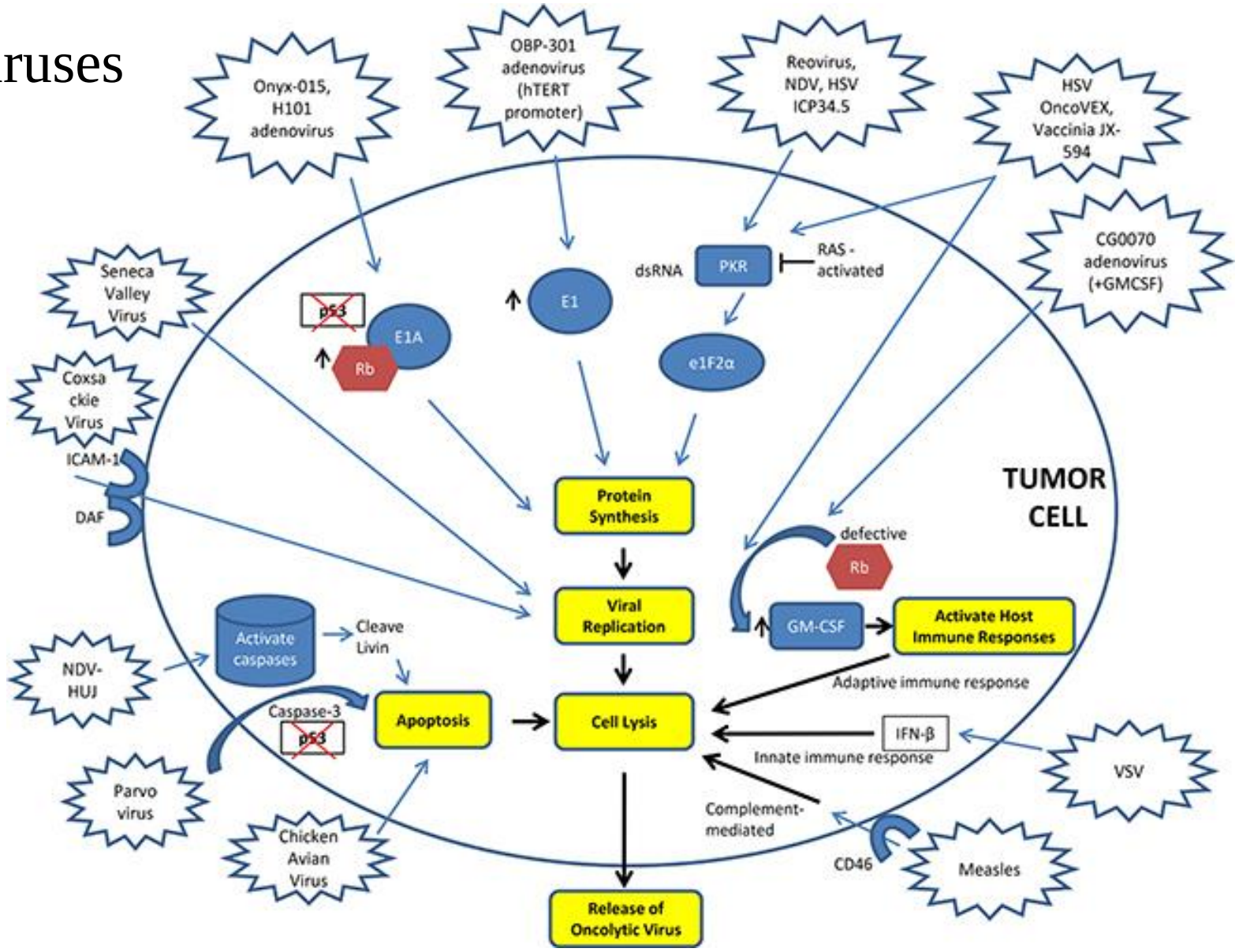
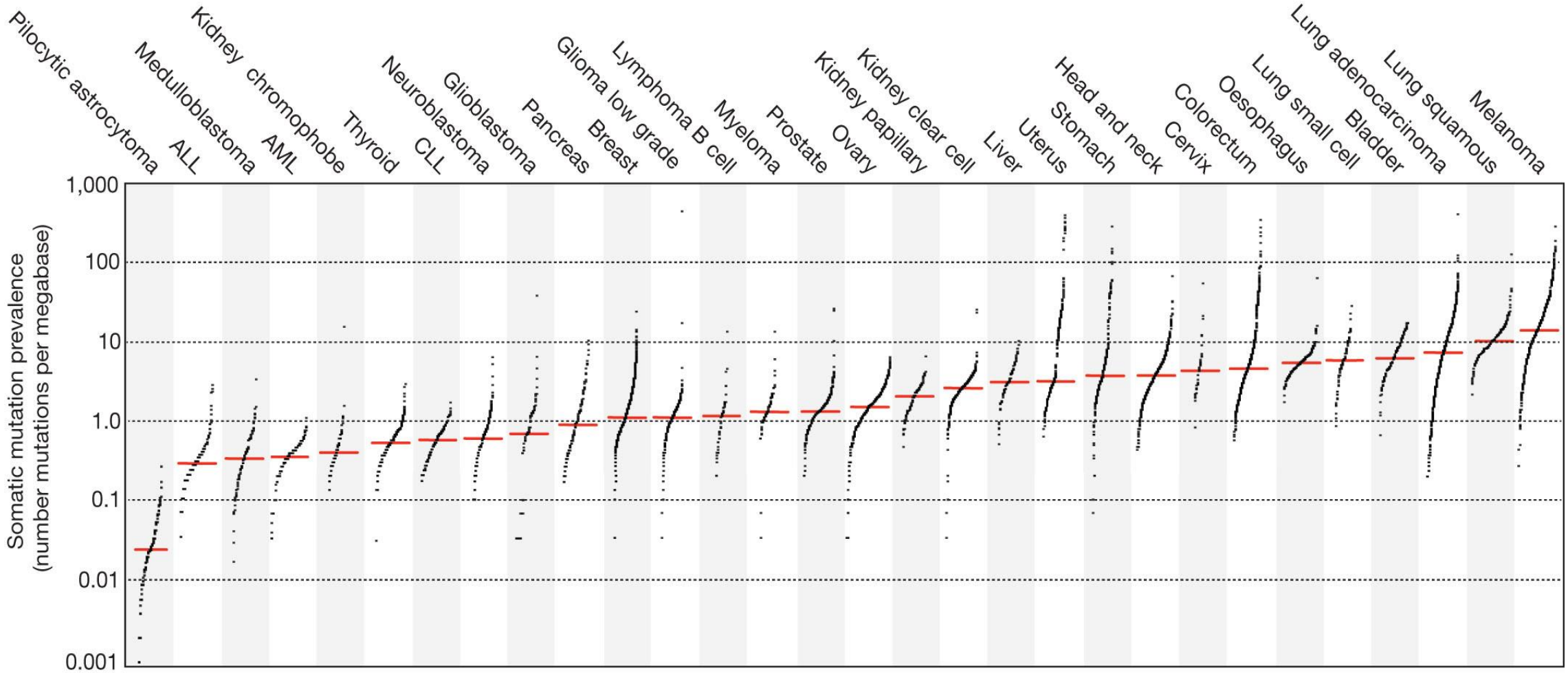


Figure 1: Mechanisms of action of oncolytic viruses. DAF – Decay Accelerating Factor, GM-CSF – Granulocyte Macrophage-Colony Stimulating Factor, HSV – Herpes Simplex Virus, hTERT – Human Telomerase, ICAM-1 – Intercellular Adhesion Molecule-1, ICP – Infectious Cell Protein, INF-β – Interferon beta, NDV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

The prevalence of somatic mutations across human cancer types.



LB Alexandrov *et al. Nature*, (2013)

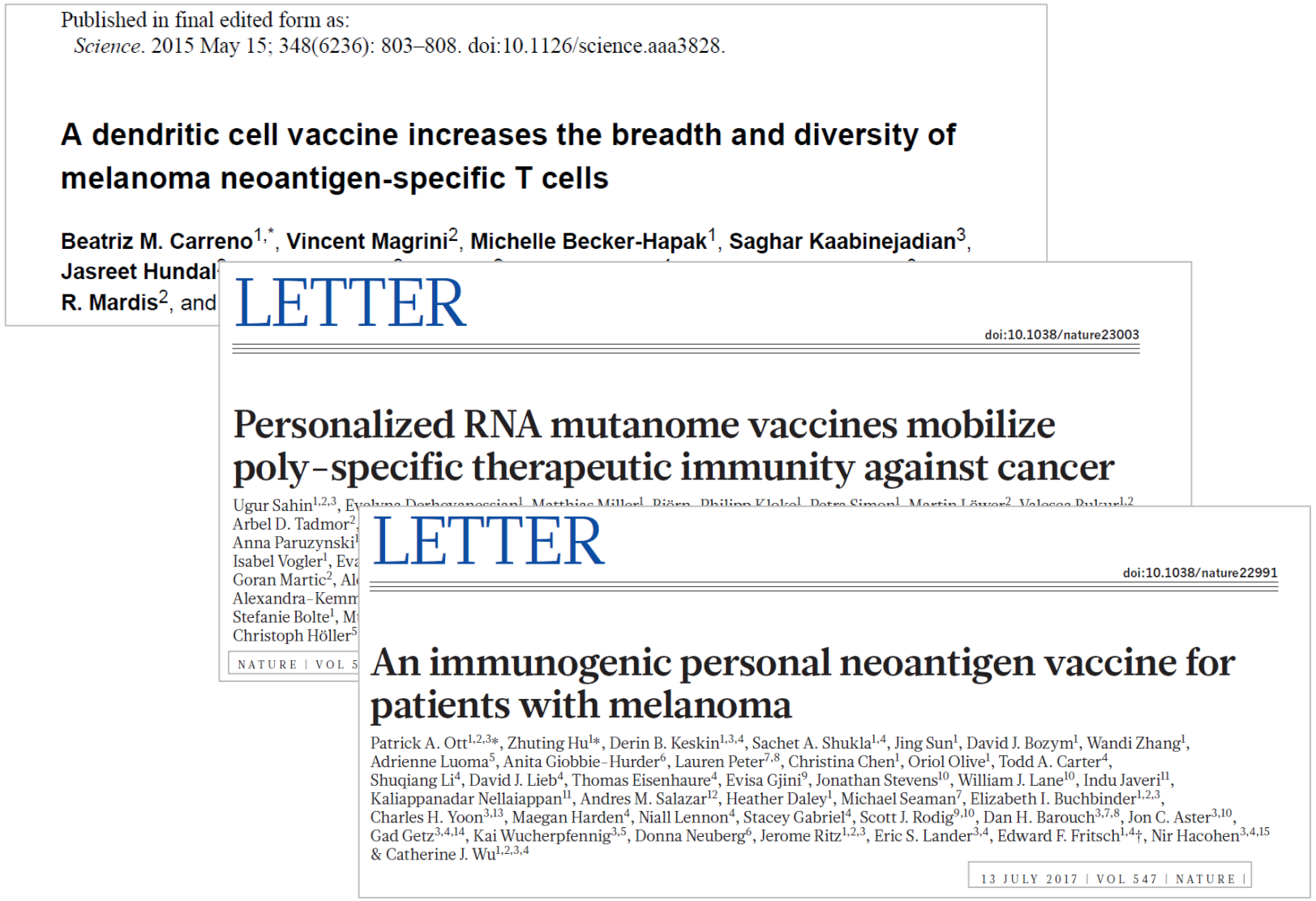
nature

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



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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[3], R. Mardis[2], and

LETTER

doi:10.1038/nature23003

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

Ugur Sahin[1,2,3], Evelyna Derhovanessian[1], Matthias Miller[1], Björn, Philipp Kloke[1], Petra Simon[1], Martin Löwer[2], Valesca Pukurl[1,2], Arbel D. Tadmor[2], Anna Paruzynski[1], Isabel Vogler[1], Eva, Goran Martic[2], Al, Alexandra-Kemm, Stefanie Bolte[1], M, Christoph Höller[5]

NATURE | VOL 5

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]

13 JULY 2017 | VOL 547 | NATURE |

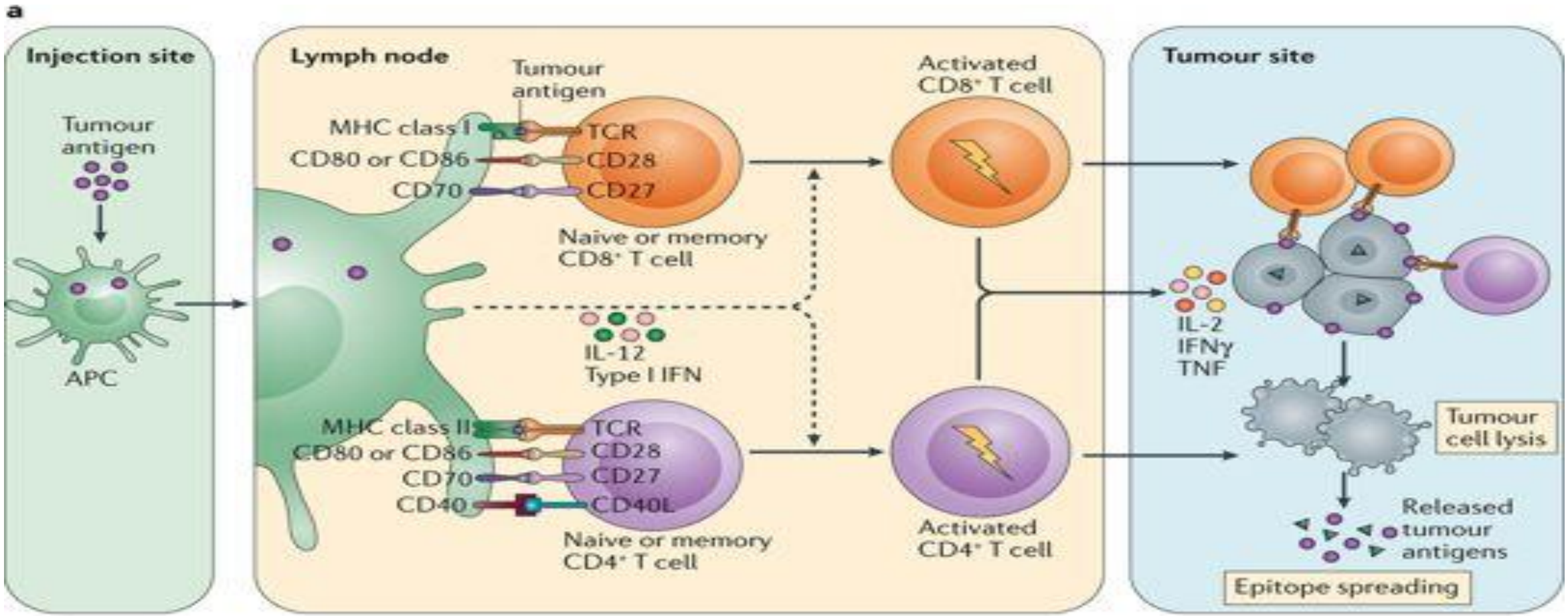
•Neoantigens have emerged as targets of effective tumour-directed T cell responses. Increased neoantigen load is associated with improved patient outcomes.

•**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.

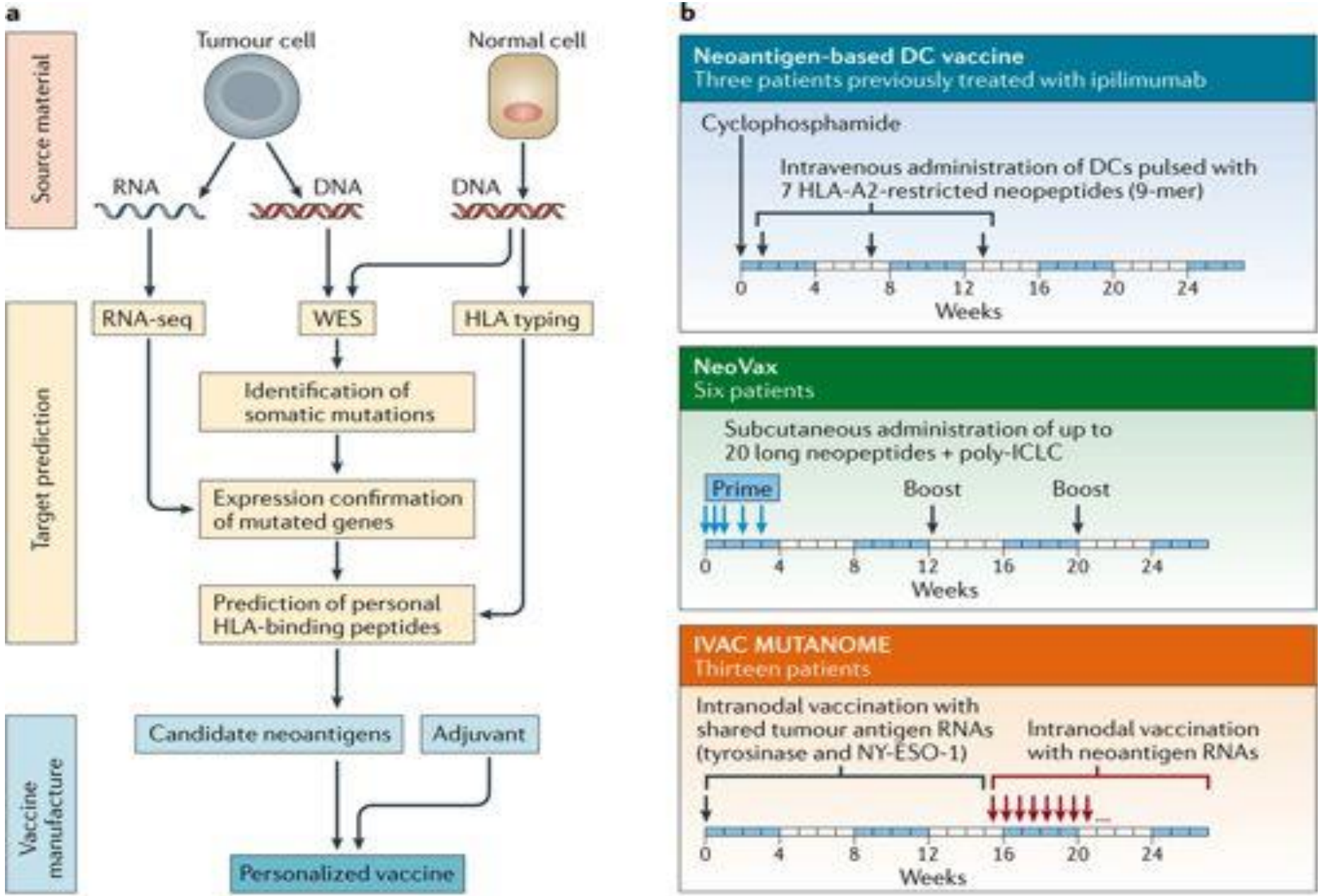
•A crucial aspect of a vaccine targeting neoantigens is the selection of epitopes that can be presented *in vivo* by tumour or antigen-presenting cells. HLA-binding prediction, high-resolution mass spectrometry and understanding of antigen processing are important research areas for further discovery.

•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*

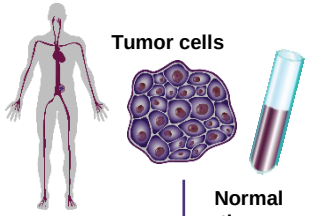


Z. Hu, P. Ott, C. Wu *Nat Rev Immunol 2018*

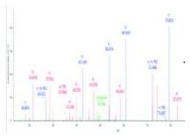


Z. Hu, P. Ott, C. Wu *Nat Rev Immunol 2018*

Computational identification of neoantigens is a multistep-process



Mass Spec.

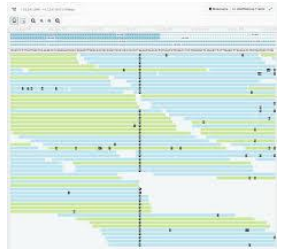


Ranked neoepitopes

DNA + RNA Sequencing

Data produced:
DNA Sequence
(30 Gigabytes!)

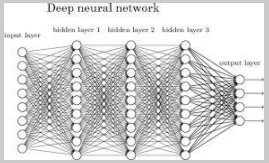
Variant Identification



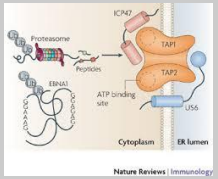
- SNV
- MNV
- FS
- Indel

Data produced:
variant call file (vcf)

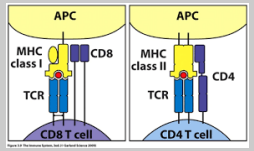
Neoantigen identification



Deep machine learning

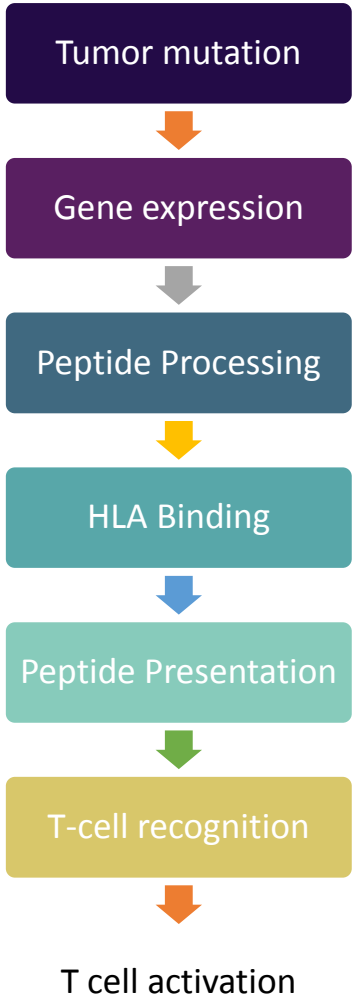


Antigen processing models



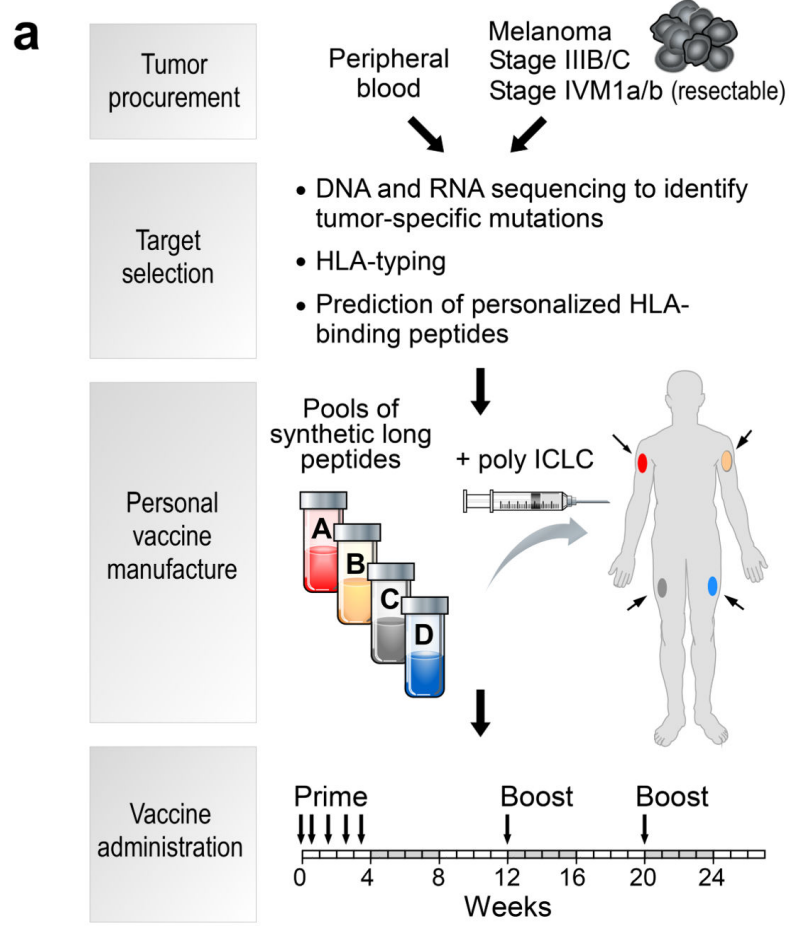
MHC I Binding prediction

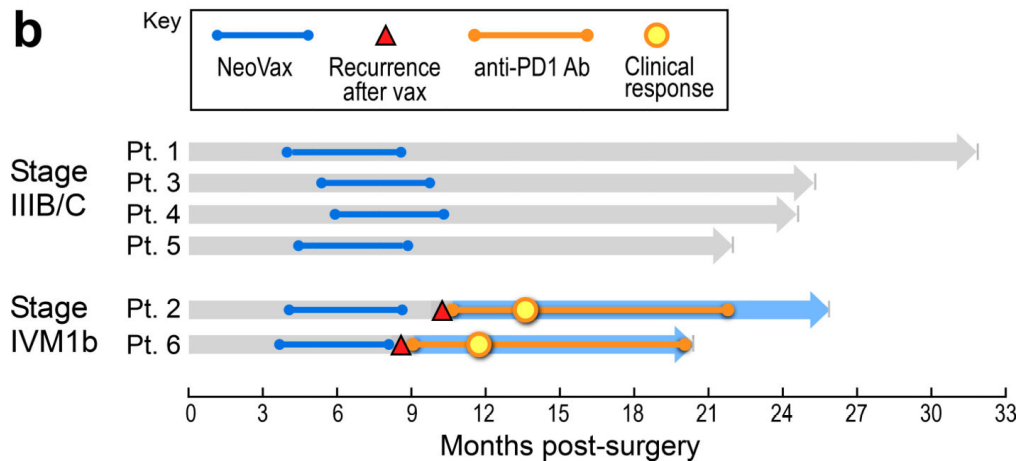
There is a need for better prediction models



- Only a fraction of identified mutations are expressed and translated
- Only a fraction of the expressed mutated peptides is presented on the HLA
- Only a fraction of these neoepitopes are immunogenic and recognized by autologous T cells
- No one knows what makes a peptide immunogenic

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



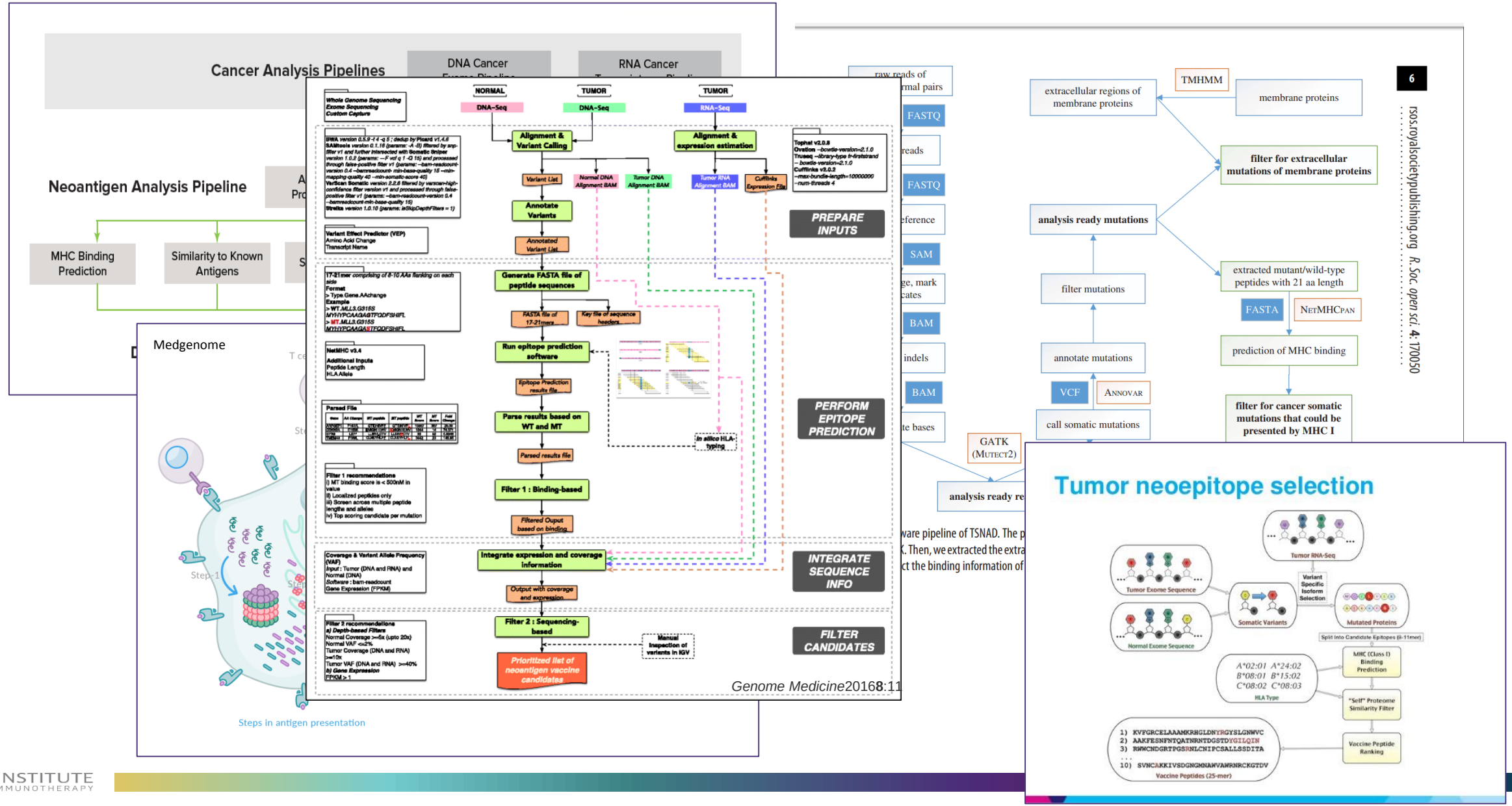


A. Somatic mutations were identified by WES of melanoma and germline DNA and their expression confirmed by tumor RNA-sequencing. Immunizing peptides were selected based on HLA binding predictions. Each patient received up to 20 long peptides in 4 pools.

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

Neoepitope pipelines are becoming more common, diverse and complex



TESLA : a community-based effort to optimizing neoepitope discovery

Nadine Defranoux, PhD

PARKER INSTITUTE for CANCER IMMUNOTHERAPY

CANCER RESEARCH INSTITUTE

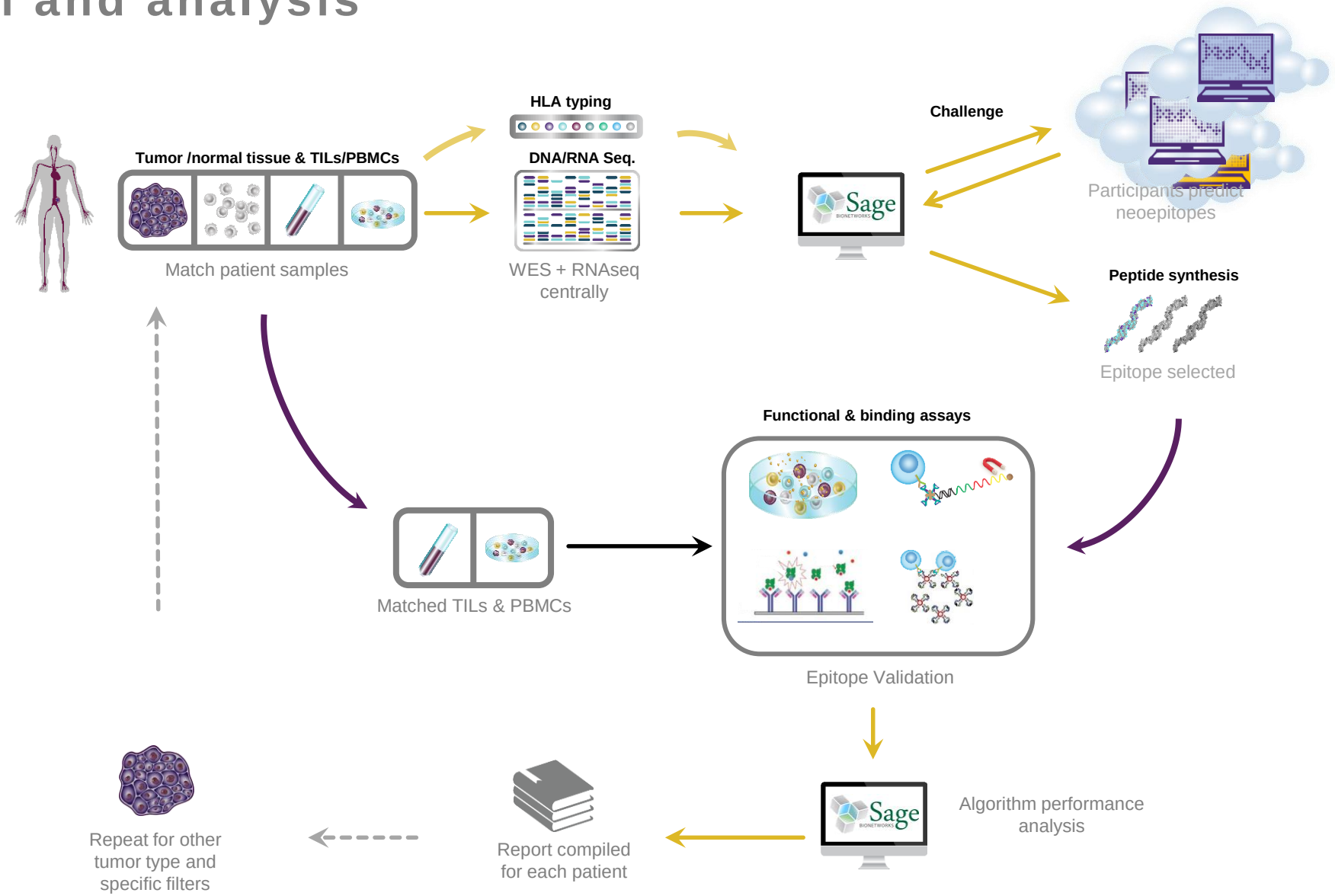
Sage BIONETWORKS

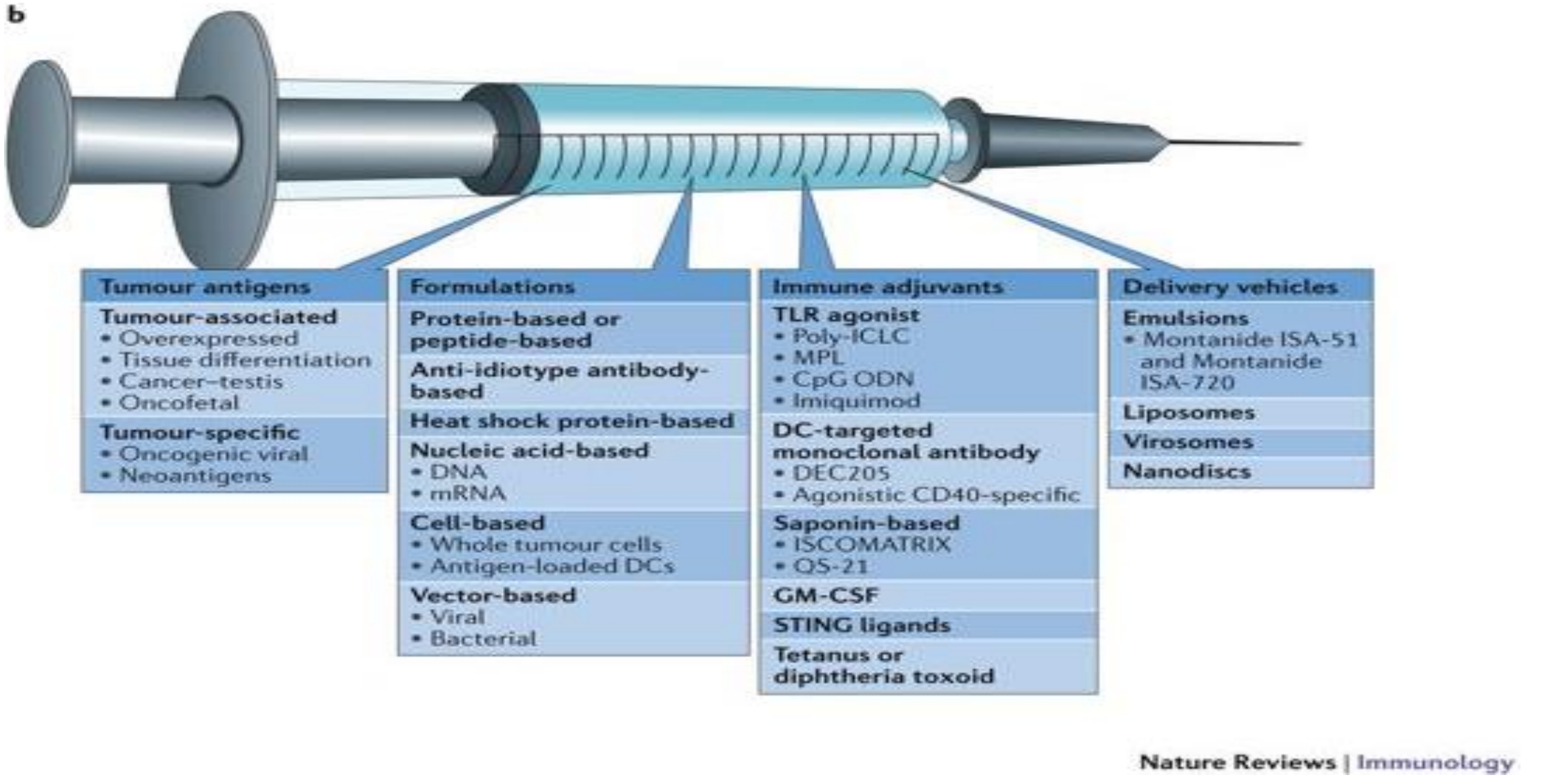
The Tumor neoEpitope SeLection Alliance

- TESLA aims to :
  - Bring together key players in the field of neoantigen discovery
  - Elucidate current differences in prediction methodologies
  - Generate high quality epitope validation sets that provide a basis for participating groups to assess and improve their prediction pipelines
  - Identify the best algorithm features that predict which tumor neoantigens are recognized by T cells and stimulate an immune response
  - Assess and expand the viability of epitope prediction methods to a broad array of cancer types

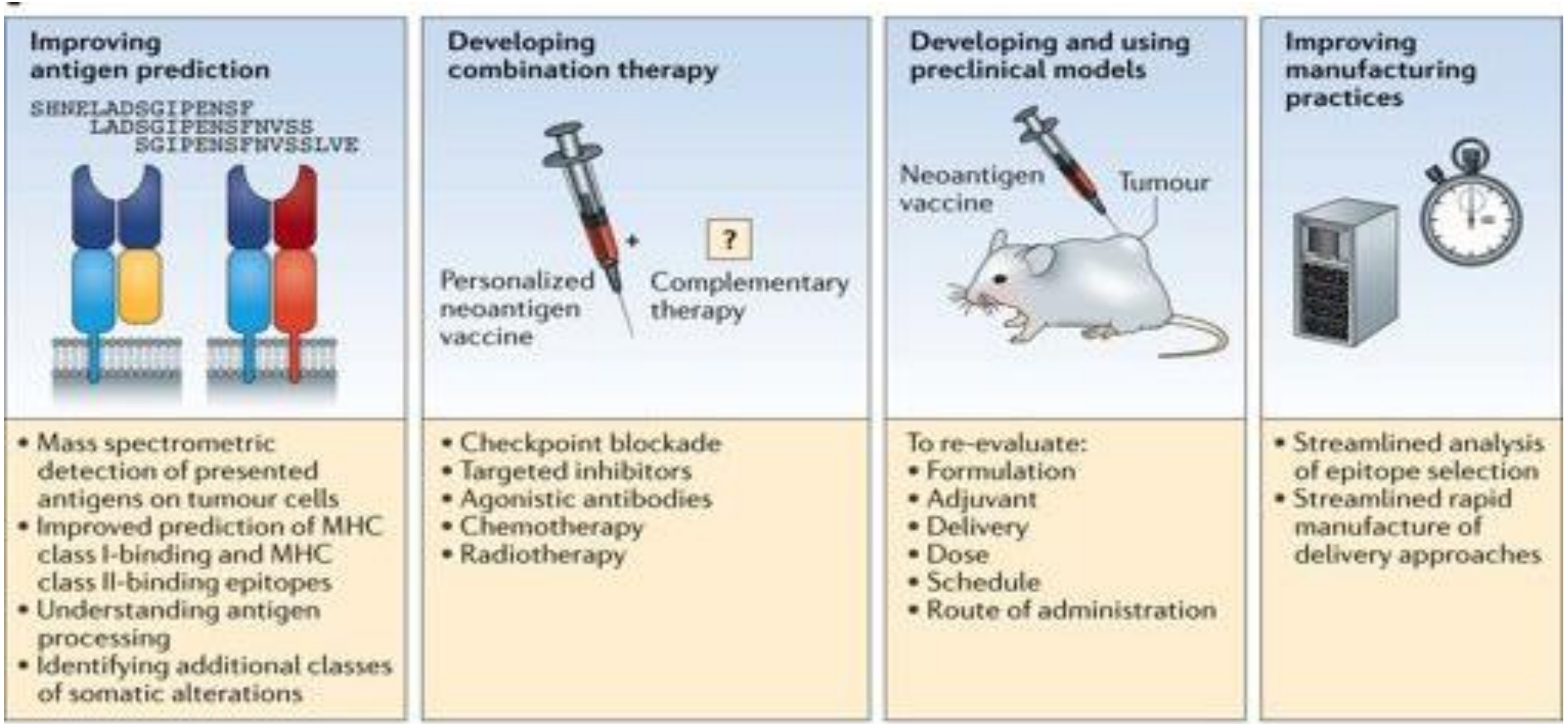
- TESLA is not:
  - Competition to determine ‘the best’ pipeline
  - A clinical program to validate predicted neoepitopes in patients.

TESLA: from sample acquisition to neoepitope prediction, validation and analysis





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



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)

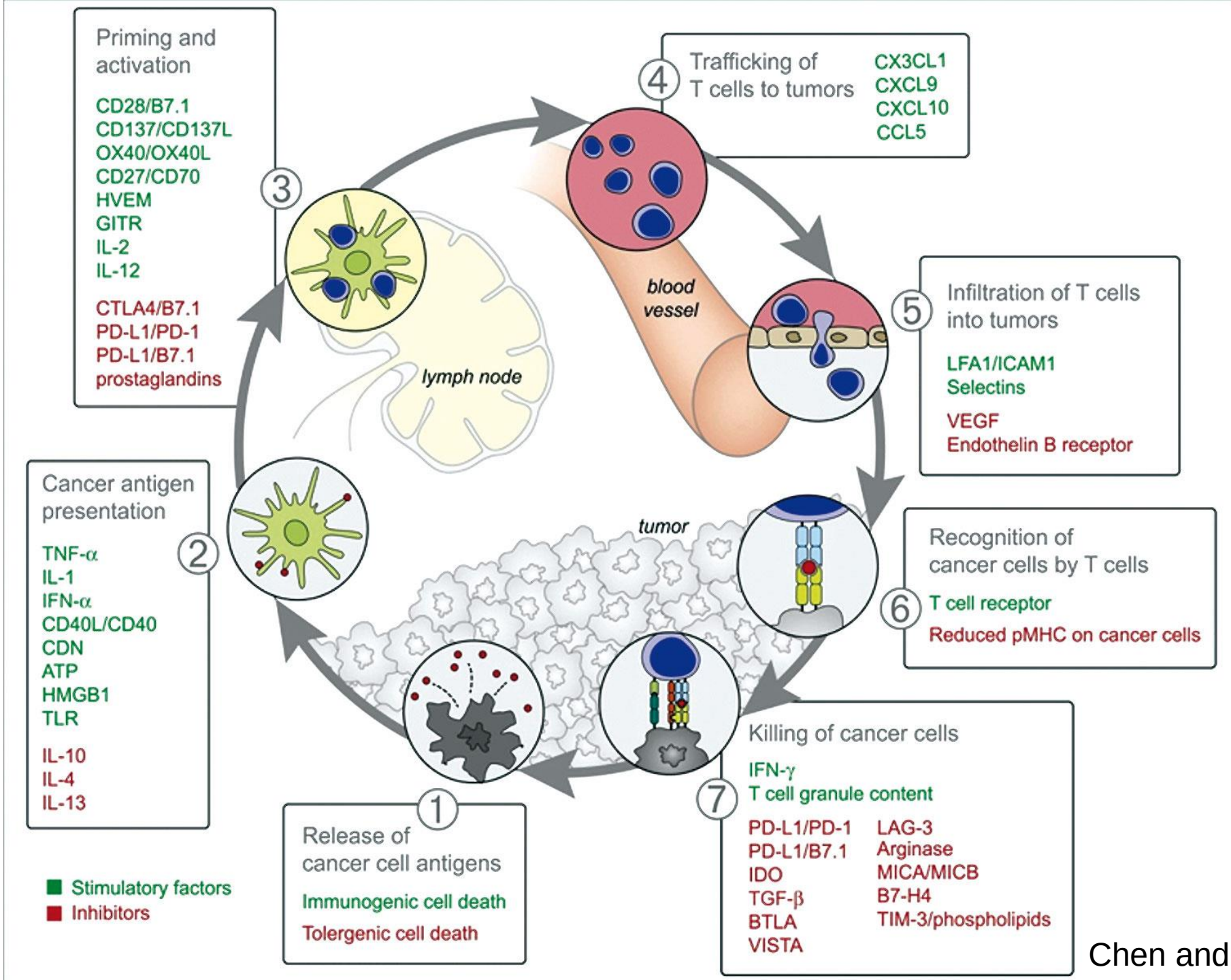
• Developments in imaging and other screening methods have made possible the detection of pre-malignant lesions.

•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