PÉCSI TUDOMÁNYEGYETEM
ÁLTALÁNOS ORVOSTUDOMÁNYI KAR
Immunológiai és Biotechnológiai Intézet

Biotechnology 2018

Biological therapies

Vaccine development & Cancer vaccines

Najbauer József

Lectures 17-18; 2018. 04. 05.

Lecture outline

1. Vaccine evolution
2. Vaccine immunology
3. Vaccine antigens
4. Vaccine adjuvants
5. Vaccine development
6. HIV and AIDS
7. Approaches to cancer immunotherapy

1. Vaccine evolution

Key concepts

- Vaccines have made the second most major contribution to the control and eradication of infectious diseases after the distribution of clean water
- Modern vaccine concepts stem from early empirical approaches to variolation and vaccination
- The germ theory opened the door to a more relevant knowledge-based vaccine development process
- Since the late 18th century, several important techniques to produce effective vaccines have been developed:
  - Attenuation and inactivation of pathogens at end of the 19th century
  - Toxoids and bacterial cancer immunotherapy in the 1920s
  - Use of adjuvants in the 1920s
  - Embryonated eggs to grow viruses in the 1930s
  - Cell cultures to grow viruses in the 1950s
  - Vaccines based on split pathogens or subunits in the 1970s
  - Recombinant DNA approach in the 1980s
  - Conjugation of polysaccharides to protein carriers in the 1980s
  - Reassortment of viral genes in the 1990s
  - Dendritic cell vaccines for cancer treatment in 2010

Bonanni & Ignacio Santos, Perspectives Vaccinol, 2011

Hospitalized victims during the polio outbreak of the 1950s

March of Dimes Foundation.

Figure 1.1 During the polio epidemics of the 1950s, entire wards were filled with people obliged to rely on an ‘iron lung’ due to paralysis of the respiratory muscles. Some patients would remain this way for the rest of their lives.

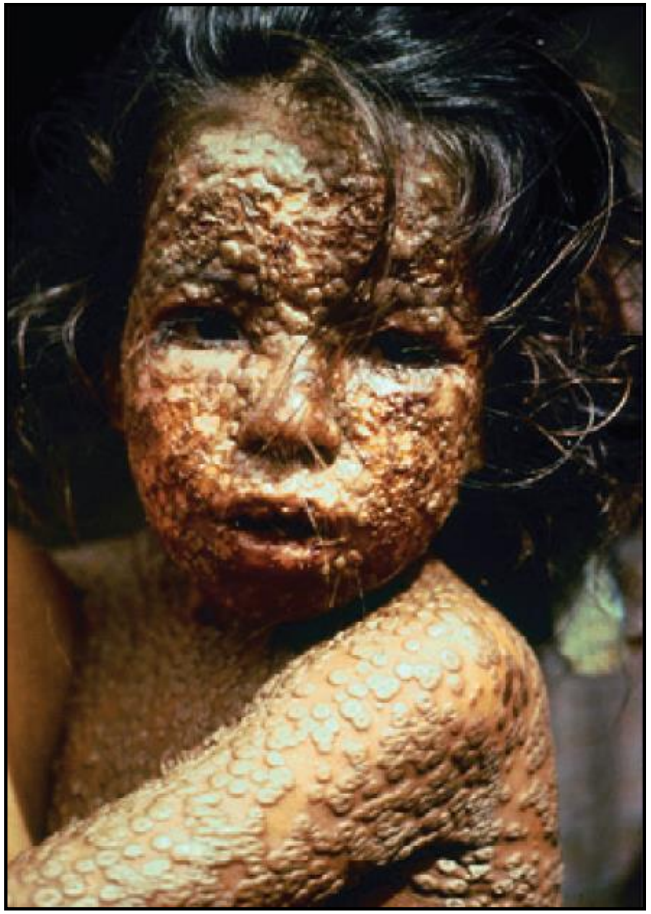
Bonanni & Ignacio Santos, Perspectives Vaccinol, 2011

Child with polio

Figure 1.10 Polio has been eradicated in most countries of the world; however, outbreaks still occur in developing countries.

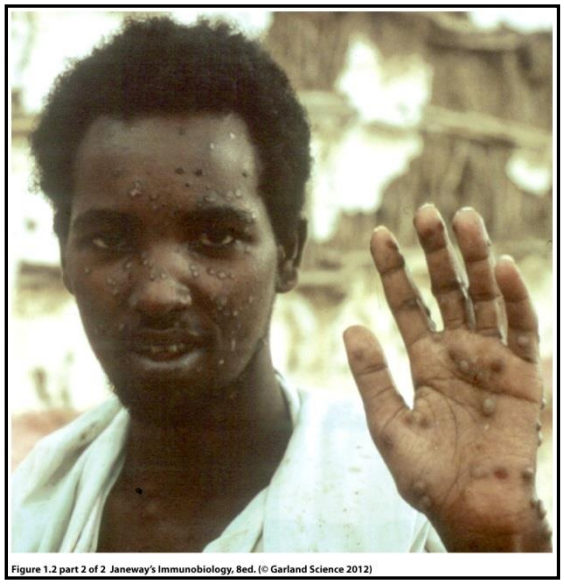
Bonanni & Ignacio Santos, Perspectives Vaccinol, 2011

Child with smallpox



Centers for Disease Control and Prevention
Bonanni & Ignacio Santos, Perspectives Vaccinol, 2011

The last case of smallpox



Ali Maow Maalin contracted and survived the last case of smallpox in Somalia in 1978. The WHO announced in 1979 that smallpox had been eradicated.

Eradication of smallpox by vaccination

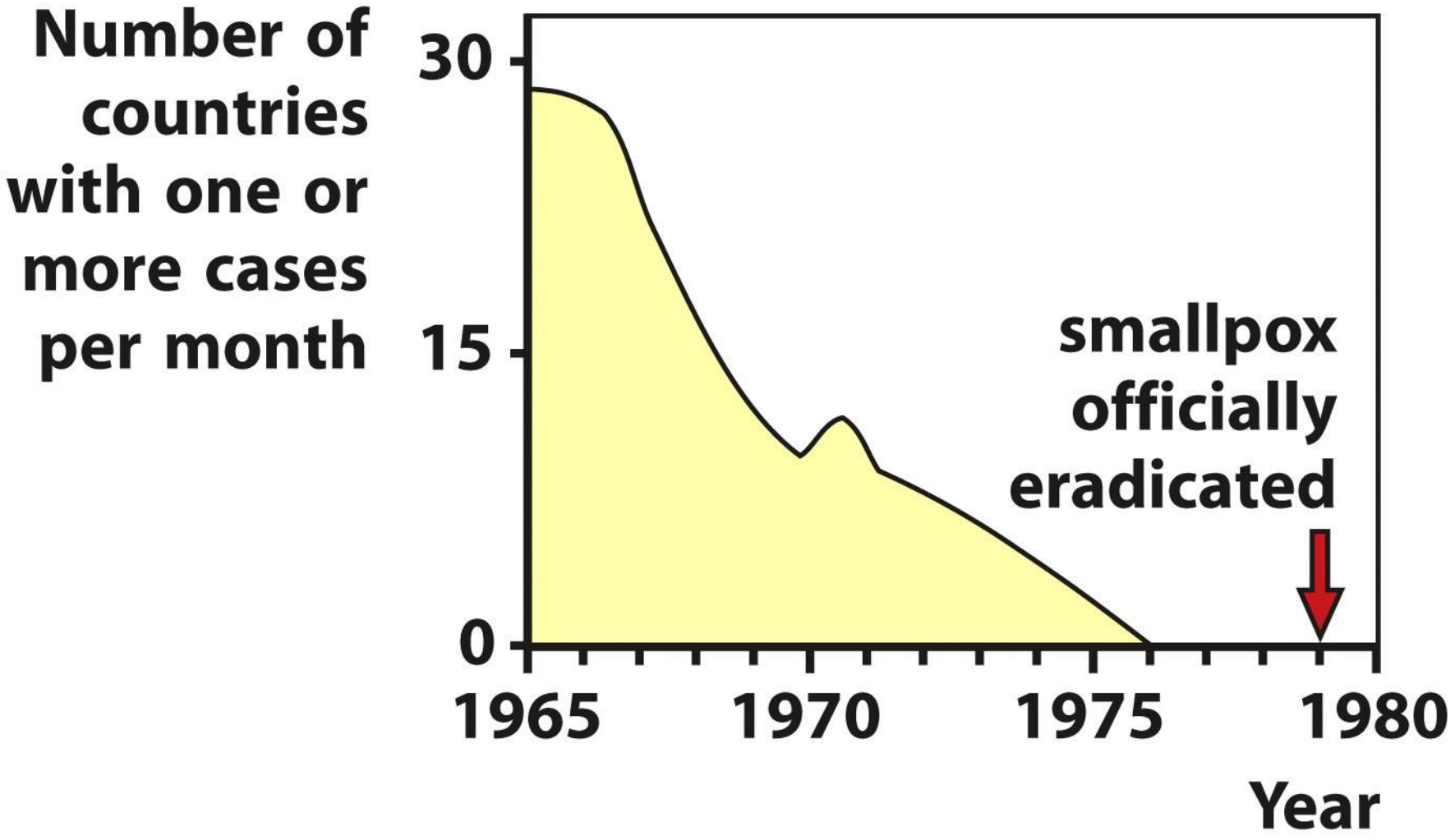


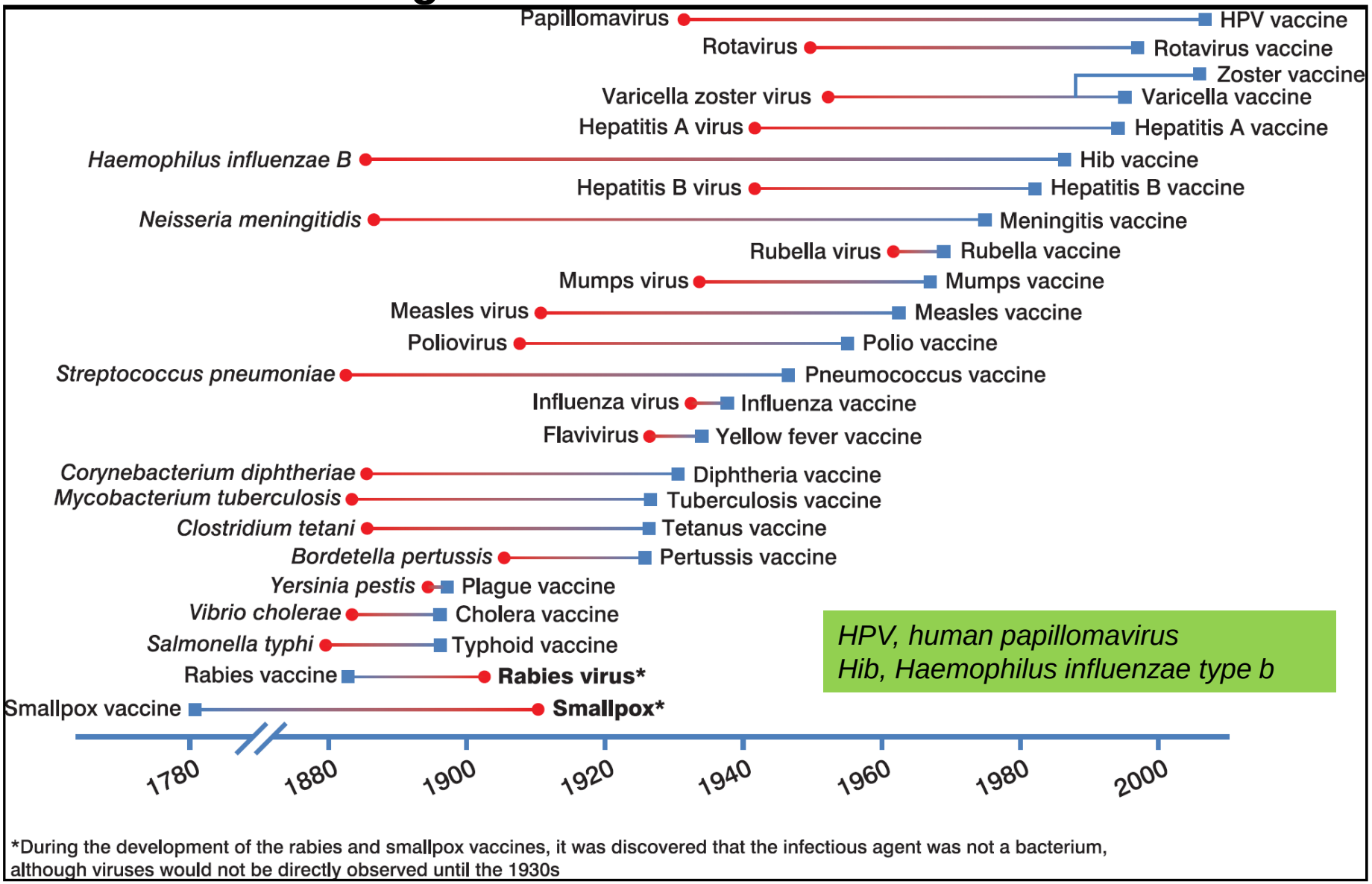
Figure 1.2 part 1 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Smallpox inoculation procedure in the 18th century

Collection of the University of Michigan Health System, gift of Pfizer Inc. UMHS.23.

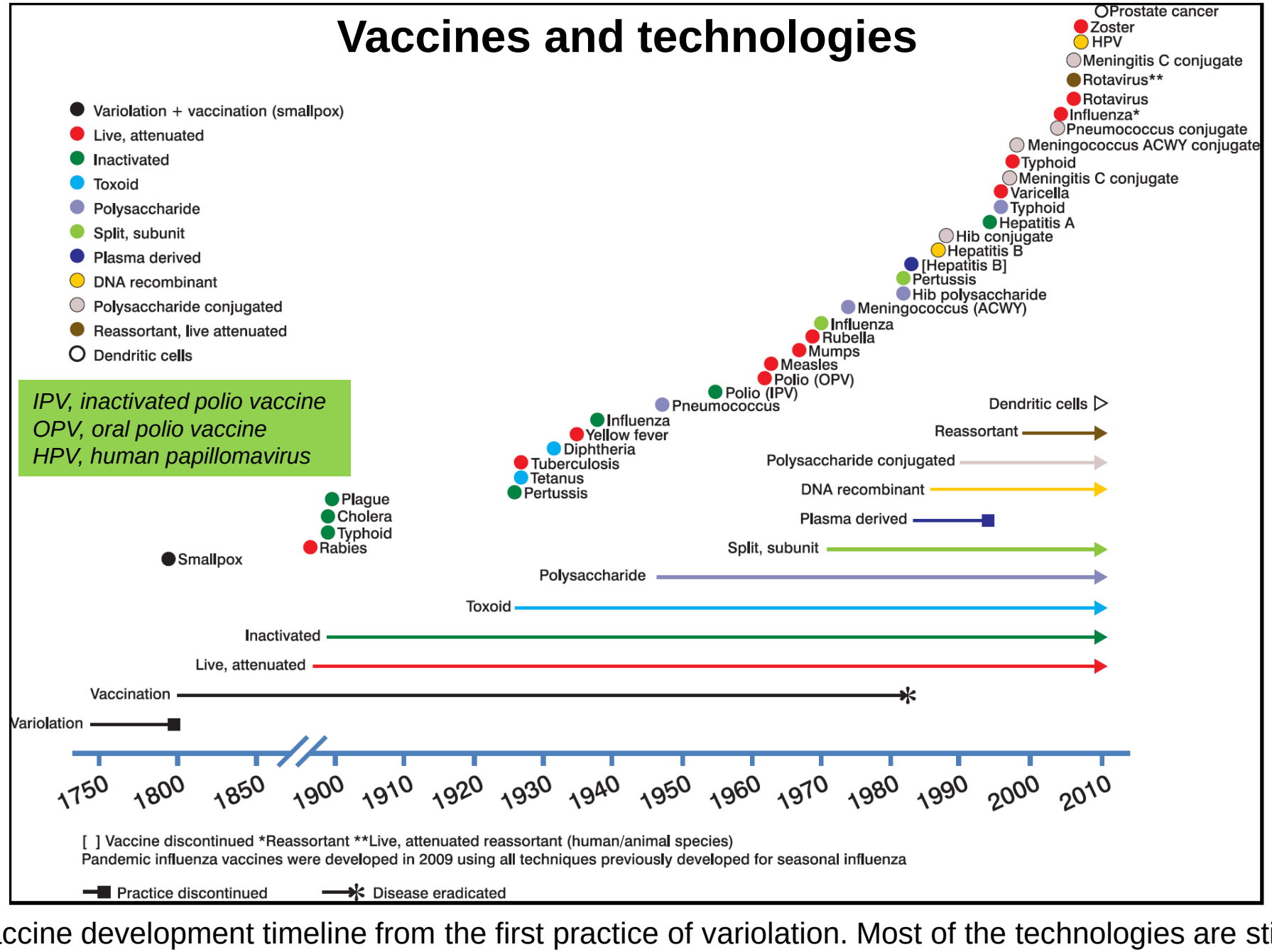
Figure 1.4 In 1796, Edward Jenner, a general practitioner and surgeon, inoculated 8-year-old James Phipps with material from cowpox blisters of a milkmaid. The boy developed a mild fever and was subsequently immune to smallpox.

Pathogen isolation and vaccines



Bonanni & Ignacio Santos, Perspectives Vaccinol, 2011

Depending on availability of appropriate technology, there may be considerable variations in time between pathogen identification ● and development of a vaccine ■ . In the case of smallpox, a vaccine was available long before viruses as causing agents were known. The rabies vaccine was also developed before knowing the causative agent. A pathogen, like varicella zoster virus, may cause different diseases (varicella and zoster) for which separate vaccines have been developed.



Vaccine development timeline from the first practice of variolation. Most of the technologies are still used for the development of vaccines. Plasma-derived vaccines have not been used in most countries since the 1990s

Tetanus case

Neonatal tetanus is still a risk in the developing world.

Centers for Disease Control and Prevention.

Bonanni & Ignacio Santos, Perspectives Vaccinol, 2011

Effectiveness of vaccines for some common infectious diseases

TABLE 1–1 Effectiveness of Vaccines for Some Common Infectious Diseases

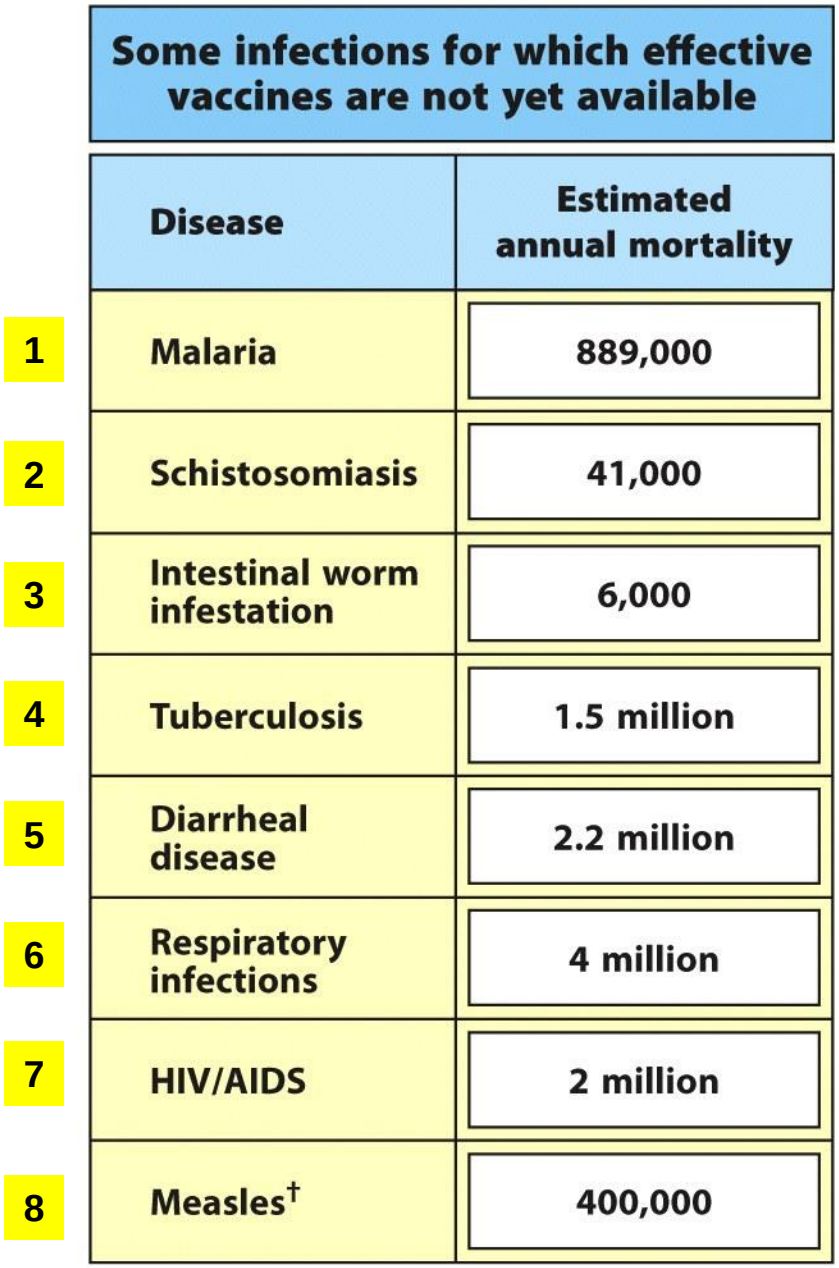
Disease (A)	Maximum Number of Cases (year) (B)	Number of Cases in 2009 (C)	Percentage Change (D)
Diphtheria	206,939 (1921)	0	–99.99
Measles	894,134 (1941)	61	–99.99
Mumps	152,209 (1968)	982	–99.35
Pertussis	265,269 (1934)	13,506	–94.72
Polio (paralytic)	21,269 (1952)	0	–100.0
Rubella	57,686 (1969)	4	–99.99
Tetanus	1,560 (1923)	14	–99.10
Haemophilus influenzae type B	~20,000 (1984)	25	–99.88
Hepatitis B	26,611 (1985)	3,020	–87.66

This table illustrates the striking decrease in the incidence of selected infectious diseases for which effective vaccines have been developed.
Data from Orenstein WA, AR Hinman, KJ Bart, and SC Hadler. Immunization. In Mandell GL, JE Bennett, and R Dolin (eds). Principles and Practices of Infectious Diseases, 4th ed. Churchill Livingstone, New York, 1995, and Morbidity and Mortality Weekly Report 58:1458-1469, 2010.

Data for USA

Abbas, Lichtman, Pillai, Cellular and Molecular Immunology (7th ed), 2012

Some infections for which effective vaccines are not yet available



Some infections for which effective vaccines are not yet available

	Disease	Estimated annual mortality
1	Malaria	889,000
2	Schistosomiasis	41,000
3	Intestinal worm infestation	6,000
4	Tuberculosis	1.5 million
5	Diarrheal disease	2.2 million
6	Respiratory infections	4 million
7	HIV/AIDS	2 million
8	Measles†	400,000

Figure 16.22 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

2. Vaccine immunology

Key concepts

- The human immune system consists of two connected compartments - the innate and adaptive - which function via the actions of secreted and cellular effectors
- The innate and adaptive immune systems work sequentially to identify invaders and formulate the most appropriate response; this interaction is crucially bridged by specialized antigen-presenting cells (APCs)
- The innate response, via the action of APCs, sets the scene for the subsequent adaptive response by providing information about the nature of the threat
- Primary exposure to a pathogen or antigen induces the production of a population of adaptive immune cells with antigen specificity that are retained for long periods and provide a rapid response upon subsequent exposure
- The vaccine concept is based on stimulating the body's defense mechanisms against a specific pathogen to establish this immunological memory
- Current vaccine strategies take advantage of immunological mechanisms, and often target the innate immune system and APCs to induce the desired specific adaptive immune response
- Future research is also set to examine ways of making the immune response more effective in generating cross protective responses against different subtypes or strains of pathogens exhibiting antigenic variation

Leo O et al, Perspectives Vaccinol, 2011

Immunological milestones of particular relevance to vaccinology

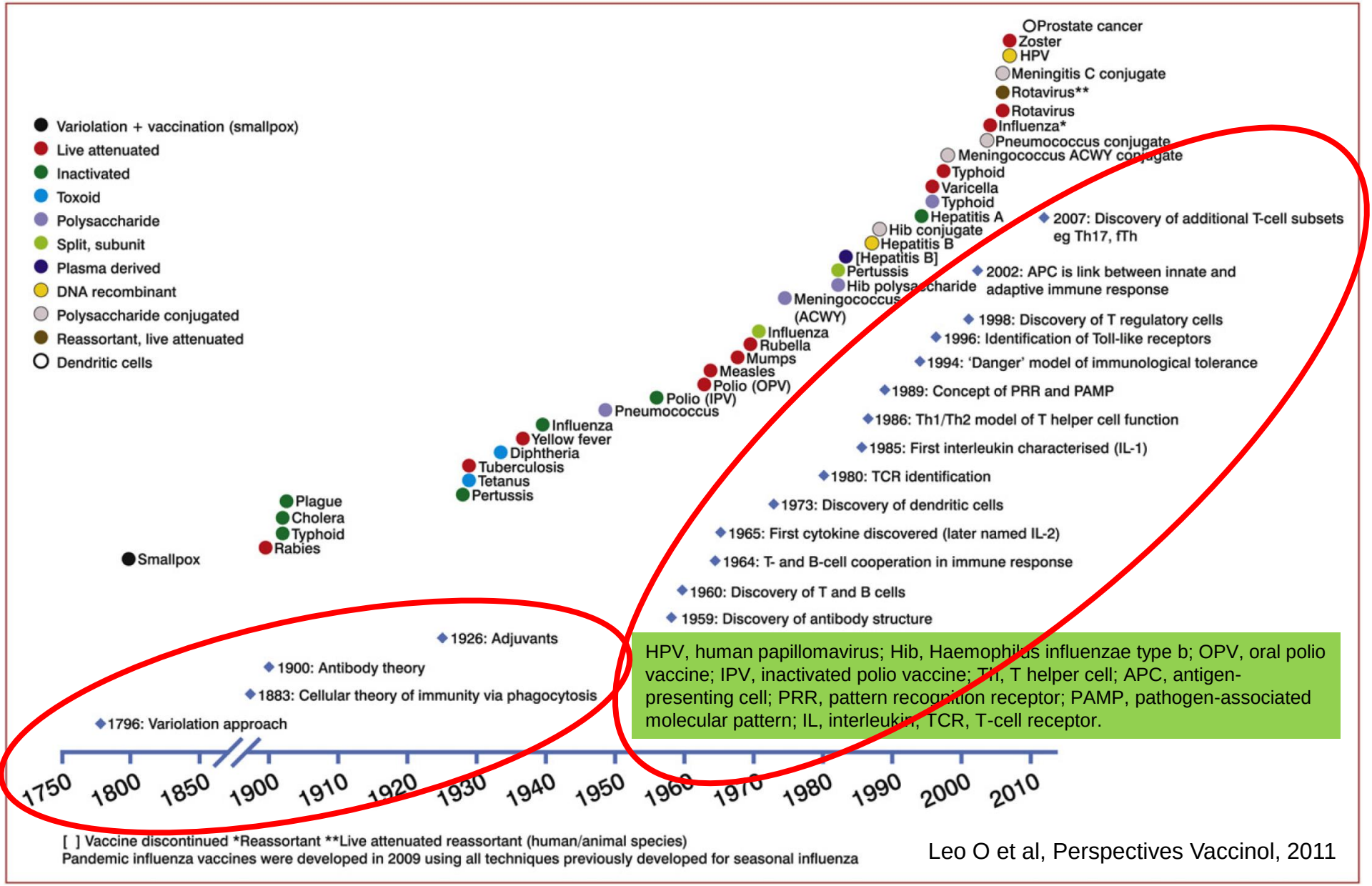


Figure 2.1 Progress in vaccine design and technology is underpinned by discoveries in immunology. This is shown by the increasing number of vaccines developed as knowledge of immunity has increased.

Key cellular players of the immune system

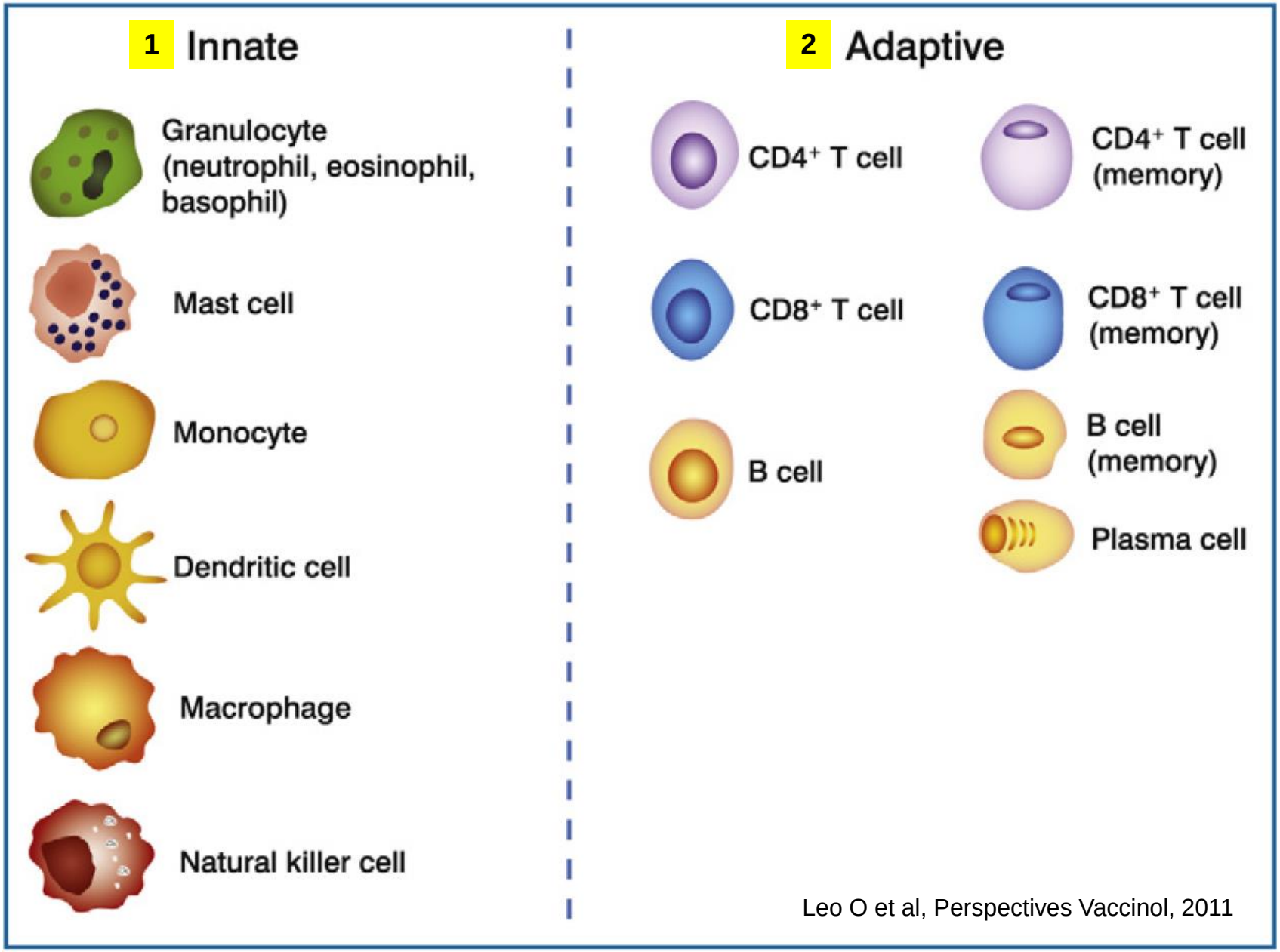
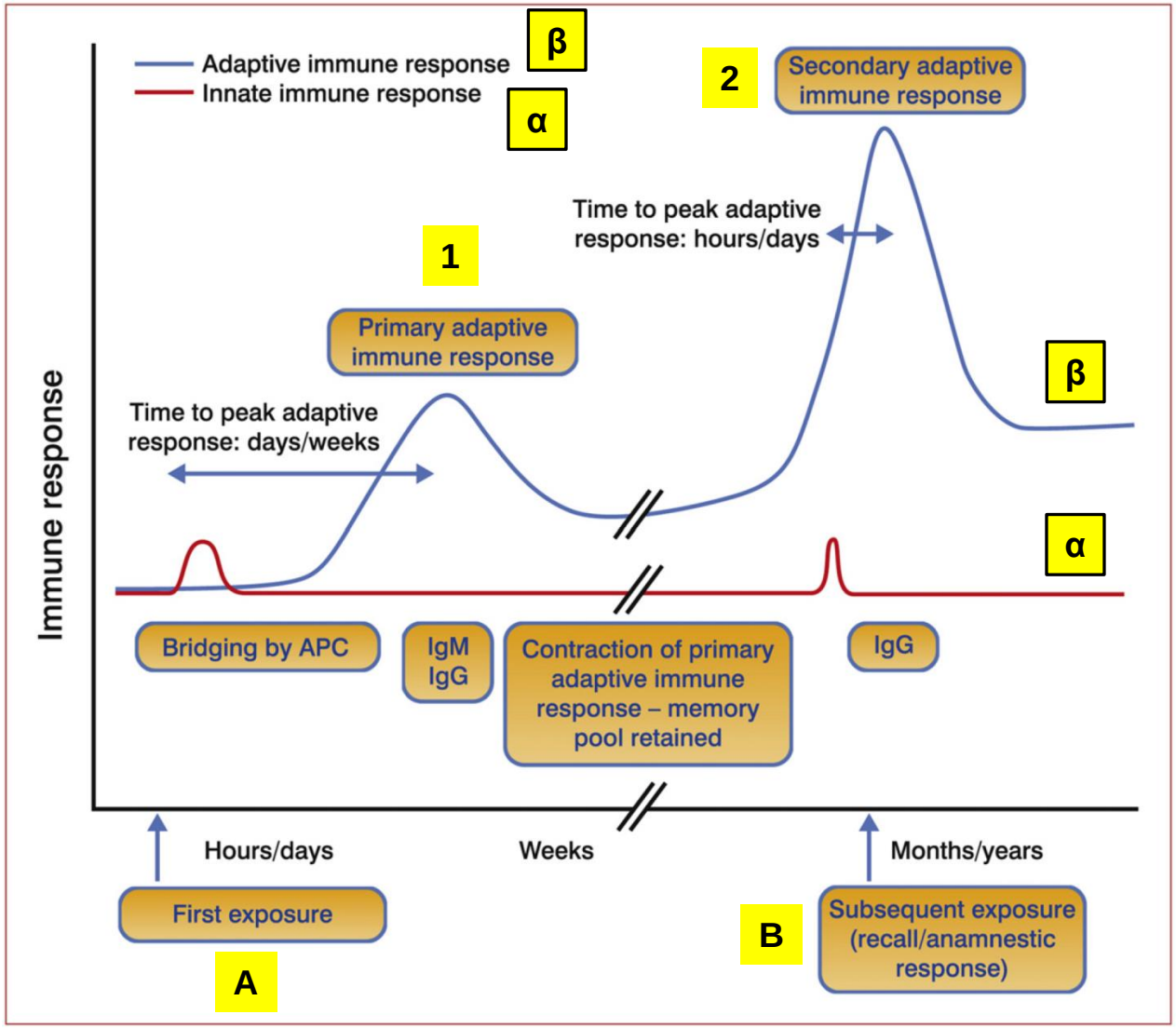


Figure 2.2 The innate and adaptive immune systems are populated by many different cells that vary in their roles and responsibilities. CD, cluster of differentiation.

The kinetics of primary and recall (memory) immune responses



APC, antigen-presenting cell
IgM, immunoglobulin M
IgG, immunoglobulin G

Leo O et al,
Perspectives Vaccinol, 2011

Figure 2.8 On first exposure to a pathogen or antigen (referred to as 'priming' in vaccination), the innate immune system must detect, process and translate the threat into a form that can be understood by the adaptive immune system. This occurs via the bridging actions of APCs and takes days/weeks. Following resolution of the challenge, a specialiszed 'memory' cell population remains. The cells within this population are maintained for a long time (months/years) and may remain within the host for the rest of their host's life. On subsequent exposure to the same antigen (referred to as 'boosting' in vaccination), the innate immune response is triggered as before but now the memory cell populations are able to mount a greater and more rapid response as they do not need to undergo the same activation process as naïve cells.

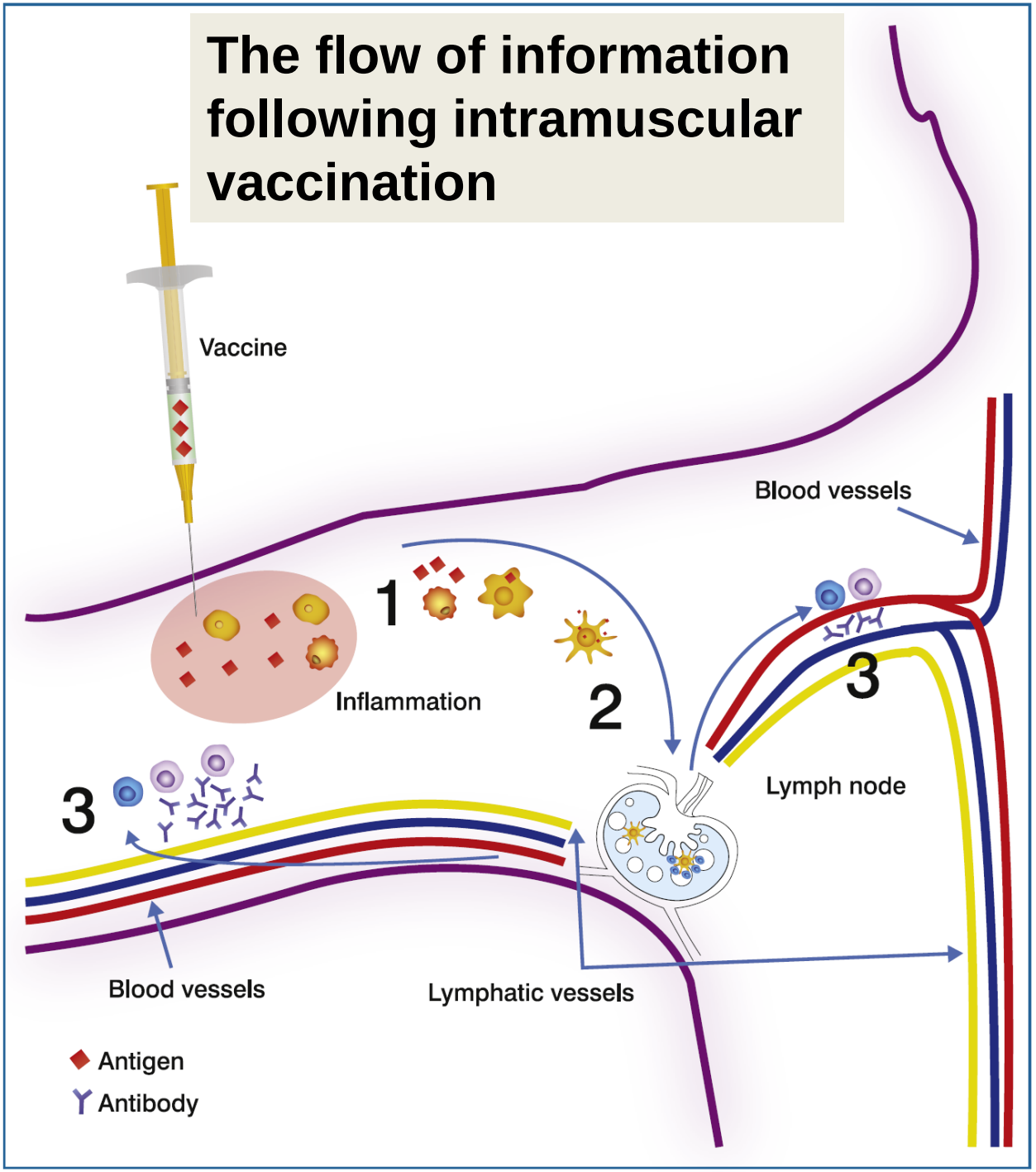


Figure 2.10

- An antigen delivered by a vaccine is taken up by macrophages and immature APCs (**1**).
- APCs migrate to the lymph node draining the site of vaccination (**2**).
- The adaptive immune response is now initiated and effectors, such as CD4+ effector T cells, cytotoxic T cells and soluble antibodies (**3**), are produced which travel throughout the bloodstream and back to the site of vaccination.

APC, antigen-presenting cell

Leo O et al, Perspectives Vaccinol, 2011

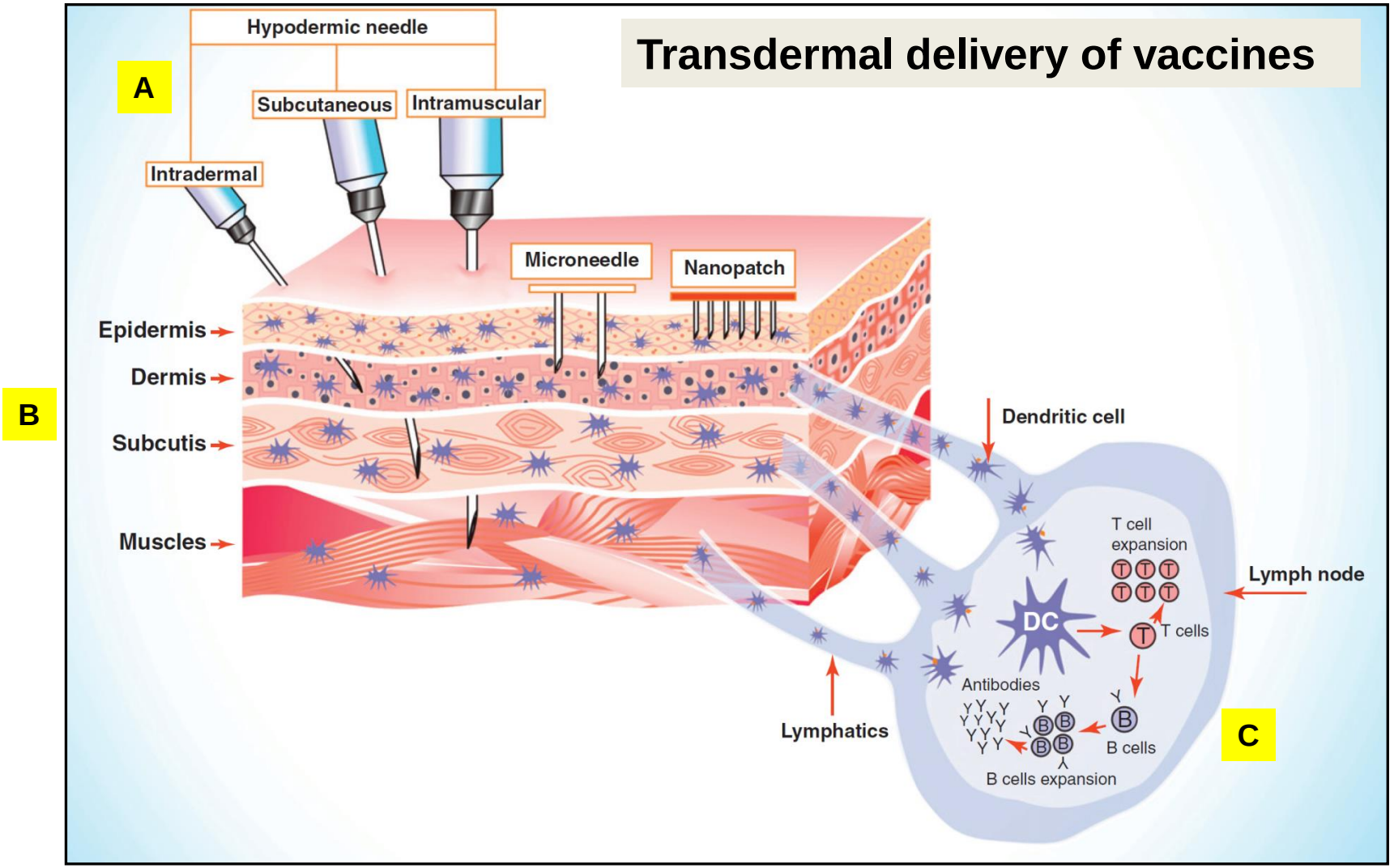


FIGURE 1 Classical and transdermal needle-driven delivery in relation to immunology of the skin. The skin is made up of a multilayered epidermis, which serves as the external physical barrier, and the dermis, which contains the blood and lymphatic vessels as well as the nerves. Hypodermal administration can deposit a cargo deep into the muscles (intramuscular), into the subcutis (subcutaneous) or into the dermis (intradermal). Microneedle and nanopatch methods, which use microscopic projections, deposit their cargo either into or just beyond the epidermis, but not deep enough to reach the nerve endings, which are responsible for the sensation of pain. The dendritic cells (DC) of the skin are compartmentalized, with Langerhans' cells and dermal dendritic cells populating the epidermis and the dermis, respectively. Upon antigen capture, these cells traffic to local lymph nodes to stimulate an immune response involving T and B cells. The high density of DC per unit volume and the huge surface area (1.5–2.0 m^2) of the skin makes it a highly immune competent and attractive site with excellent potential for vaccine delivery. The illustrations are not to scale.

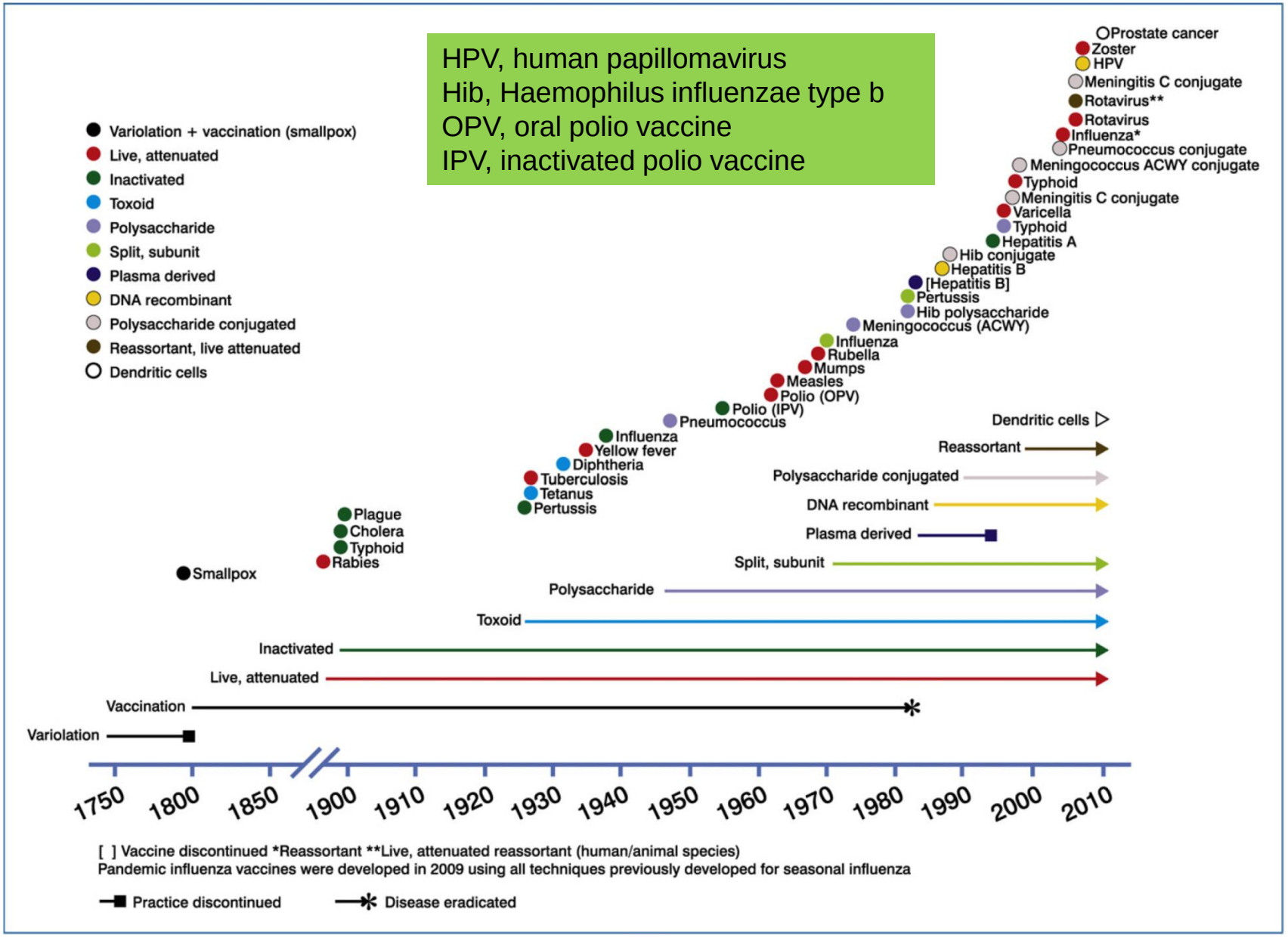
3. Vaccine antigens

Key concepts

- Many vaccines are comprised of whole viruses or bacteria and therefore contain many, often poorly defined, antigens as well as other microbial molecules important in triggering innate and/or adaptive immune responses
- Where the whole pathogen approach is not feasible or desirable, other approaches are considered, such as subunit antigens that are naturally derived or generated using recombinant DNA technology
- Vaccines containing fewer defined antigens may be less reactogenic but also less immunogenic thus necessitating the inclusion of adjuvants
- Key pathogen virulence determinants usually make excellent antigens for inclusion in vaccines, e.g. viral ligands such as haemagglutinins or inactivated bacterial toxins
- The final choice of antigen is often determined by what is achievable immunologically and technologically, and what is optimal from a safety perspective
- An immunogen is an antigen capable of inducing an adaptive immune response; an epitope is the highly specific structure or site on an antigen that is recognized by either the surface B-cell receptor, T-cell receptor or soluble antibody

Strugnell R et al, Perspectives Vaccinol, 2011

Vaccines and technologies



Strugnell R et al, Perspectives Vaccinol, 2011

Figure 3.1 Vaccine development timeline from the first practice of variolation - deliberate infection of humans with material derived from human smallpox pustular material. Most of the other technologies are still used for the development of vaccines. Plasma-derived vaccines have not been used in most countries since the 1990s.

Approaches to vaccine antigen selection

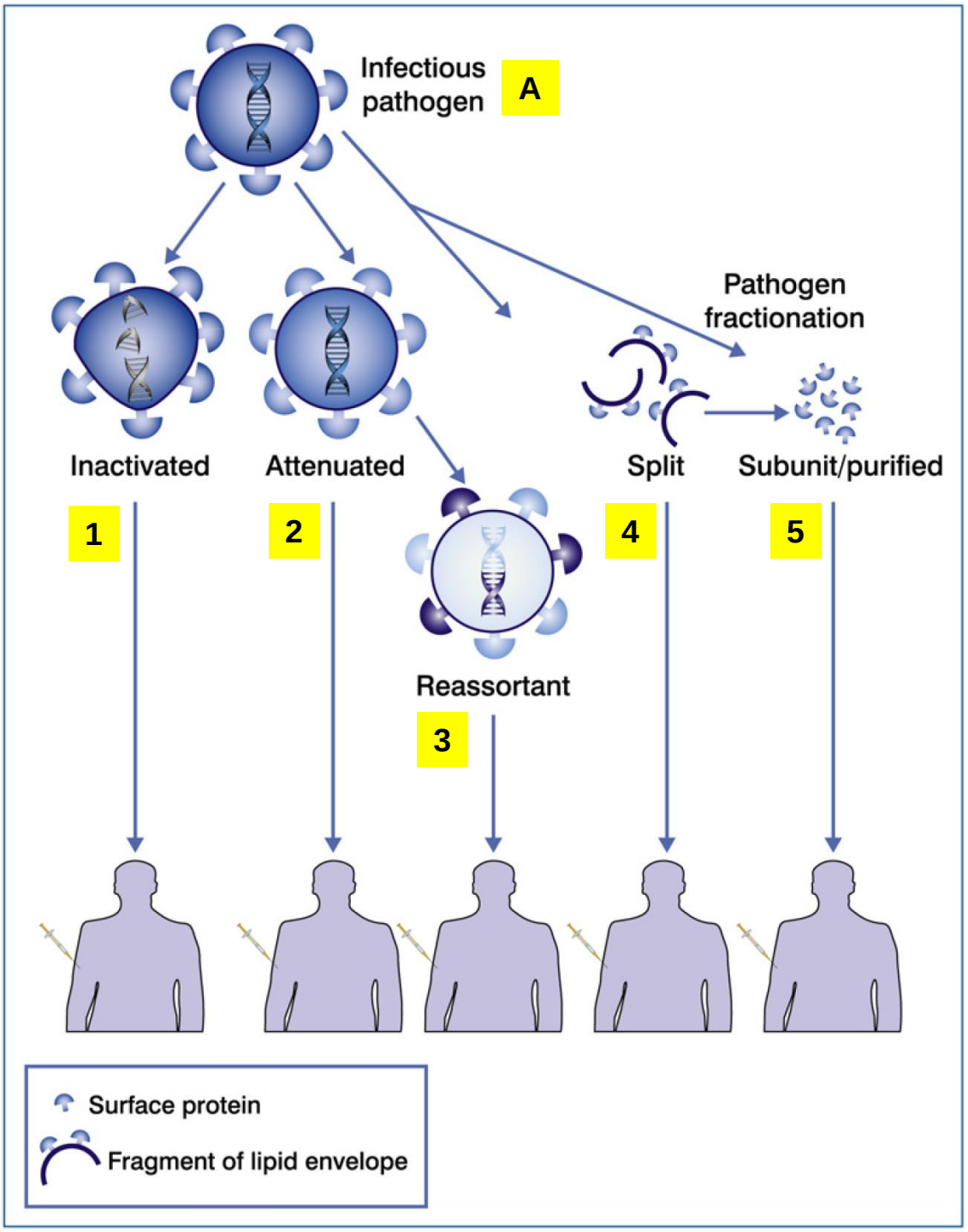
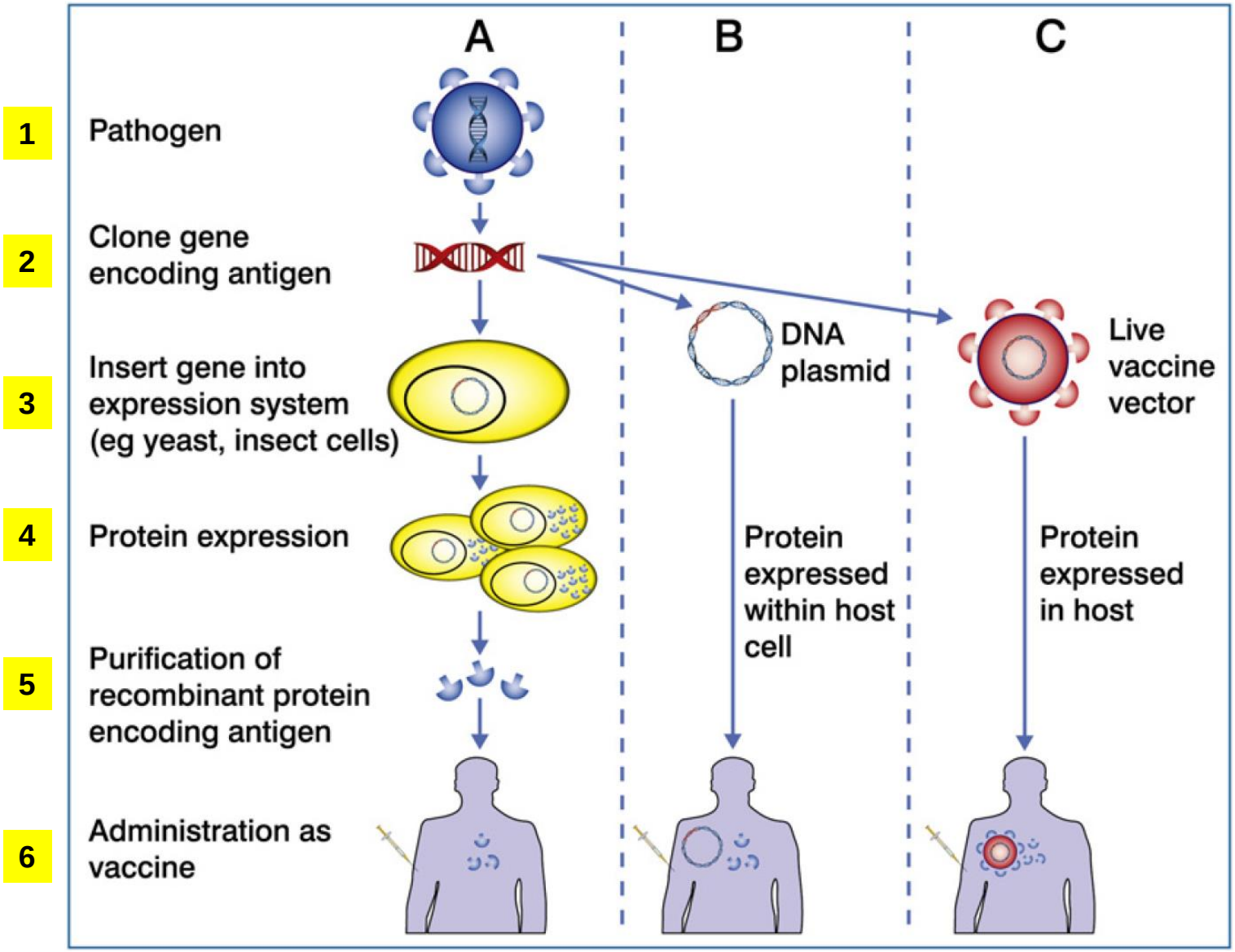


Figure 3.2 Whole pathogen-based vaccines need to undergo attenuation or inactivation processes, while subunit vaccines rely on purified fractions of pathogens derived by physical disruption of whole organisms.

Strugnell R et al, Perspectives Vaccinol, 2011

Recombinant DNA approaches to vaccine antigens



Strugnell R et al, Perspectives Vaccinol, 2011

Figure 3.3 Protein antigens are produced using recombinant DNA technology, where the DNA sequence coding for the antigenic protein is inserted into an expression system that is then able to produce large quantities of that specific antigen in vitro (**panel A**) or following administration to the host, eg using a DNA plasmid (**panel B**) or a live vaccine vector (**panel C**) as the expression system.

Characteristics of live and killed vaccines

TABLE 3.1. CHARACTERISTICS OF LIVE AND KILLED VACCINES

Live attenuated 1	Killed/inactivated 2
Examples: OPV, MMR, VZV, some influenza, BCG	Examples: IPV, HAV, whole-cell pertussis
Mimic the natural infection and retain most defensive triggers/immunogenic elements; however, may retain immune evasion factors	Usually require adjuvants due to reduced immunogenicity/ missing defensive triggers
Strong priming usually achieved with 1–2 doses	Multiple doses usually needed for priming
Long-term persistence of immunity	*Booster* doses may be needed to maintain long-term immunity
May induce some mild disease symptoms	Do not induce disease symptoms
Rare revision to virulence; unsuitable for immunocompromised patients	No risk of reactivation, non-infectious
Potential for immunological interference with other live vaccines	Low risk of immunological interference
Less stable over time, heat labile	Relatively stable over time, better resistance to cold chain deviation
Response affected by recent administration of blood/blood-derived products or presence of maternal antibody in an infant	Generally not affected by administration of blood/ blood-derived products

OPV, oral polio vaccine; MMR, measles, mumps and rubella vaccine; VZV, varicella zoster virus vaccine; BCG, Bacille Calmette–Guérin (against severe forms of tuberculosis); IPV, inactivated polio vaccine; HAV, hepatitis A virus vaccine.

('revision' - it should be 'reversion' instead)

Strugnell R et al, Perspectives Vaccinol, 2011

4. Vaccine adjuvants

Key concepts

- Adjuvantation of vaccines is a well-established concept and practice
- Adjuvants enhance and modulate immune responses to antigens. This is particularly important when the antigens are purified and lack intrinsic innate and/or adaptive immune triggers
- Adjuvants differ in the types and magnitude of immune responses they elicit, hence they must be selected in view of the immune response required to induce immunity to a given pathogen or antigen
- Combinations of adjuvants can take advantage of the properties of each individual component of an adjuvant composition
- Adjuvants are a key tool in developing efficacious vaccines to meet many vaccine challenges

Garçon N et al, Perspectives Vaccinol, 2011

Use of adjuvants in vaccines

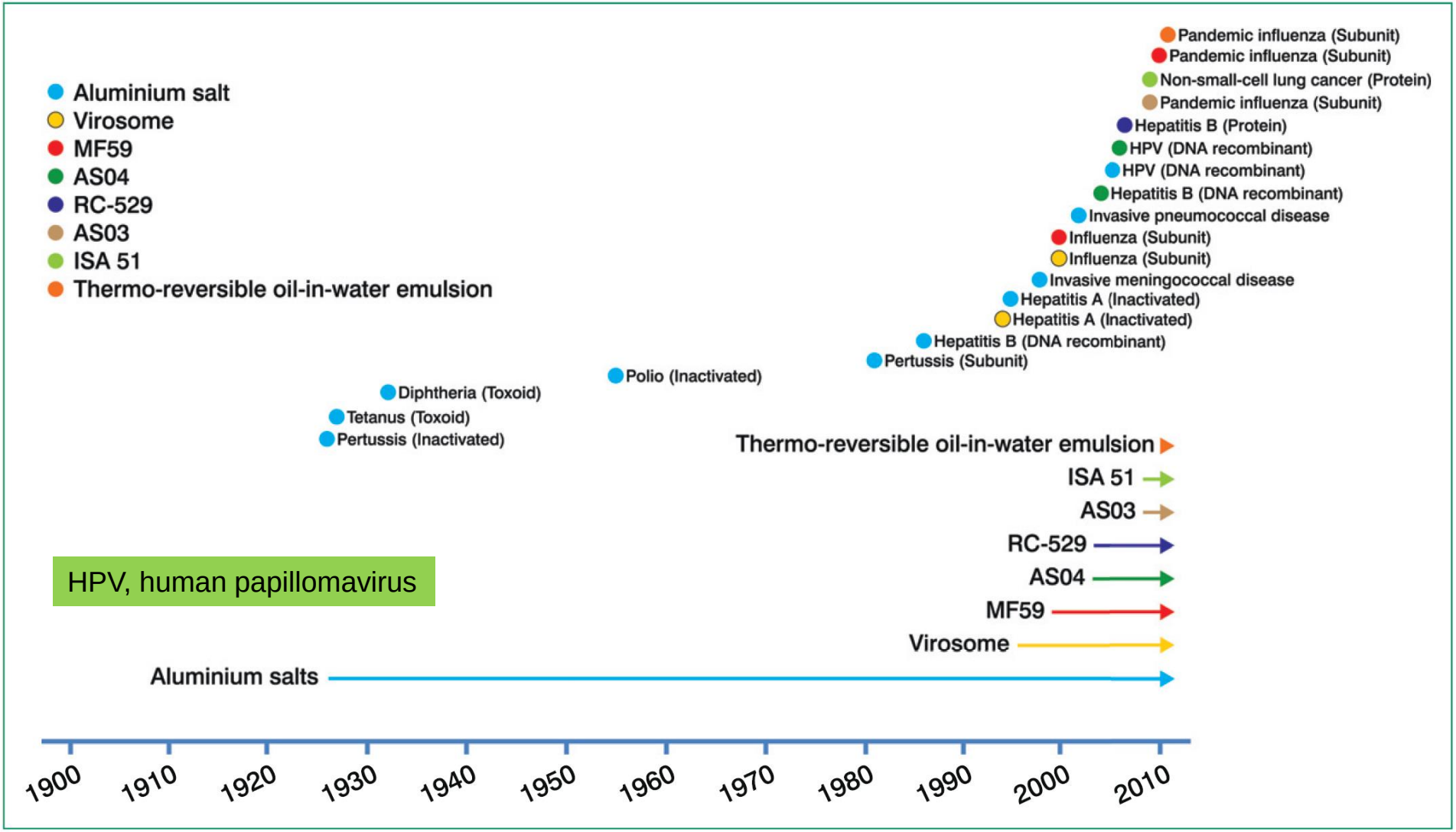


Figure 4.1 As with all areas of vaccine development, the availability and variety of adjuvanted vaccines has increased with a greater understanding of immunology. The antigen approach employed for the individual disease is given in parentheses. Aluminium salts were the only adjuvant used in licensed vaccine formulations for human vaccines until the 1990s. Several new adjuvants have been developed and used since.

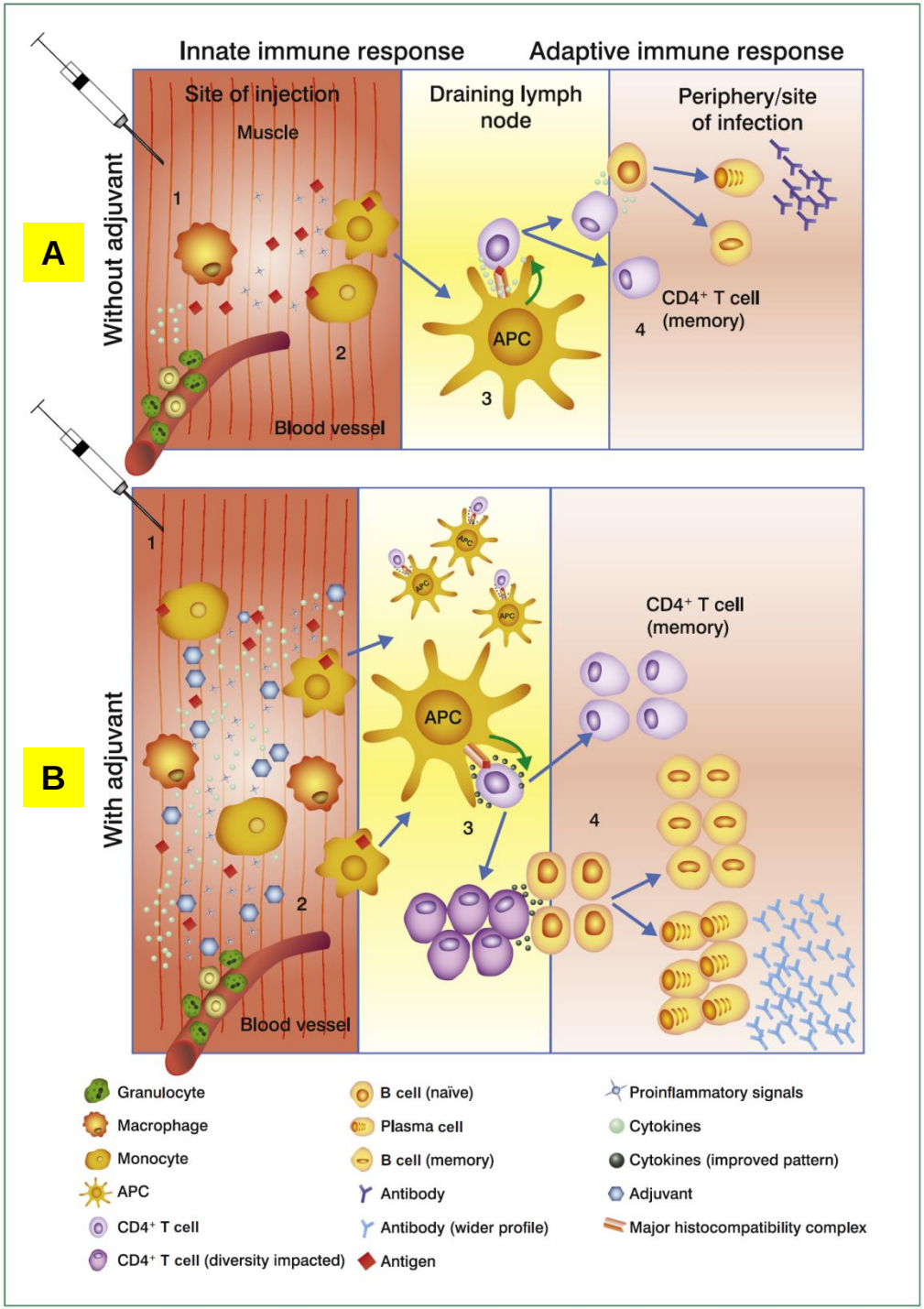
Garçon N et al, Perspectives Vaccinol, 2011

Possible impact of adjuvants on immune mechanisms

1. Recognition of PAMPs
2. Presentation of antigens to T-cell receptor
3. Recognition of co-stimulatory signals
4. Intracellular signalling processes in APCs

APC, Antigen-presenting cell
PAMP, Pathogen-associated molecular pattern

Garçon N et al, Perspectives Vaccinol, 2011



Adjuvants: general mode of action

Compared with the same antigen in a non-adjuvanted formulation, the expected benefits of adjuvants are:

- An increased recruitment of innate cells at the site of injection
- An increased number of activated APCs migrating to the draining lymph node
- An increased uptake of the antigen by APCs with a subsequent enhancement and modulation of the adaptive immune response

Garçon N et al, Perspectives Vaccinol, 201

Properties of adjuvants

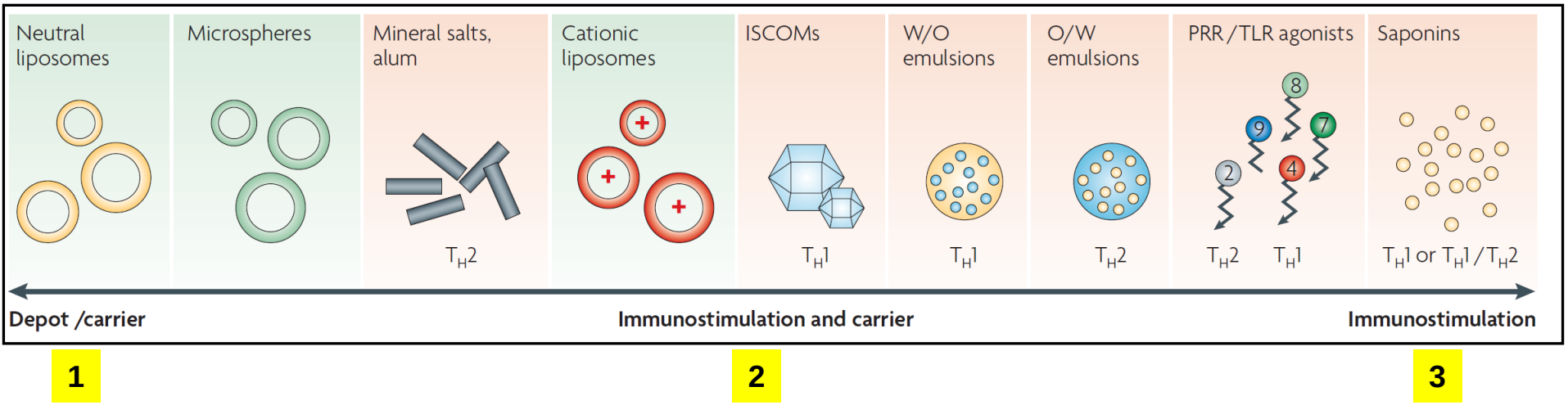


Figure 3 The main type of adjuvants with respect to their depot/carrier and immunostimulatory properties are shown. Some compounds can possess both characteristics whereas others possess only one. In addition, some of the adjuvants shown (red background) can have immunomodulatory properties beyond their ability to trigger global immune stimulation, by directing responses specifically towards a T helper (TH) 1 or TH2 response. A third dimension (not represented here) is the specific targeting ability of adjuvants, although carrier/depot activity and ligand specificity can contribute to targeting. ISCOMs, immunostimulating complexes; O/W, oil-in-water emulsion; PRR, pattern-recognition receptor; TLR, Toll-like receptor; W/O, water-in-oil emulsion.

Guy B, Nature Rev Microbiol, 2008

5. Vaccine development

Key concepts

- Vaccine development is a complex multistep process
- From concept to licensure, it takes many years to develop a vaccine
- Following authorization to market, it may be necessary to provide evidence of economic value prior to governments approving the implementation of a new vaccination program
- Safety is a major issue for any vaccine; it is assessed at every step of vaccine development and safety surveillance continues indefinitely after licensure
- Sometimes an adverse reaction is observed after a vaccination. It is important to determine whether a temporal association between the adverse event and the vaccination is causal, rather than a random chance occurrence (coincidental). Otherwise, vaccination programs are halted for risks that are only theoretical, thus endangering people's health through not being vaccinated
- Clinical and epidemiological studies indicate that licensed vaccines have a benefit-risk profile where the benefits of vaccines clearly outweigh the risks of adverse effects

Leroux-Roels & Bonanni, Perspectives Vaccinol, 2011

Vaccine safety is important at all stages of development

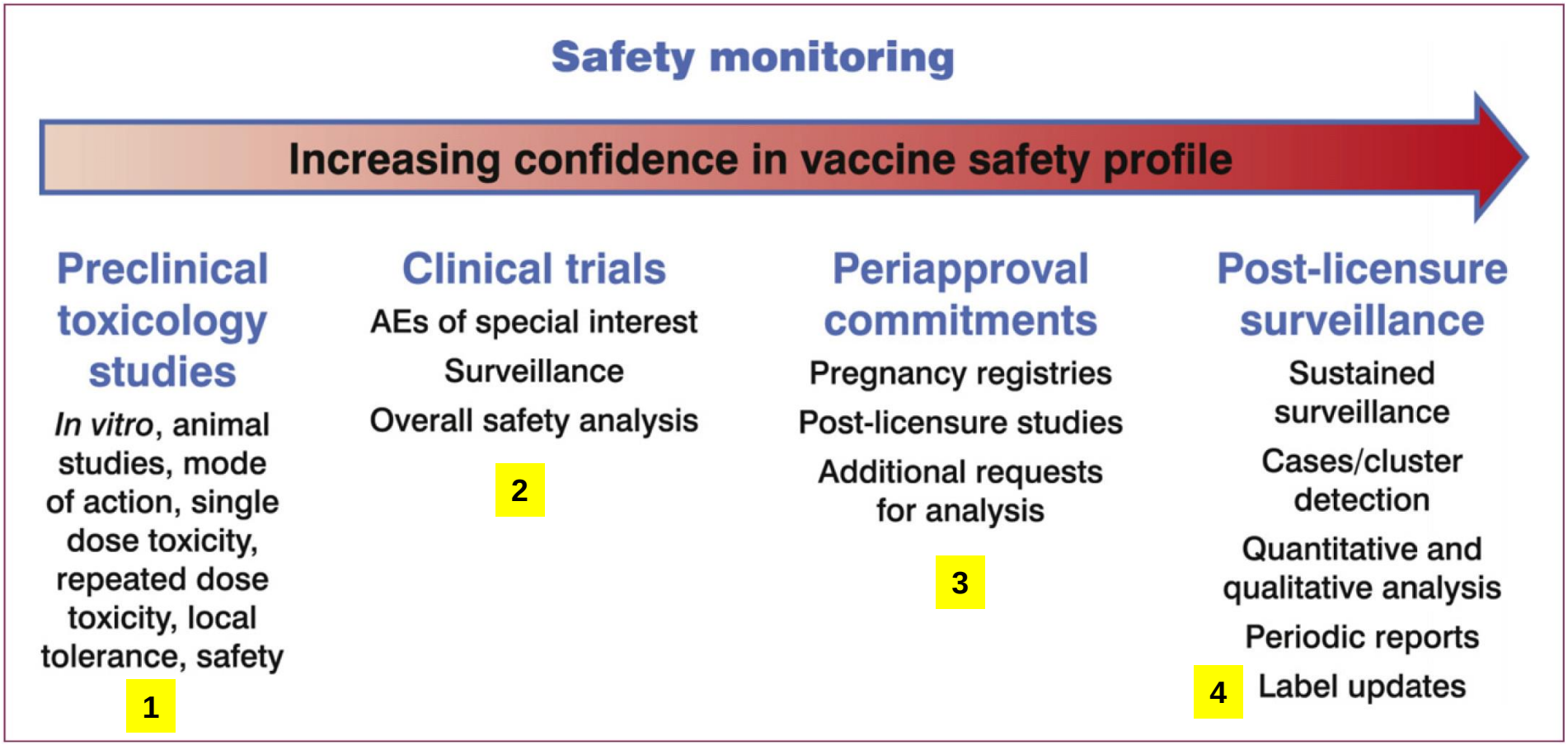


Figure 5.2 Safety is assessed at all points of the vaccine development process from preclinical toxicology studies using cell cultures and animal models through to rigorous assessment in clinical studies. Post-licensure, safety is still of prime concern and is the major focus of post-licensure surveillance studies. AEs, adverse events.

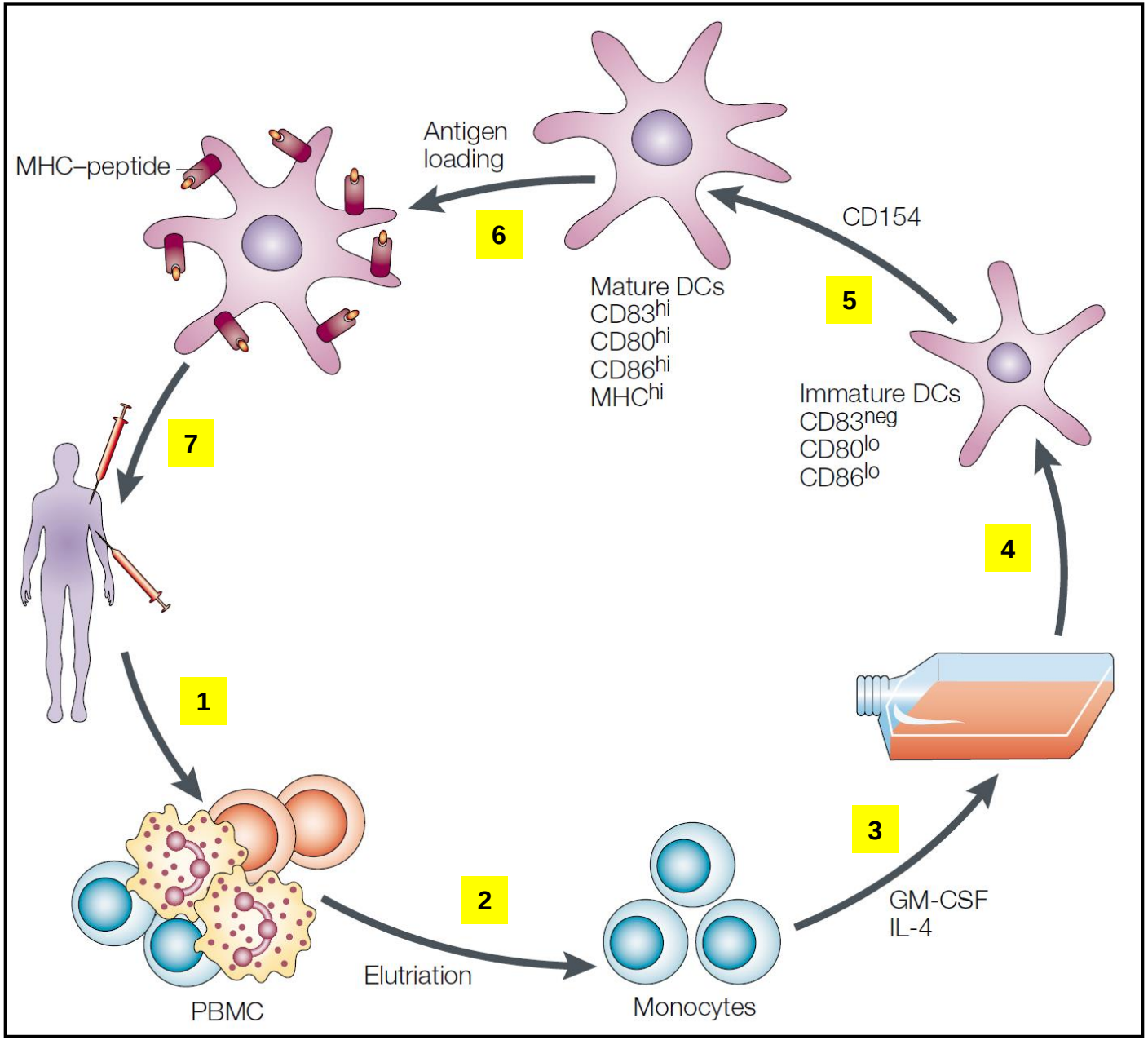
Leroux-Roels & Bonanni, Perspectives Vaccinol, 20

Dendritic cell vaccines

DC vaccines hold great promise for the treatment of cancer, HIV and other chronic infections. Utilising the patient's own DCs, this is truly an individualised biomedical intervention.

Stanberry and Strugnell, Perspectives Vaccinol, 2011

Strategy for immunization with autologous peptide-pulsed DCs



Berzofsky JA et al, Nature Rev Immunol, 2001

7. HIV and AIDS

Once upon a time, there was a world without AIDS—it seems so long ago. In the past 3 decades since first recognition of the new virus and syndrome, millions of lives have been lost or thwarted by debilitating illness or by orphanhood, and there is a sure promise of many millions of damaged lives to come.

June E. Osborn, JAMA (Editorial), 2008

Isolation of HIV

Françoise Barré-Sinoussi, PhD

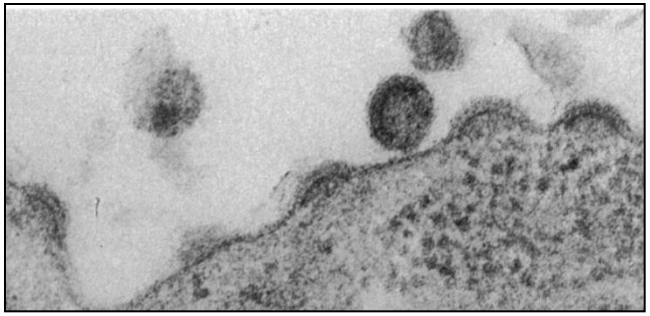
Luc Montagnier, PhD

Hampton, JAMA, 2008

Nobel Prize
Physiology or Medicine, 2008

Isolation of a T-Lymphotropic Retrovirus from a Patient at Risk for Acquired Immune Deficiency Syndrome (AIDS)

Abstract. *A retrovirus belonging to the family of recently discovered human T-cell leukemia viruses (HTLV), but clearly distinct from each previous isolate, has been isolated from a Caucasian patient with signs and symptoms that often precede the acquired immune deficiency syndrome (AIDS). This virus is a typical type-C RNA tumor virus, buds from the cell membrane, prefers magnesium for reverse transcriptase activity, and has an internal antigen (p25) similar to HTLV p24. Antibodies from serum of this patient react with proteins from viruses of the HTLV-I subgroup, but type-specific antisera to HTLV-I do not precipitate proteins of the new isolate. The virus from this patient has been transmitted into cord blood lymphocytes, and the virus produced by these cells is similar to the original isolate. From these studies it is concluded that this virus as well as the previous HTLV isolates belong to a general family of T-lymphotropic retroviruses that are horizontally transmitted in humans and may be involved in several pathological syndromes, including AIDS.*



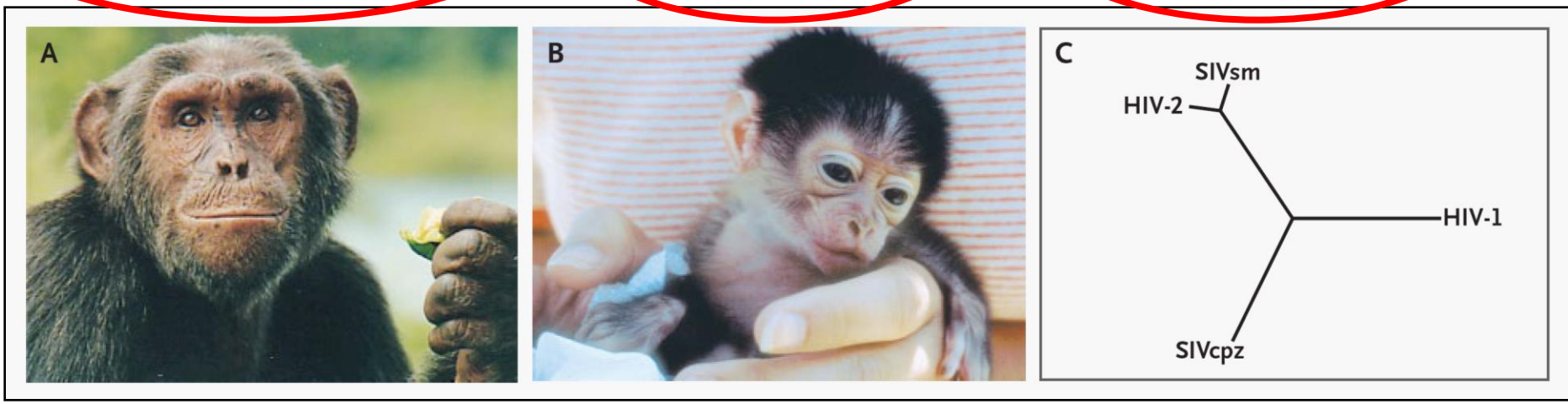
Barré-Sinoussi et al,
Science, 1983

Origin of HIV

HIV-1
Pan troglodytes troglodytes
(subspecies of chimpanzee)

HIV-2
Cercocebus atys
(sooty mangabey)

Radial phenogram
HIV and SIV



Zoonosis: disease communicable from animals to humans

Chimpanzee-to-human transmission events (≥3): HIV-1 (M, N, O)

Sooty mangabey-to-human transmission events (≥7): HIV-2 (A-G)

Cpz - Chimpanzee
HIV – Human immunodeficiency virus
SIV – Simian immunodeficiency virus
Sm – Sooty mangabey

Stebbing et al, New Engl J Med, 2004

The HIV pandemic

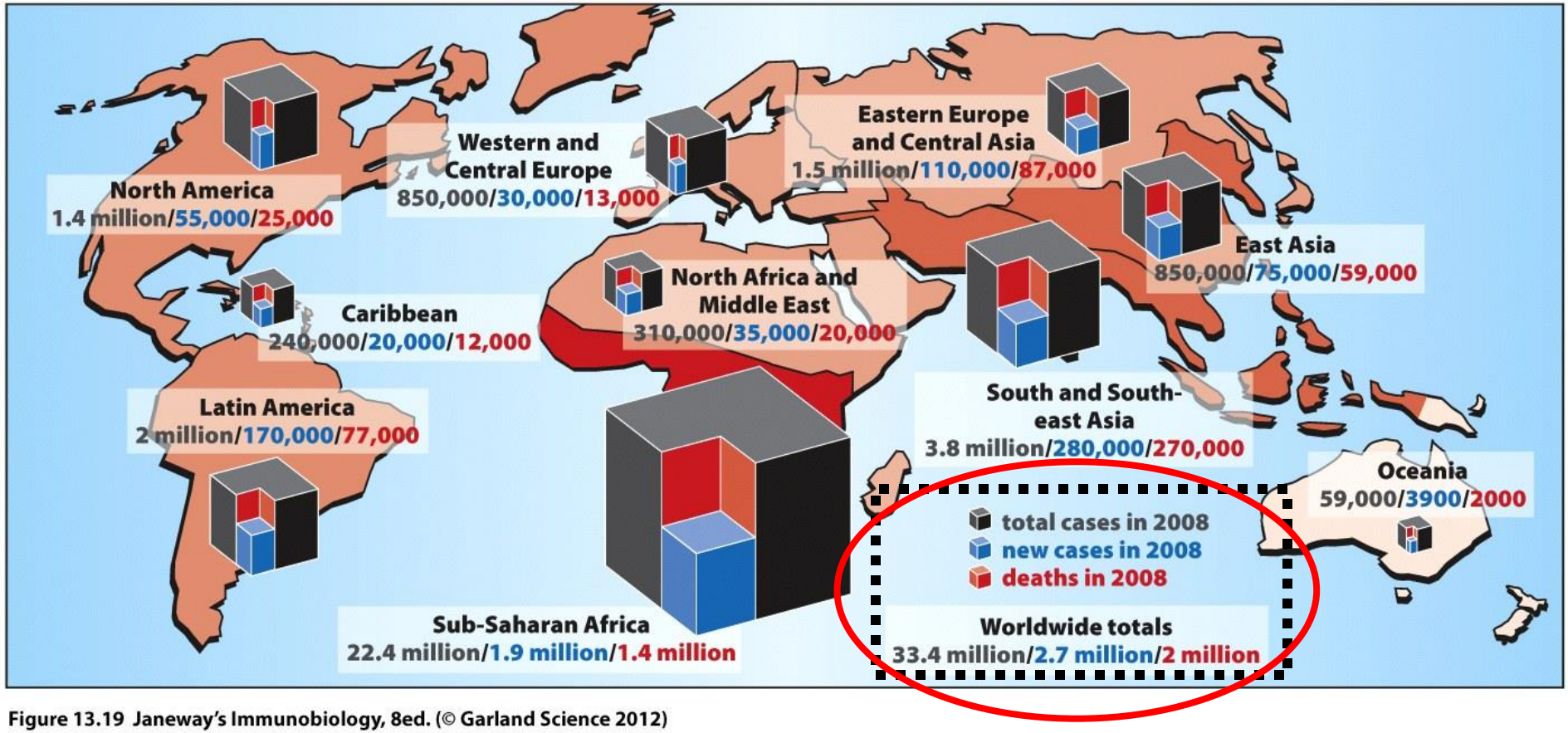
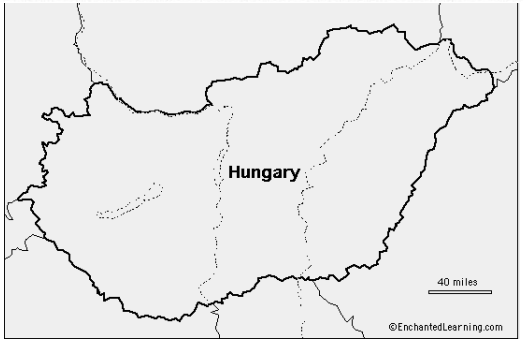


Figure 13.19 Janeway's Immunobiology, 8ed. (© Garland Science 2012)



- 37.7 million individuals (1.8 million children) living with HIV/AIDS (at the end of 2015)
- 35 million died of AIDS (by the end of 2015)
- 2.1 million new cases / year (5,700 / day)

Research expenditure, > 1 billion (10^9) US$ / year
HIV, > 310,000 entries (PubMed)
AIDS, > 250,000 entries

- 2,115 HIV positive individuals identified (cumulative total by the end of 2011)

www.who.int

Schematic structure of HIV

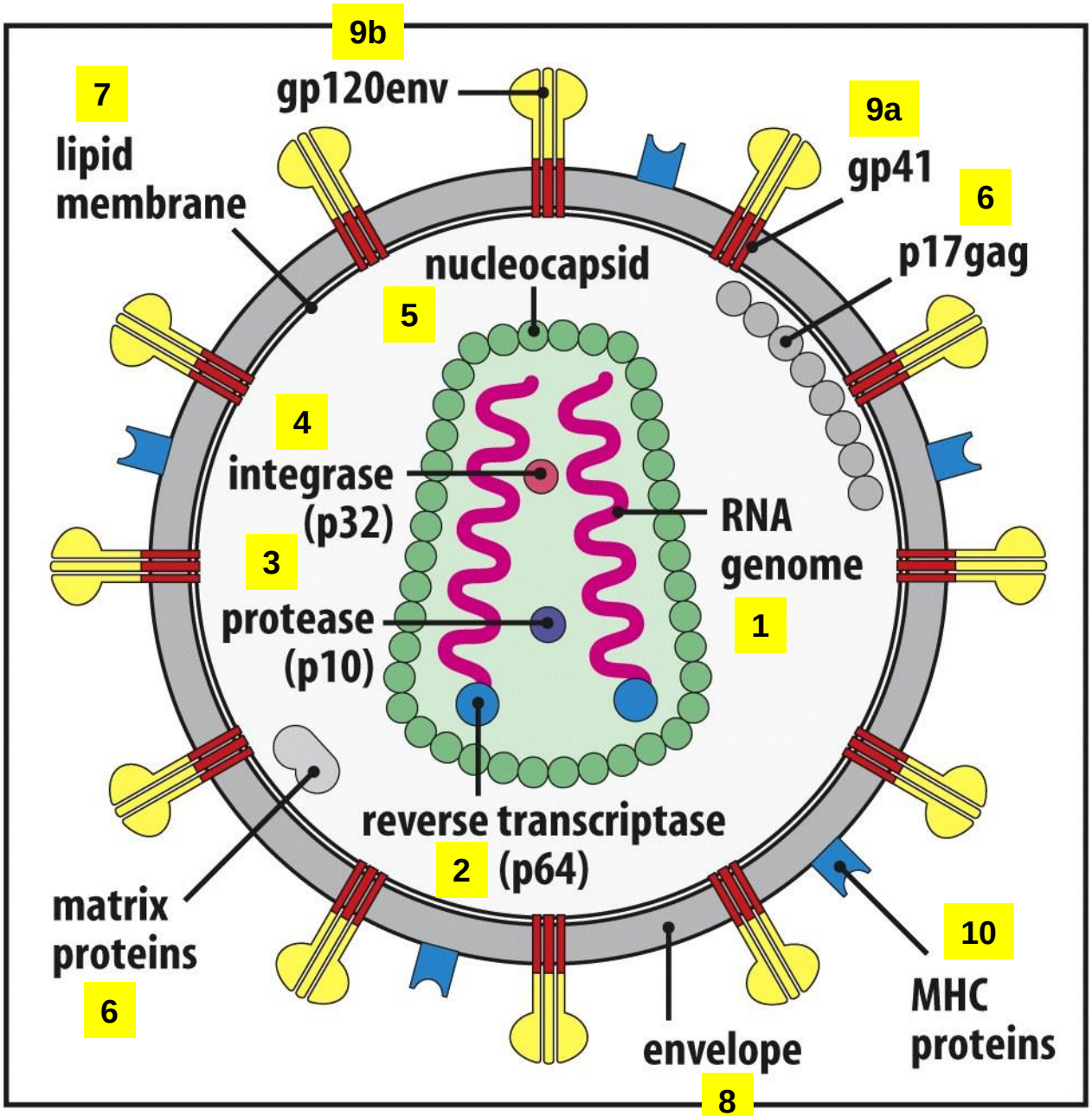


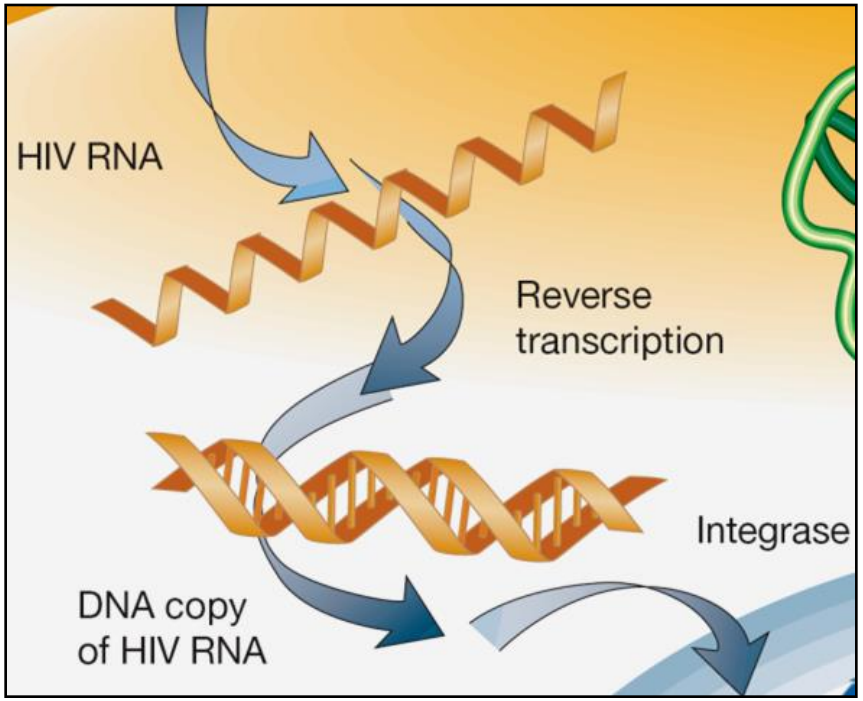
Figure 13.22 part 2 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Accelerated evolution of HIV

Number of protein-coding genes:

H. sapiens ≈ 20,000

Mutation rate in DNA replication:
$\approx 10^{-8}$-10^{-10} per bp



Reverse transcriptase: <u>error-prone enzyme</u>

HIV

RNA genome size ~9-10 kb
1 mutation / replication cycle

~10^5x higher mutation rate than in eukaryotic cells

10^{10}-10^{11} new virus particles/day/individual

~50 million people infected

Darwinian evolution of HIV at extremely fast rate

HIV vaccine strategies under study (1)

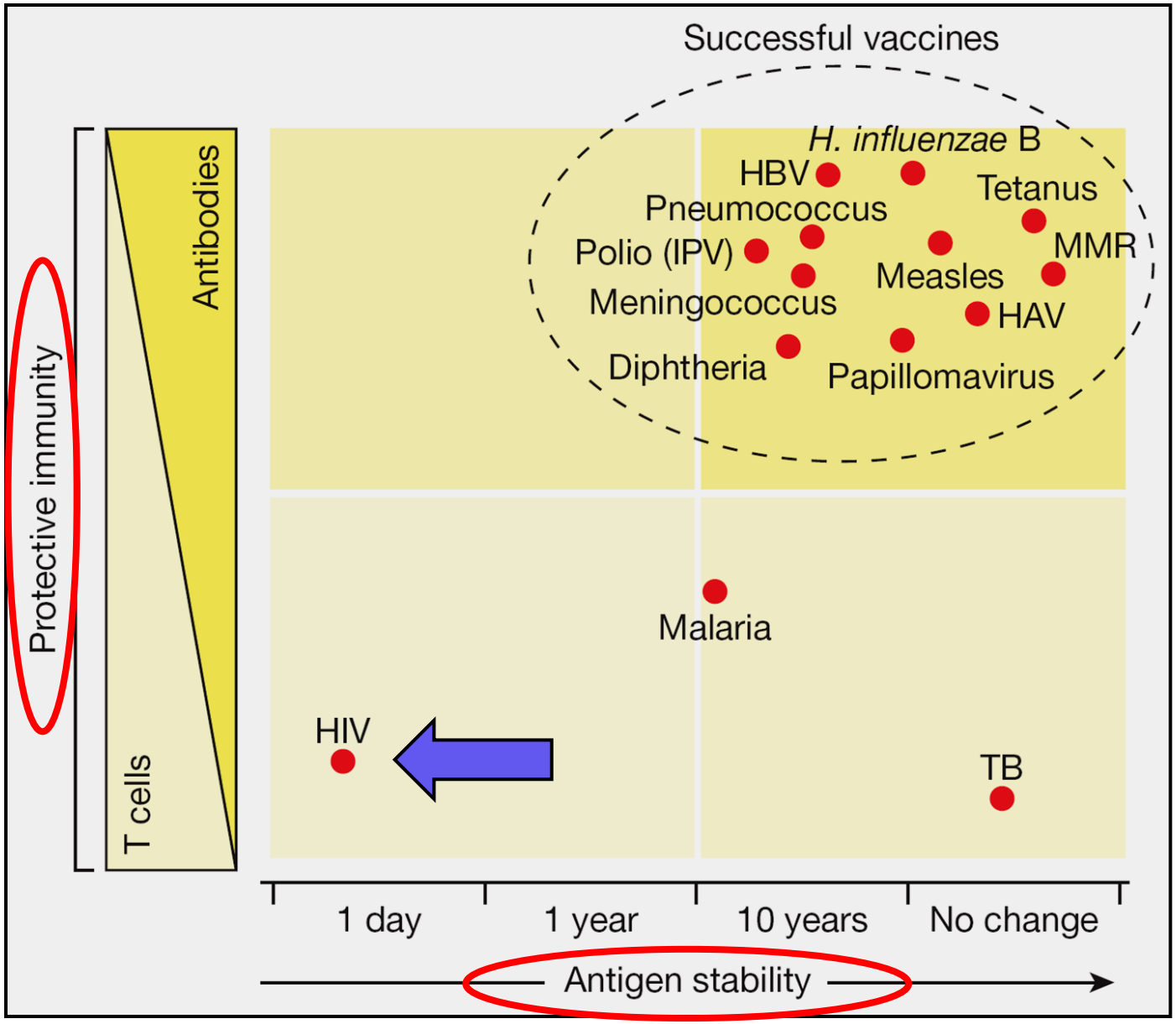
Vaccine constituents	Status	Advantages	Disadvantages
	1 **Vaccines eliciting anti-HIV antibodies**		
Viral surface proteins, such as gp120	In phase I and II trials, which examine safety	Safe and simple to prepare	Vaccine-elicited antibodies have failed to recognize HIV from patients
Whole, killed HIV	Not under study in humans	Should present HIV surface proteins in a relatively natural conformation; simple to prepare	Slight risk that preparations might include some active virus; inactivated virus might shed its proteins and become ineffective
Pseudovirions (artificial viruses containing HIV surface proteins)	Close to phase I trials	Present HIV surface proteins in a relatively natural conformation	Difficult to produce and to ensure long-term stability

HIV vaccine strategies under study (2)

2	**Vaccines eliciting cellular responses**		
Live vector viruses (non-HIV viruses engineered to carry genes encoding HIV proteins)	In phase II trials	Makers can control amount and kinds of viral proteins produced	Complicated to prepare; current vaccines elicit modest immune response
Naked DNA containing one or more HIV genes	In phase I trials	Simple and inexpensive to prepare	Some worry that integration of HIV genes into human cells could harm patients
HIV peptides (protein fragments)	In phase I trials	Simple to prepare	Do not elicit strong immune response
3	**Vaccines eliciting antibody and cellular responses**		
Combinations of elements, such as pure gp120 protein plus canarypox vector	In phase II trials	Should stimulate both arms of the immune response at once	Complicated to prepare
Live, attenuated HIV	Not under study in humans; being assessed in nonhuman primates	Most closely mimics HIV; may interfere with ability of infectious HIV to replicate	Vaccine virus could potentially cause AIDS

SOURCE: D Baltimore and C Heilman, "HIV vaccines: prospects and challenges," 1998, *Sci. Am.* **279**

HIV: vast challenge in vaccine development



Rappuoli & Aderem, Nature, 2011

HIV treatment clinic waiting room at Kisiizi Hospital in Uganda

Will HIV infection and AIDS be successfully preventable and curable diseases?

The obstacles are huge, but perhaps not insurmountable.

http://www.avert.org/media-gallery/image-1063-hiv-treatment-clinic-waiting-room-at-kisiizi-hospital-uganda

Features of effective vaccines

	Features of effective vaccines	
1	Safe	Vaccine must not itself cause illness or death
2	Protective	Vaccine must protect against illness resulting from exposure to live pathogen
3	Gives sustained protection	Protection against illness must last for several years
4	Induces neutralizing antibody	Some pathogens (such as polio virus) infect cells that cannot be replaced (e.g. neurons). Neutralizing antibody is essential to prevent infection of such cells
5	Induces protective T cells	Some pathogens, particularly intracellular, are more effectively dealt with by cell-mediated responses
6	Practical considerations	Low cost per dose Biological stability Ease of administration Few side-effects

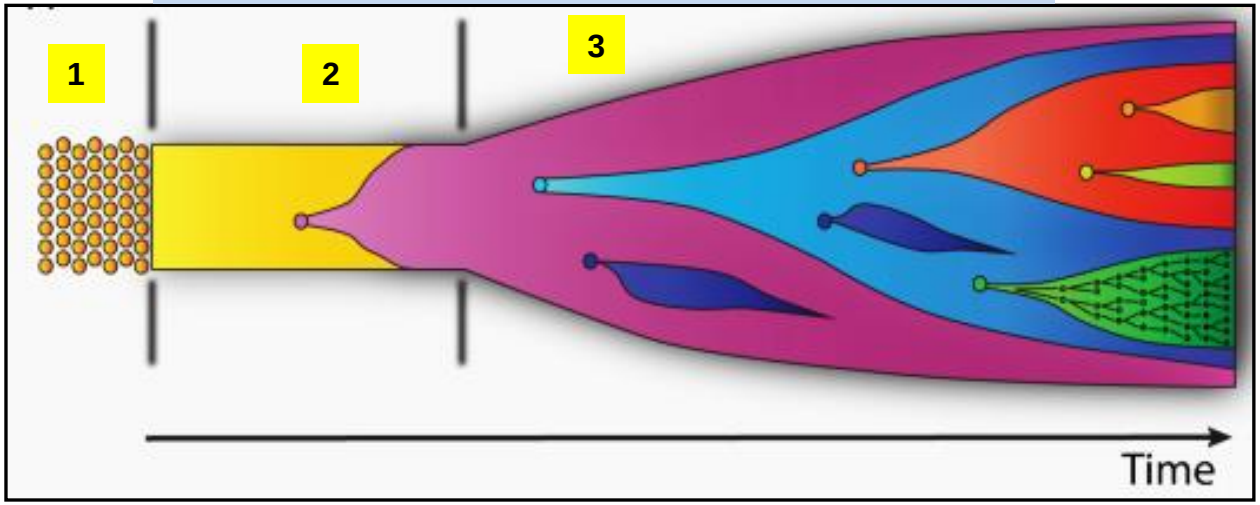
Figure 16.23 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

7. Approaches to cancer immunotherapy

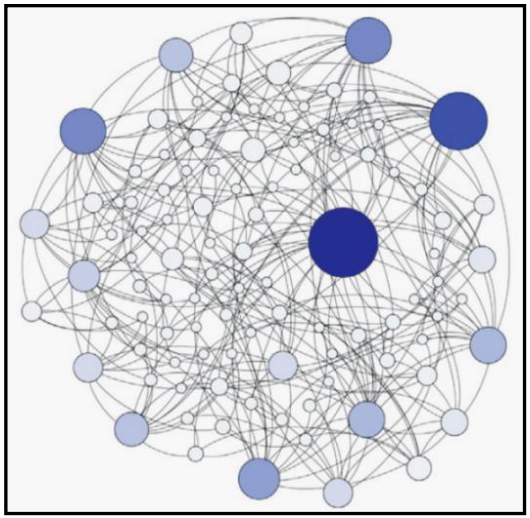
The immune system

- The immune system is a network of cells, tissues, and organs that work together to defend the body against attacks by “foreign” invaders.
- The human mature lymphoid system is comprised of 2 × 10^{12} lymphocytes and various accessory cells that include epithelial cells, monocytes/macrophages, and other antigen-presenting cells.

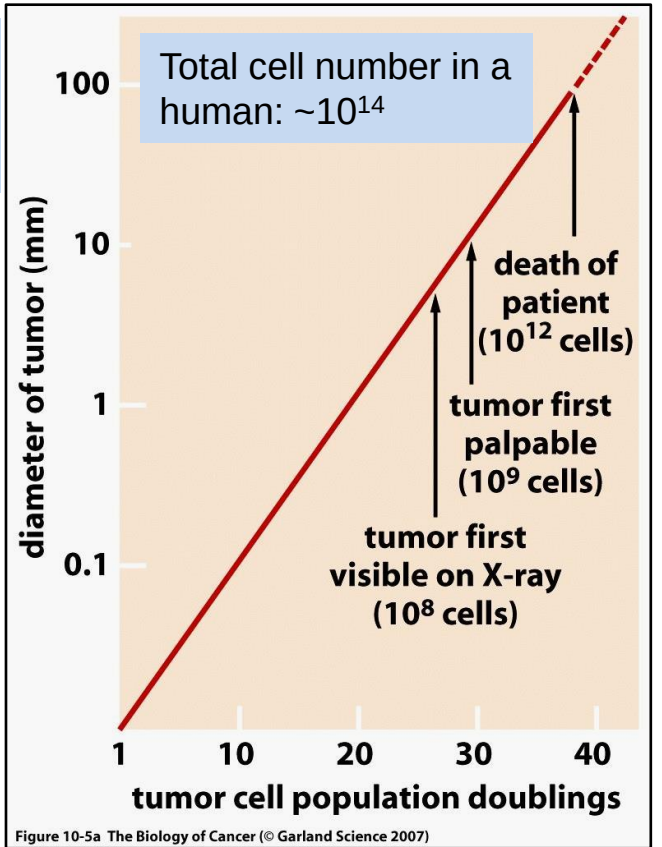
Tumor growth and evolution



1. Homeostatic tissue
2. Appearance of mutant cell clone
3. Propagation and evolution of tumor cell clones



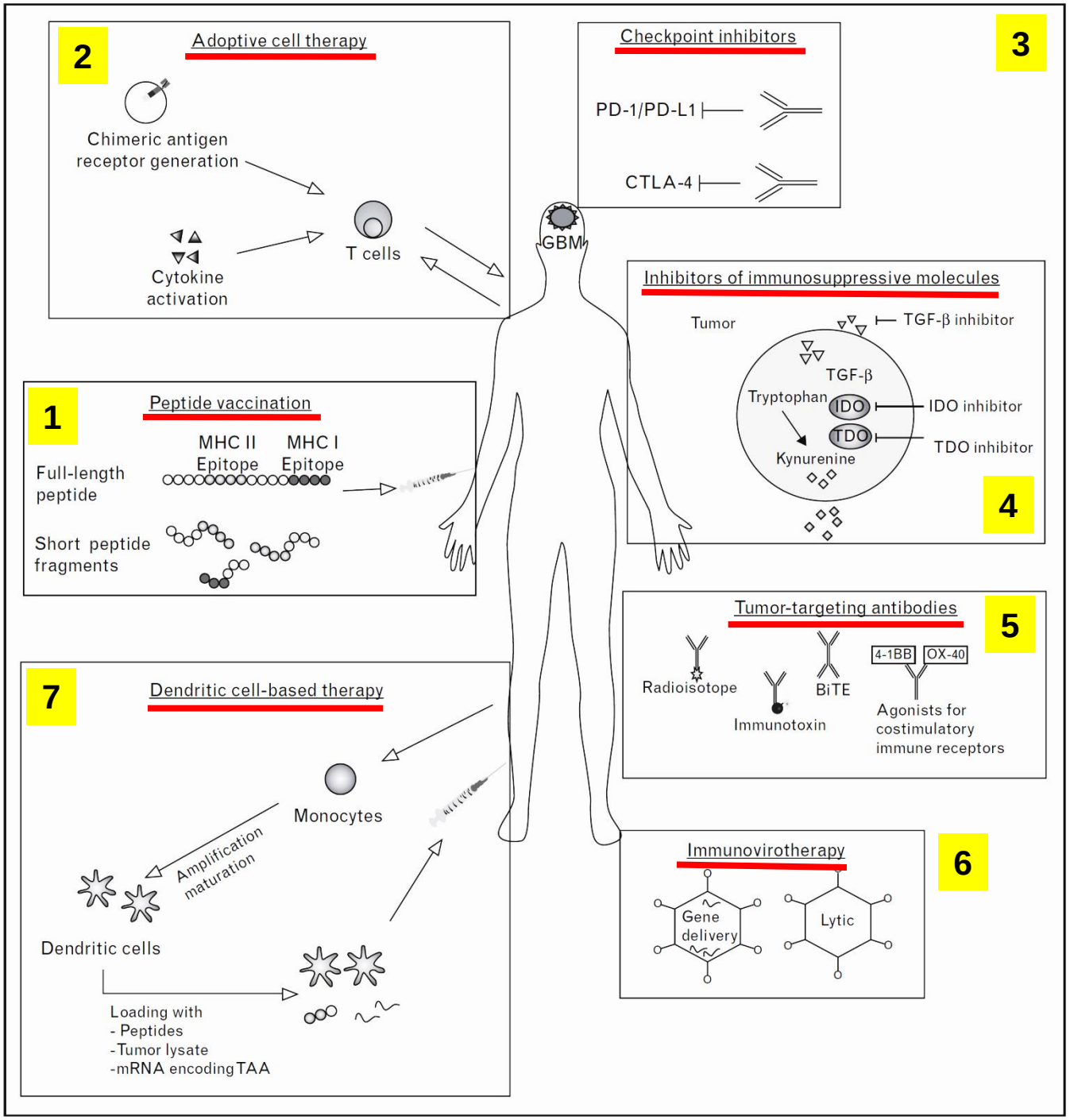
- Cruse & Lewis, Illustrated Dictionary of Immunology, 3e, 2009
- Baronchelli A et al, Trends Cogn Sci, 2013



I. Forms of immunotherapy against tumors

Basic principles

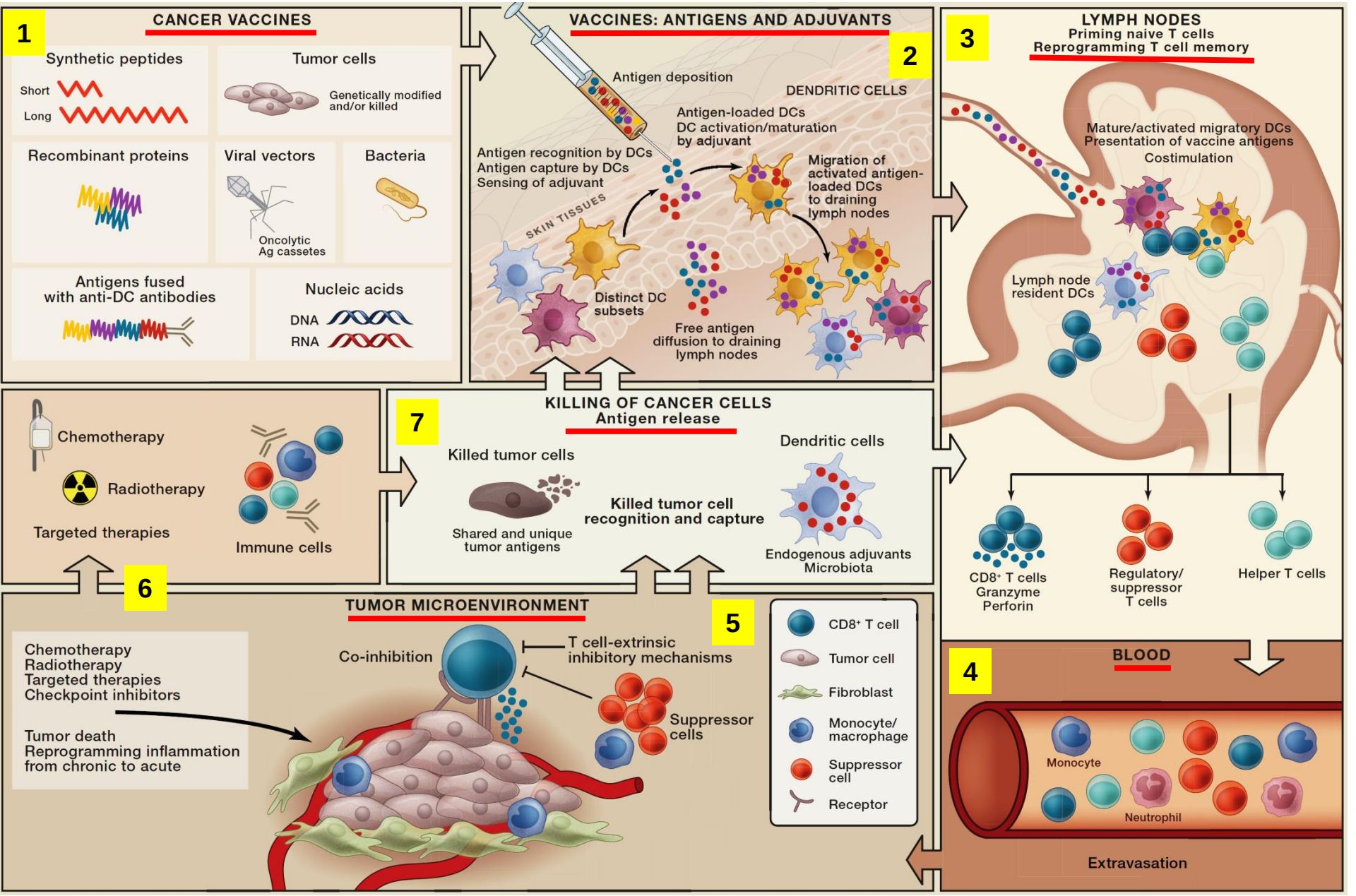
Immunotherapeutic approaches against tumors



Weiss T et al,
Curr Opin Neurol, 2015

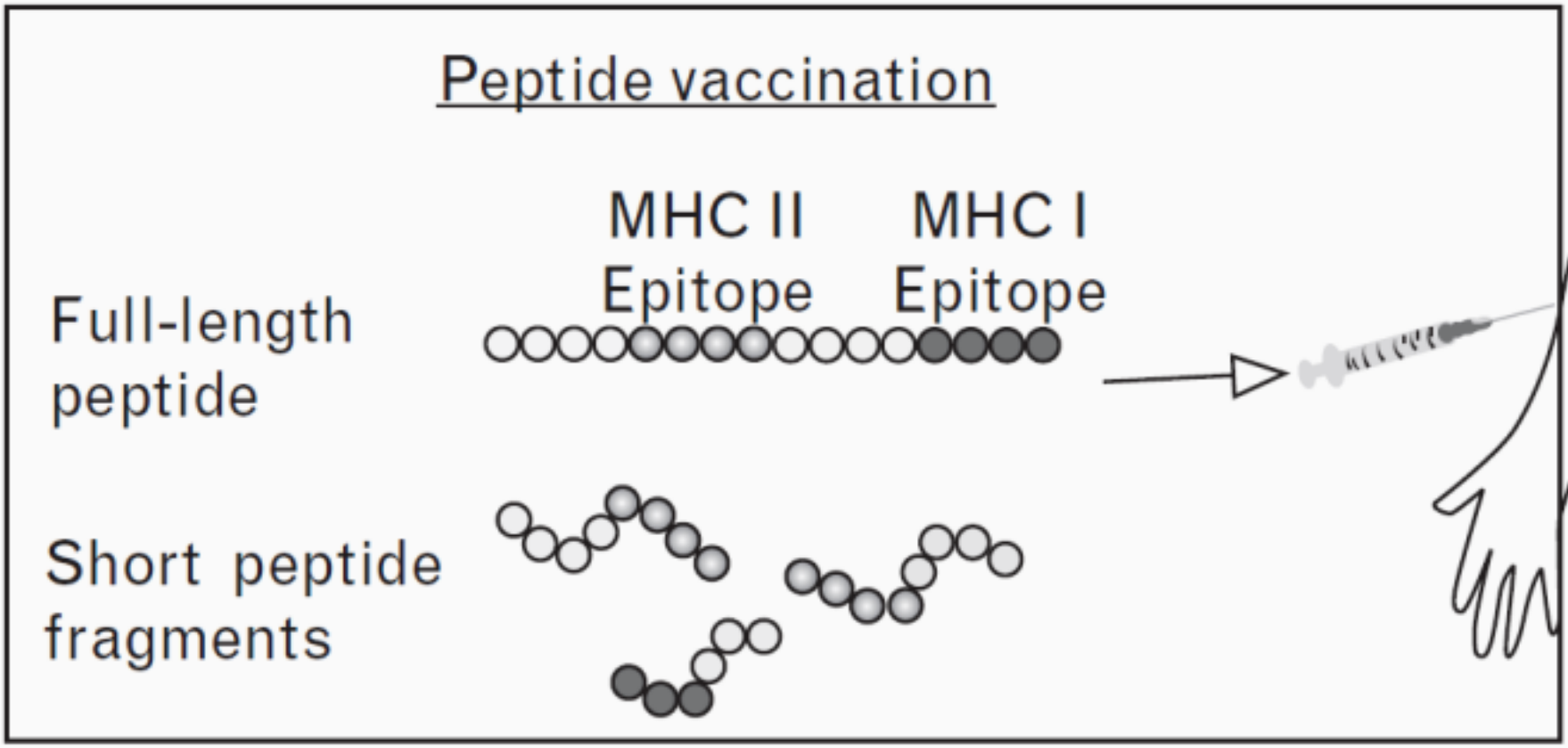
Induction of immune response against tumors

Cancer vaccines: induction of immune response



Palucka & Banchereau, Cell, 2014

Peptide vaccination



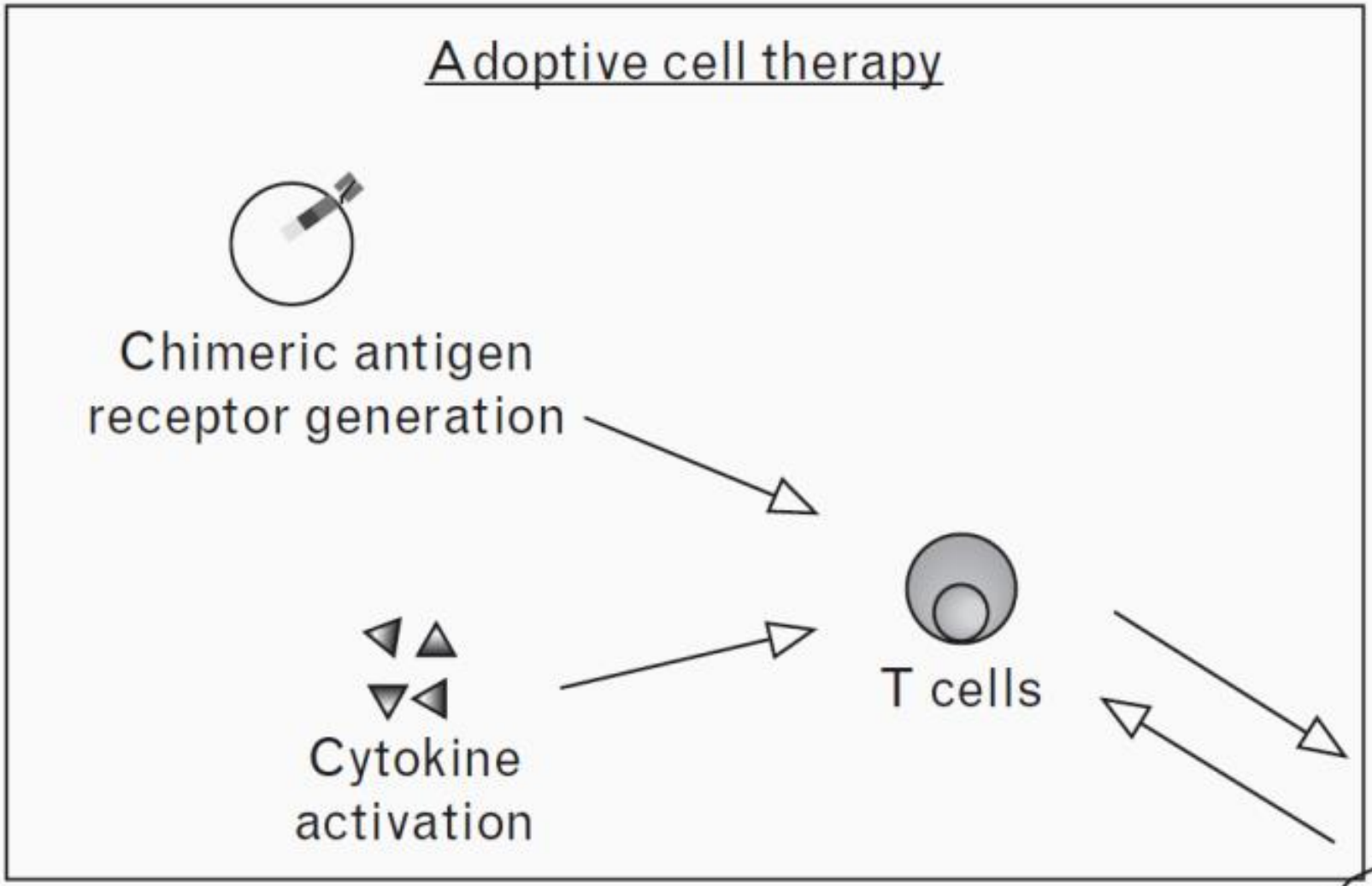
MHC, Major histocompatibility complex

Weiss T et al, Curr Opin Neurol, 2015

Peptide vaccination

- Elicitation of antigen-specific antitumor immune response by **vaccination** with **full-length tumor antigens** or **short antigenic peptide fragments** that are administered **intramuscularly, subcutaneously** or **intradermally** together with **adjuvants.**

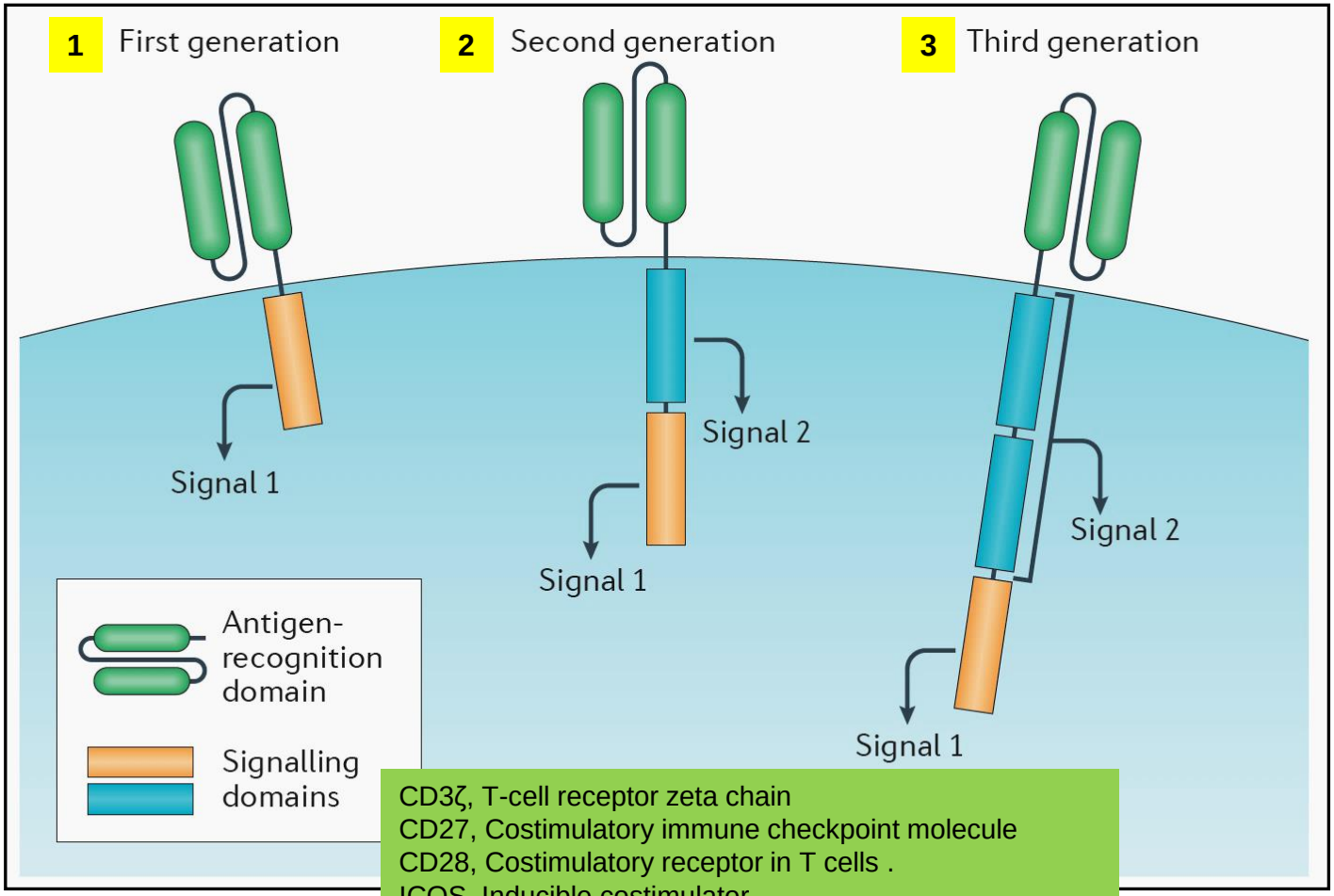
Adoptive cell therapy

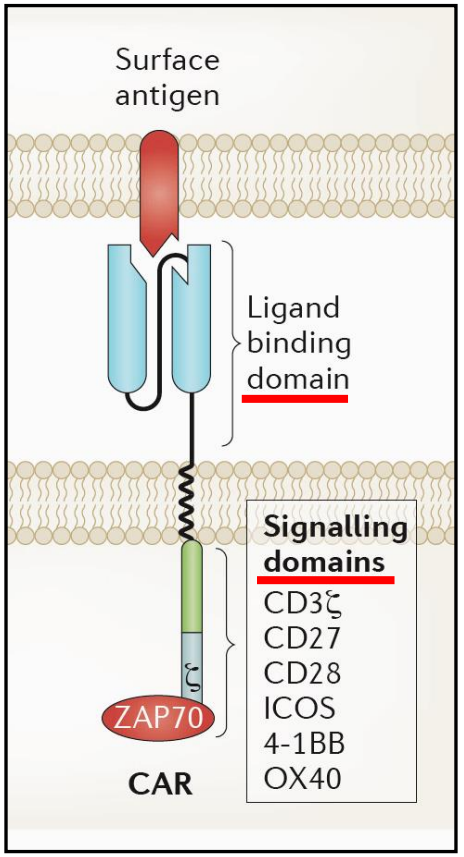


Weiss T et al, Curr Opin Neurol, 2015

CAR T-cell design

Chimeric antigen receptors (CARs) consist of an extracellular antigen-recognition domain, which is usually an antibody single-chain variable fragment (scFv), but can also be a peptide or another protein, linked to an intracellular signalling domain — usually the CD3ζ (CD3 zeta) chain of the T-cell receptor.

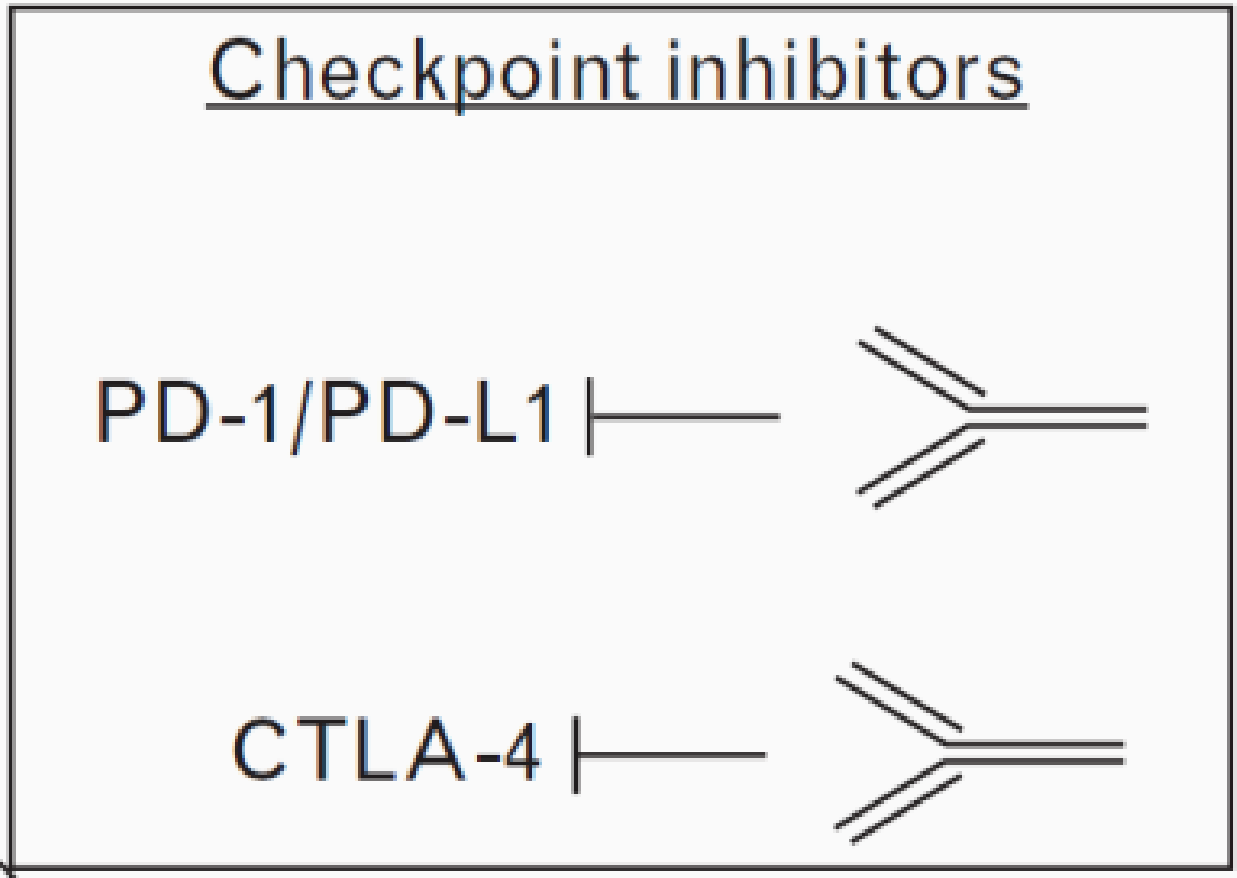




CD3ζ, T-cell receptor zeta chain
CD27, Costimulatory immune checkpoint molecule
CD28, Costimulatory receptor in T cells .
ICOS, Inducible costimulator
ZAP-70, Zeta-chain-associated protein kinase 70
4-1BB, OX-40, Costimulatory immune cell receptors

Jackson CM et al, Nature Rev Clin Oncol, 2016
Fesnak AD et al, Nature Rev Cancer, 2016

Checkpoint inhibitors

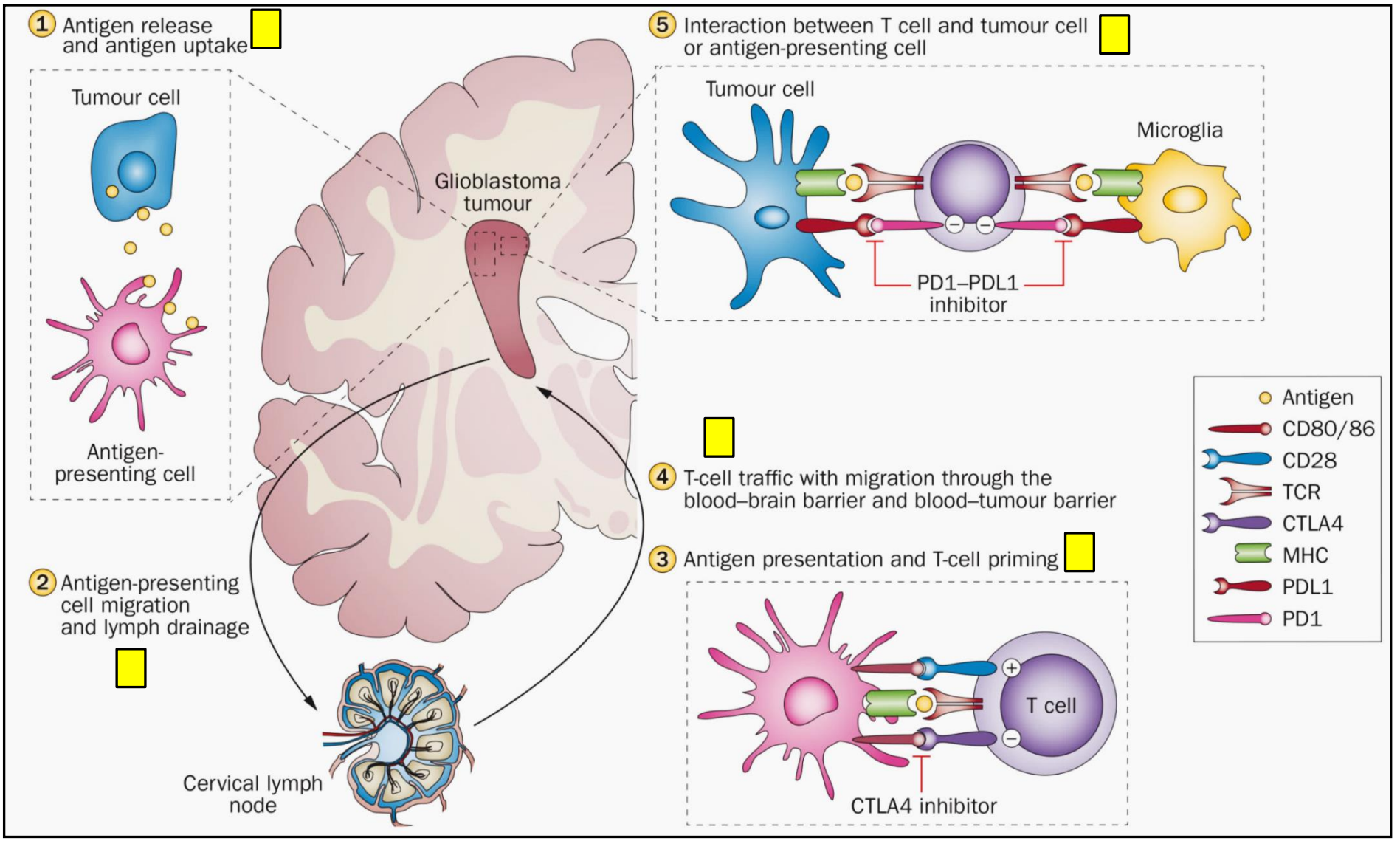


GBM

CTLA-4, Cytotoxic T lymphocyte-associated antigen-4
PD-1, Programmed death-1 (a receptor)
PD-L1, Programmed death-1 ligand

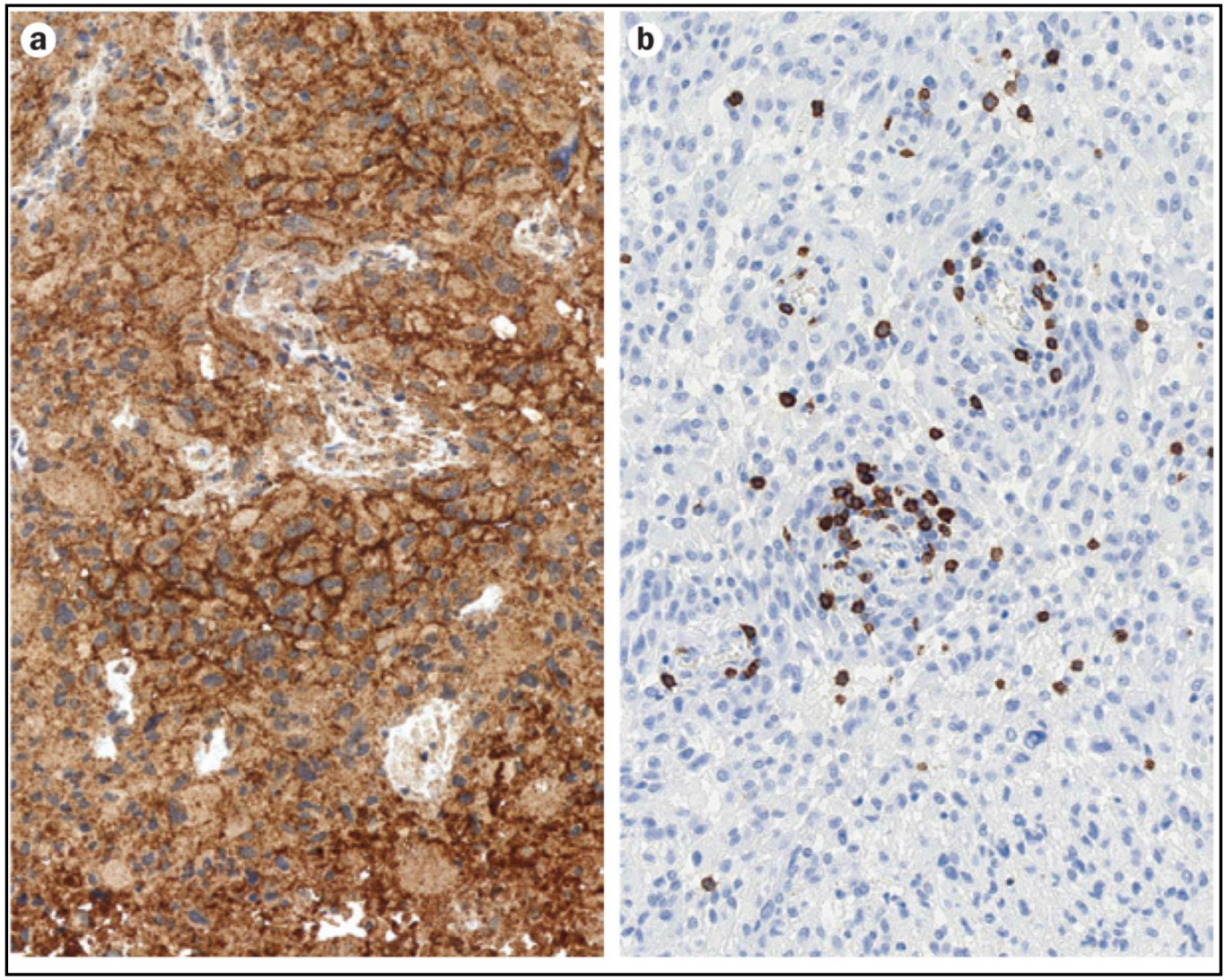
Weiss T et al, Curr Opin Neurol, 2015

Immune response against glioblastoma and immune checkpoints



Preusser M et al, Nature Rev Neurol, 2015

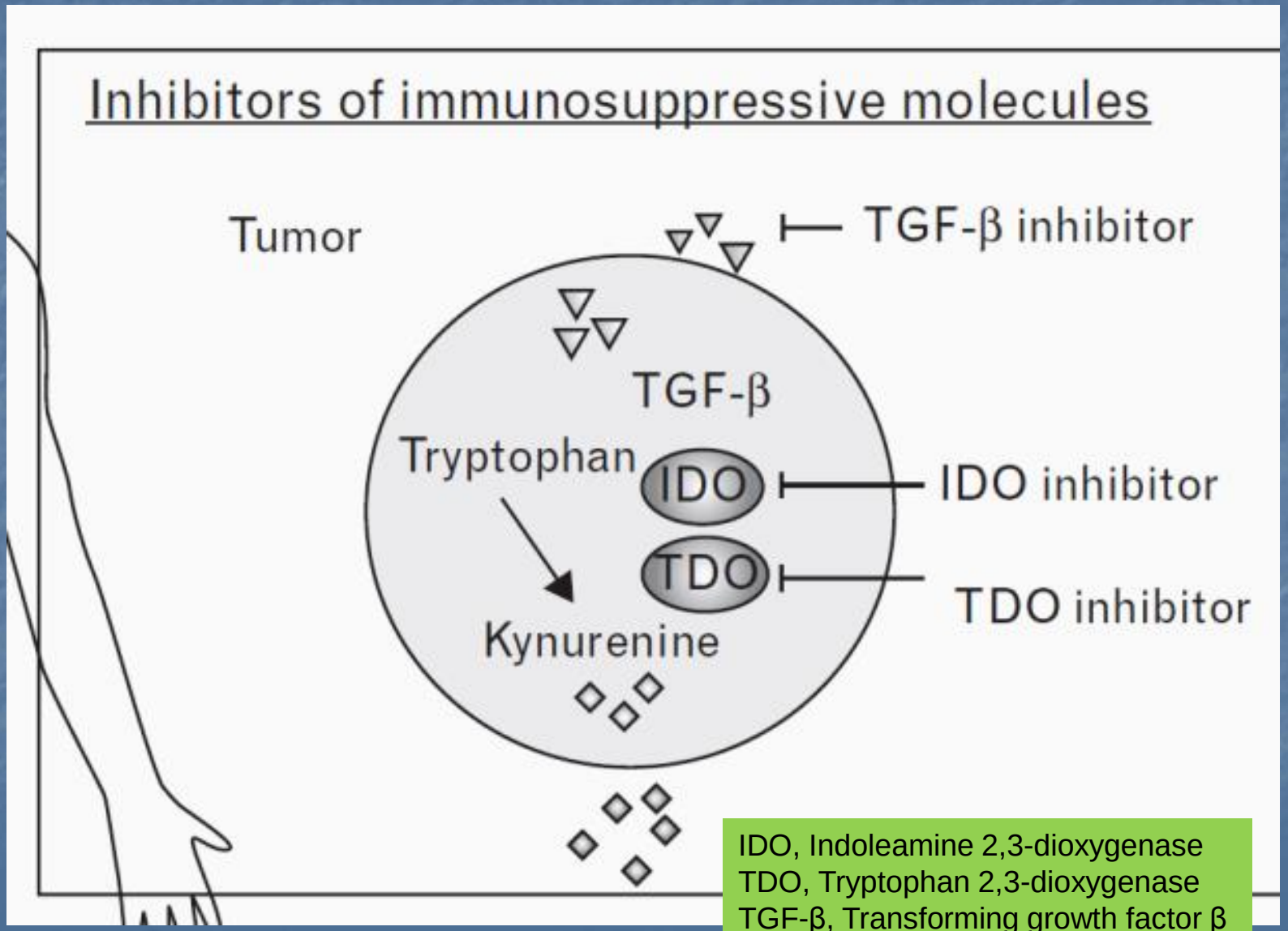
PDL1 expression and lymphocyte infiltration in glioblastoma



Expression of the immunosuppressive molecule PDL1 (**a**) and sparse infiltration with cytotoxic lymphocytes (**b**) are found in the majority of glioblastoma cases.

Preusser M et al, Nature Rev Neurol, 2015

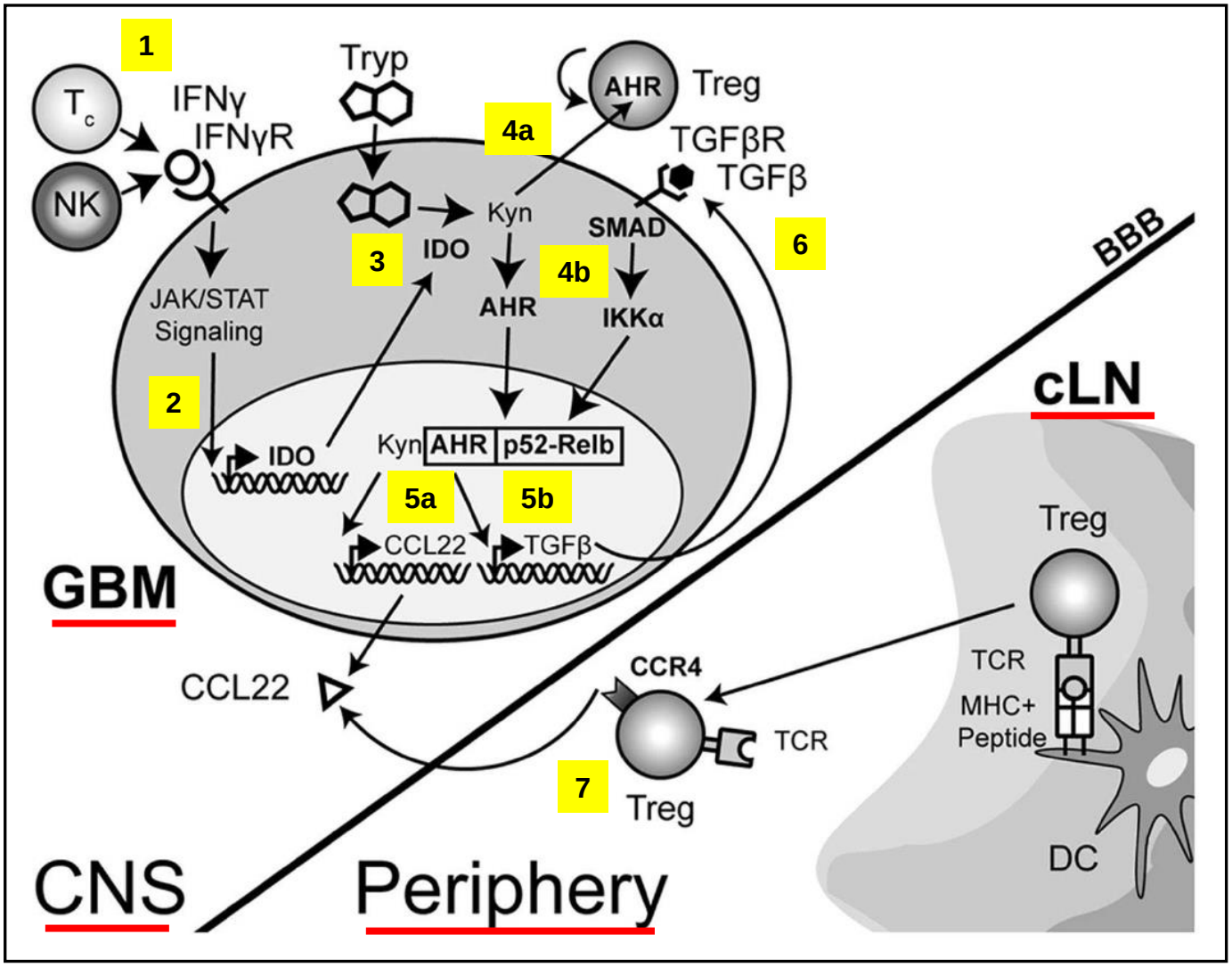
Inhibitors of immunosuppressive molecules



IDO, Indoleamine 2,3-dioxygenase
TDO, Tryptophan 2,3-dioxygenase
TGF-β, Transforming growth factor β

Weiss T et al, Curr Opin Neurol, 2015

Treg cell recruitment and expansion in brain tumors

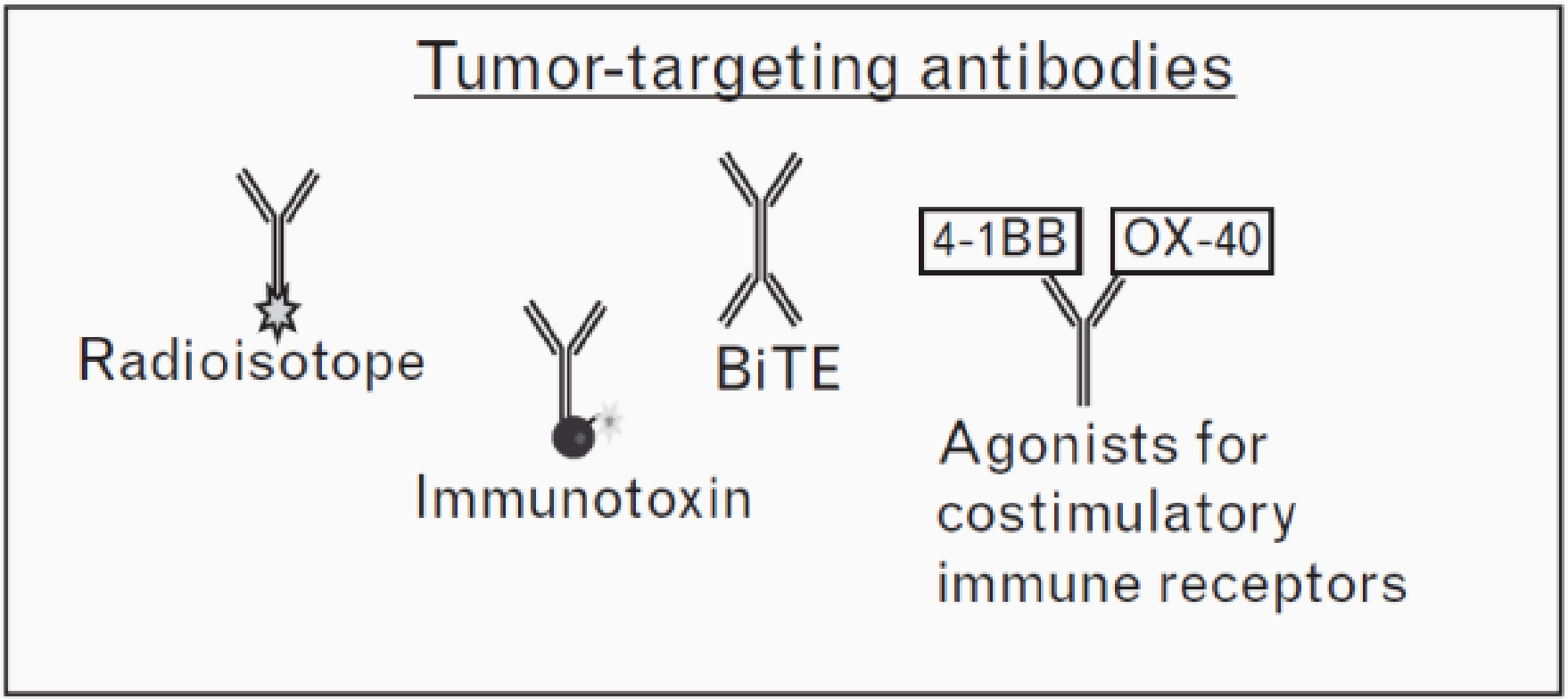


AHR, Aryl hydrocarbon receptor
BBB, Blood-brain barrier
cLN, Cervical (draining) lymph node
CNS, Cantral nervous system
GBM, Glioblastoma multiforme
IDO, Indoleamine 2,3-dioxygenase 1
IKKα, Inhibitor of nuclear factor kappa-B kinase subunit alpha
IFNγR, Interferon γ receptor
Kyn, L-Kynurenine
NK, Natural killer cell
TCR, T cell receptor
Tc, Cytotoxic T cells
TGFβR, Transforming growth factor β receptor
Treg, Regulatory T cell
Tryp, Tryptophan

Indoximod, Methylated tryptophan with immune checkpoint (IDO) inhibitory activity to increase tryptophan levels important for T cell function.

Wainwright DA et al, Front Immunol, 2013

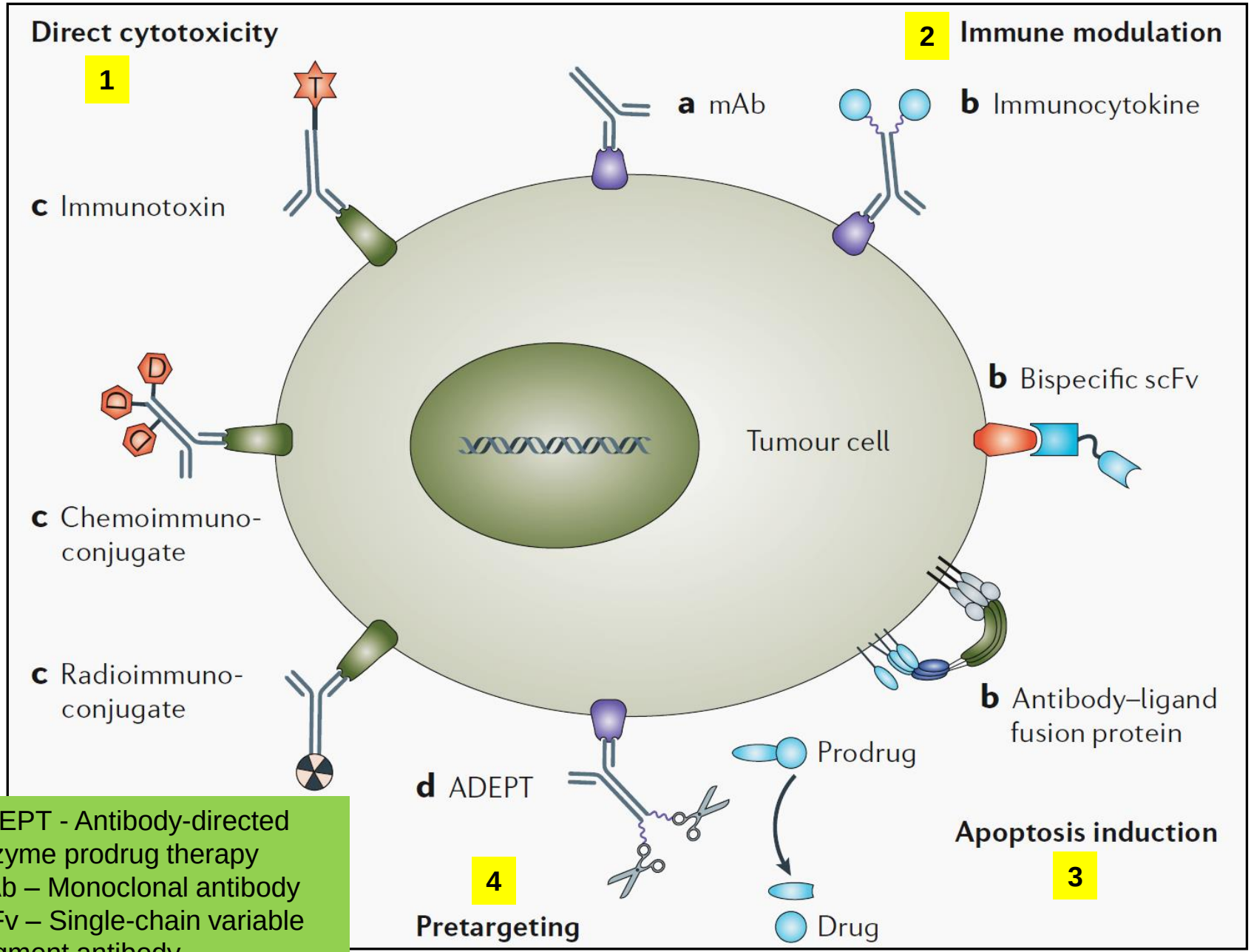
Tumor-targeting antibodies



BiTE, Bispecific T-cell engager
4-1BB, OX-40, Costimulatory immune cell receptors

Weiss T et al, Curr Opin Neurol, 2015

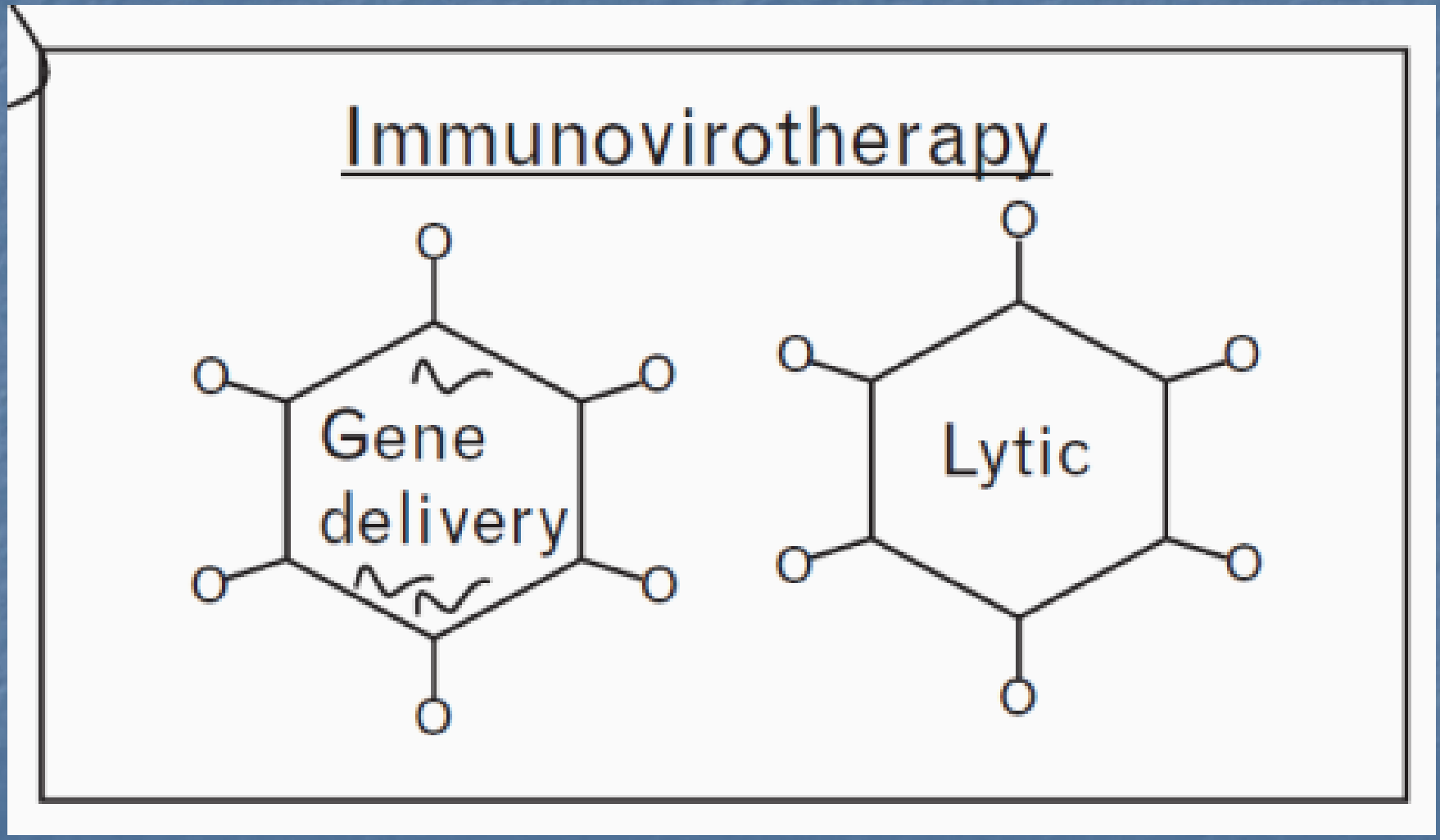
Concepts of therapeutic antibodies



ADEPT - Antibody-directed enzyme prodrug therapy
mAb – Monoclonal antibody
scFv – Single-chain variable fragment antibody

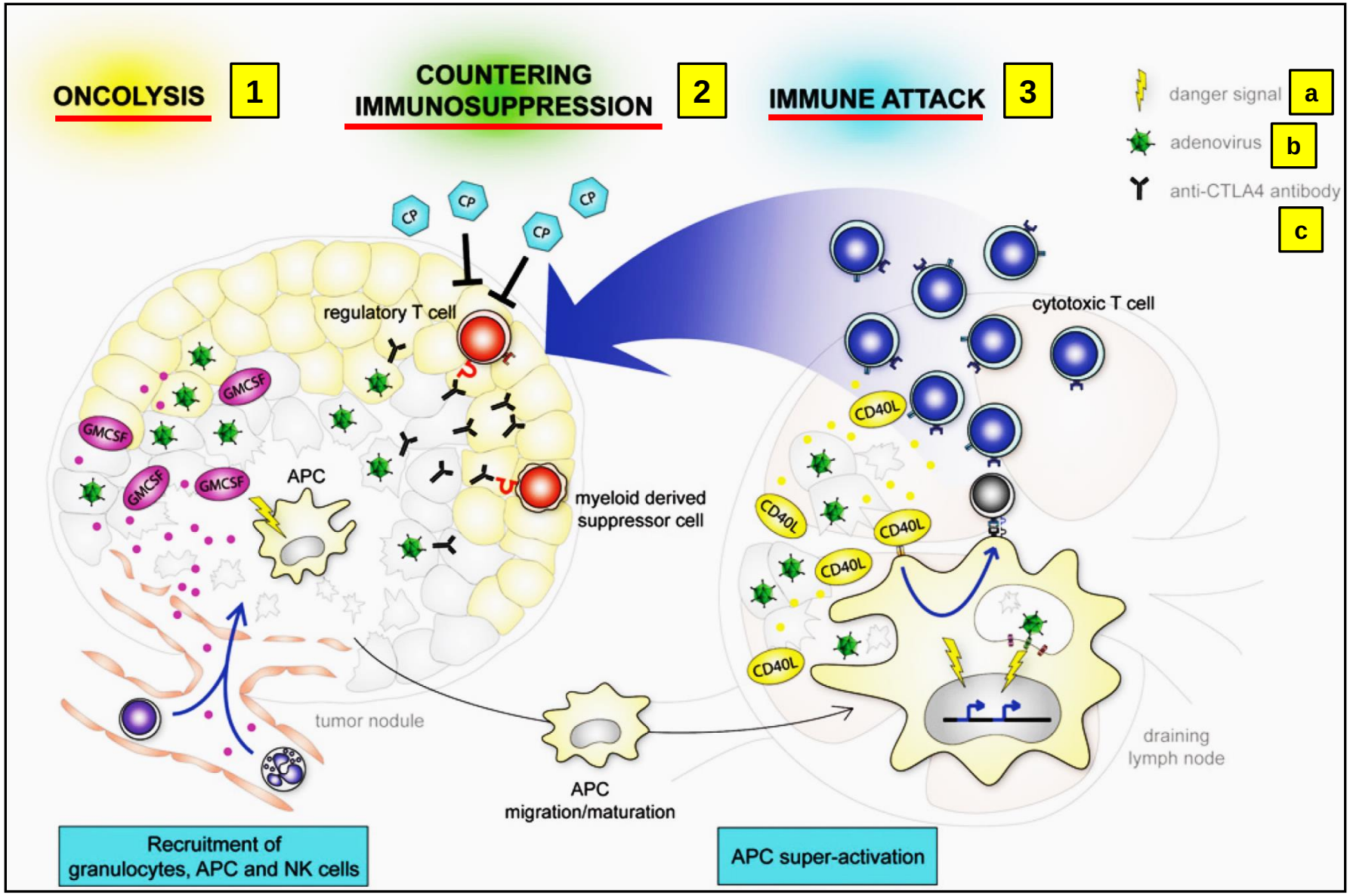
Schrama D et al, Nature Rev Drug Disc, 2006

Immunovirotherapy



Weiss T et al, Curr Opin Neurol, 2015

Immunotherapy of tumors using viruses



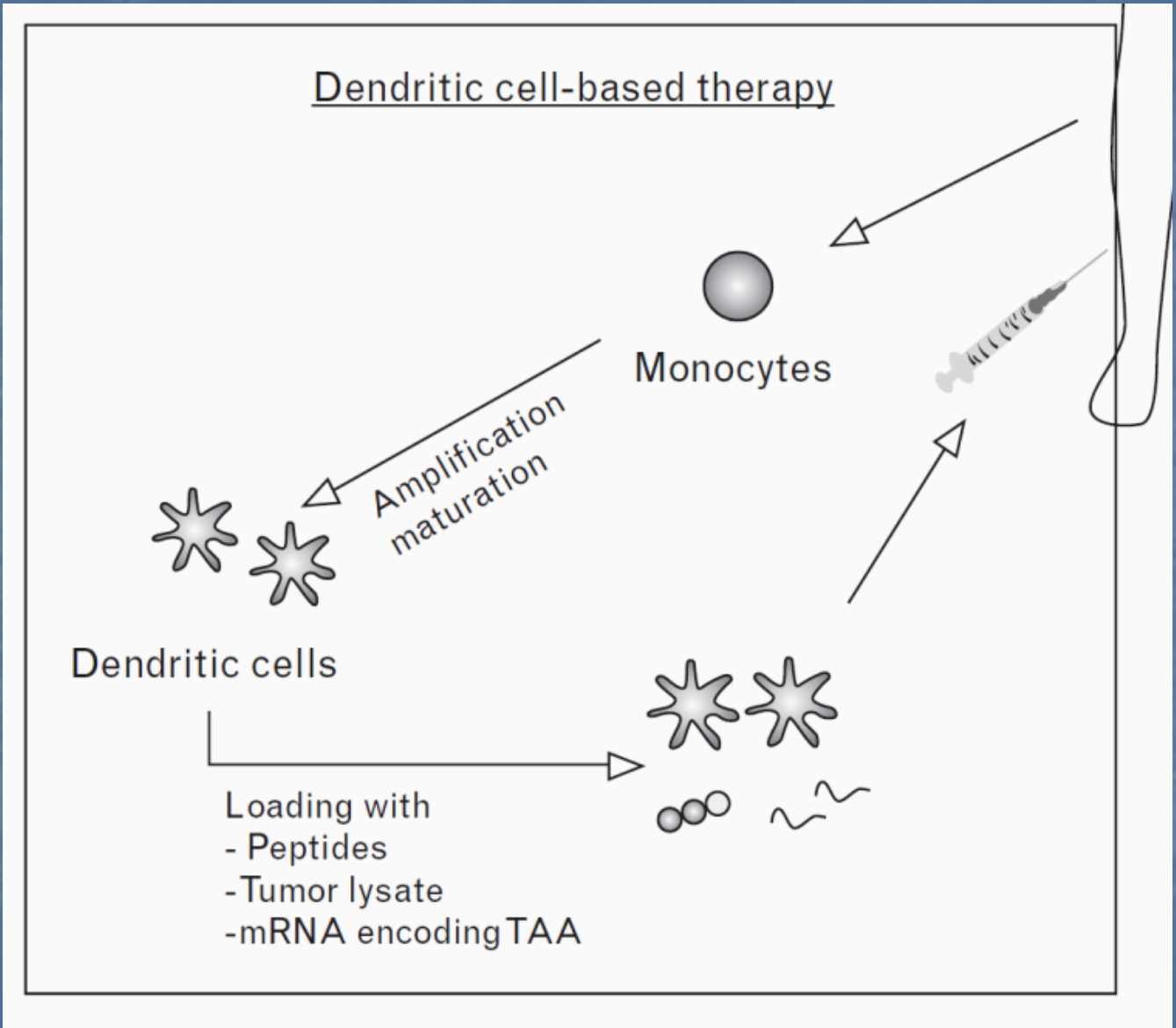
Cerullo V et al, OncoImmunol, 2012

APC – Antigen-presenting cell
CD40-CD40L - Costimulatory receptor-ligand pair (APCs-T cells)
CP - Cyclophosphamide

CTLA4 - Cytotoxic T lymphocyte-associated antigen-4 (CD152)
GMCSF - Granulocyte-macrophage colony-stimulating factor

Dendritic cell-based therapy

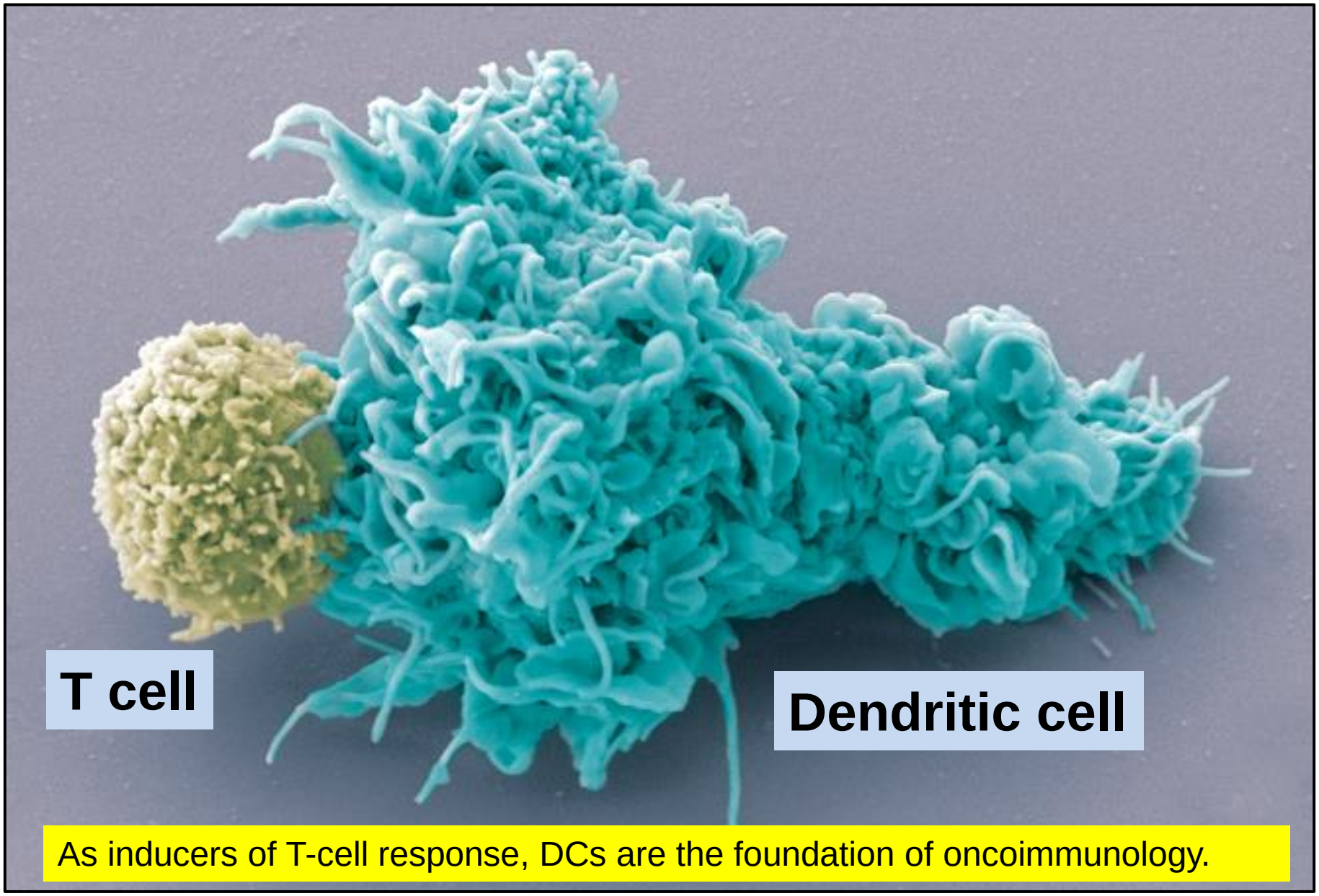
7



TAA, Tumor-associated antigen

Weiss T et al, Curr Opin Neurol, 2015

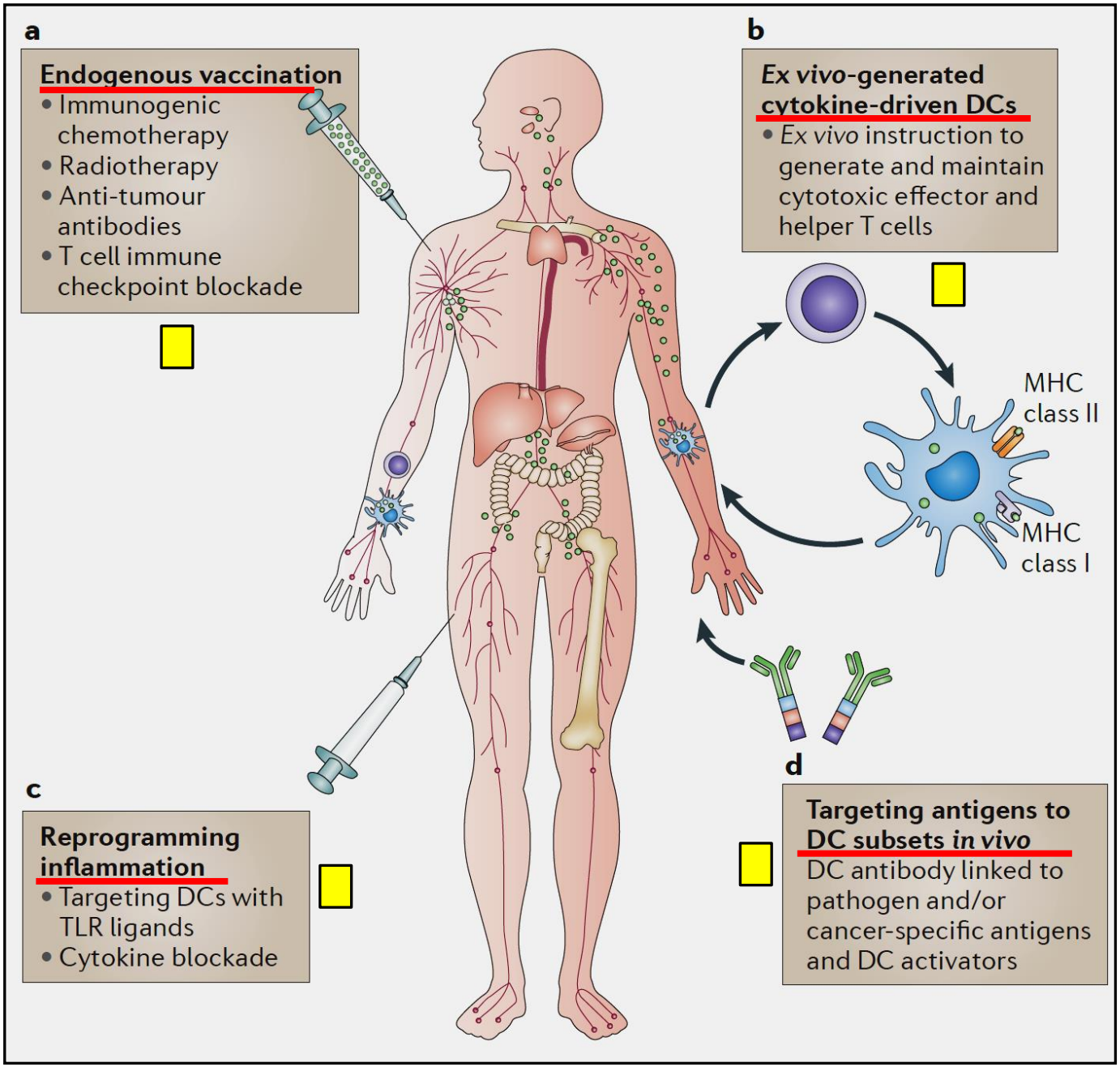
Immunological synapse



http://www.cell.com/cell_picture_show-immunology

A major communication interface between the innate and adaptive immune systems

Dendritic cells and cancer immunotherapy



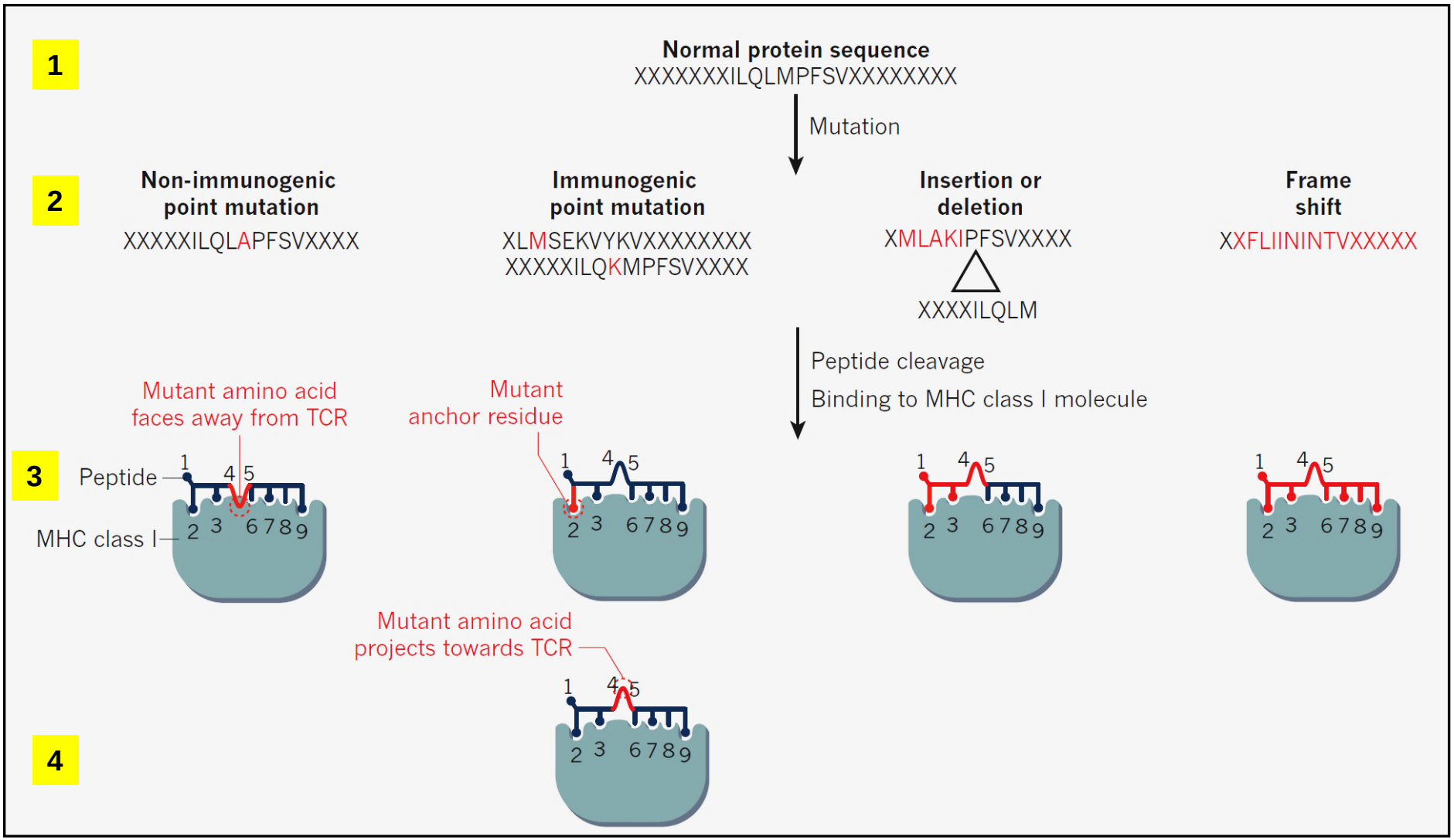
Palucka & Banchereau, Nature Rev Cancer, 2012

- Immunotherapy is proving to be an effective therapeutic approach in a variety of cancers.

- Despite the clinical success of a few vaccines for cancer prevention and antibodies against the immune regulators CTLA4 and PD-L1/PD-1, only a subset of people exhibit durable responses. This suggests that a broader view of cancer immunity is required.

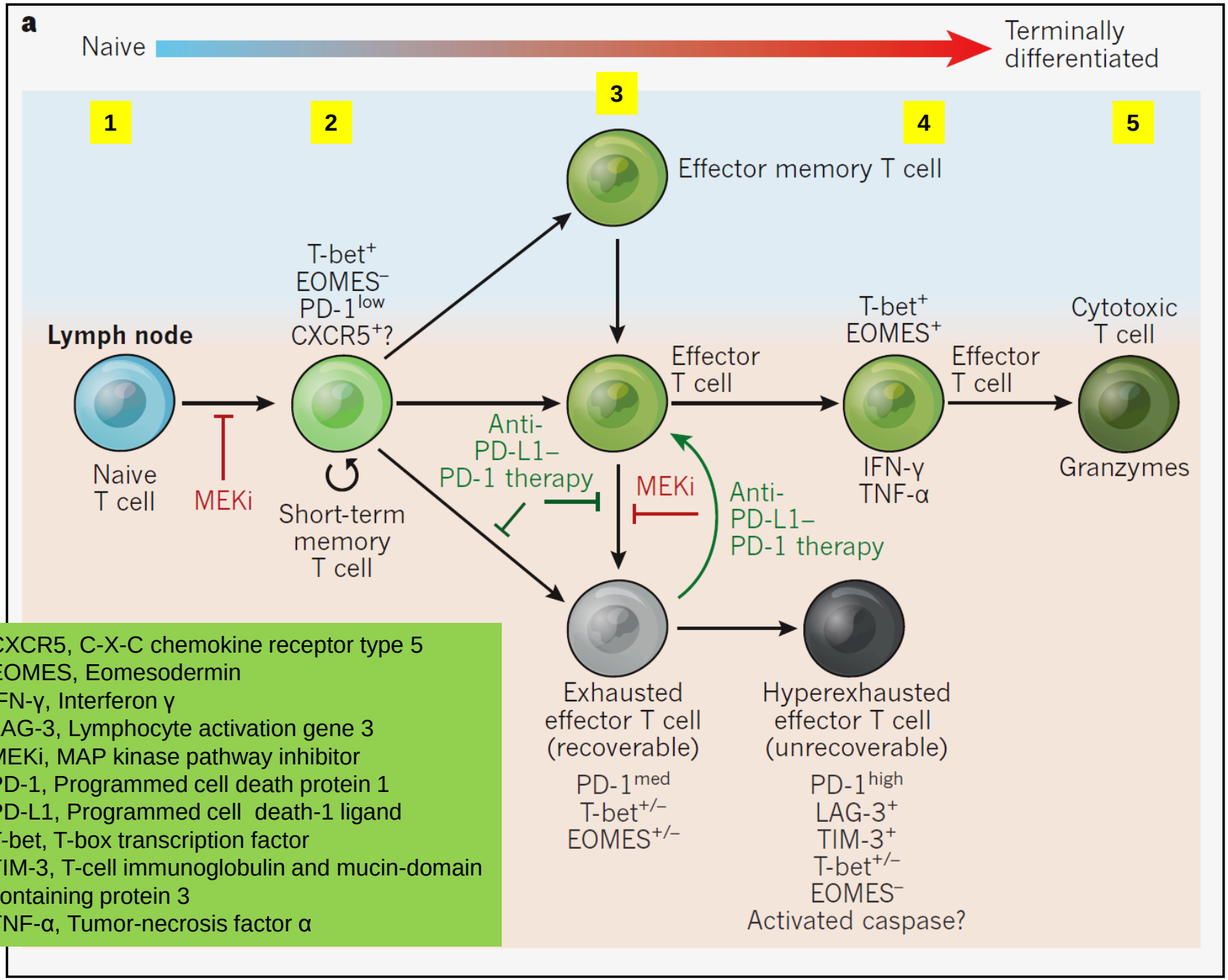
Chen & Mellman, Nature, 2017

Cancer mutations, neoantigens and immunogenicity



Chen & Mellman, Nature, 2017

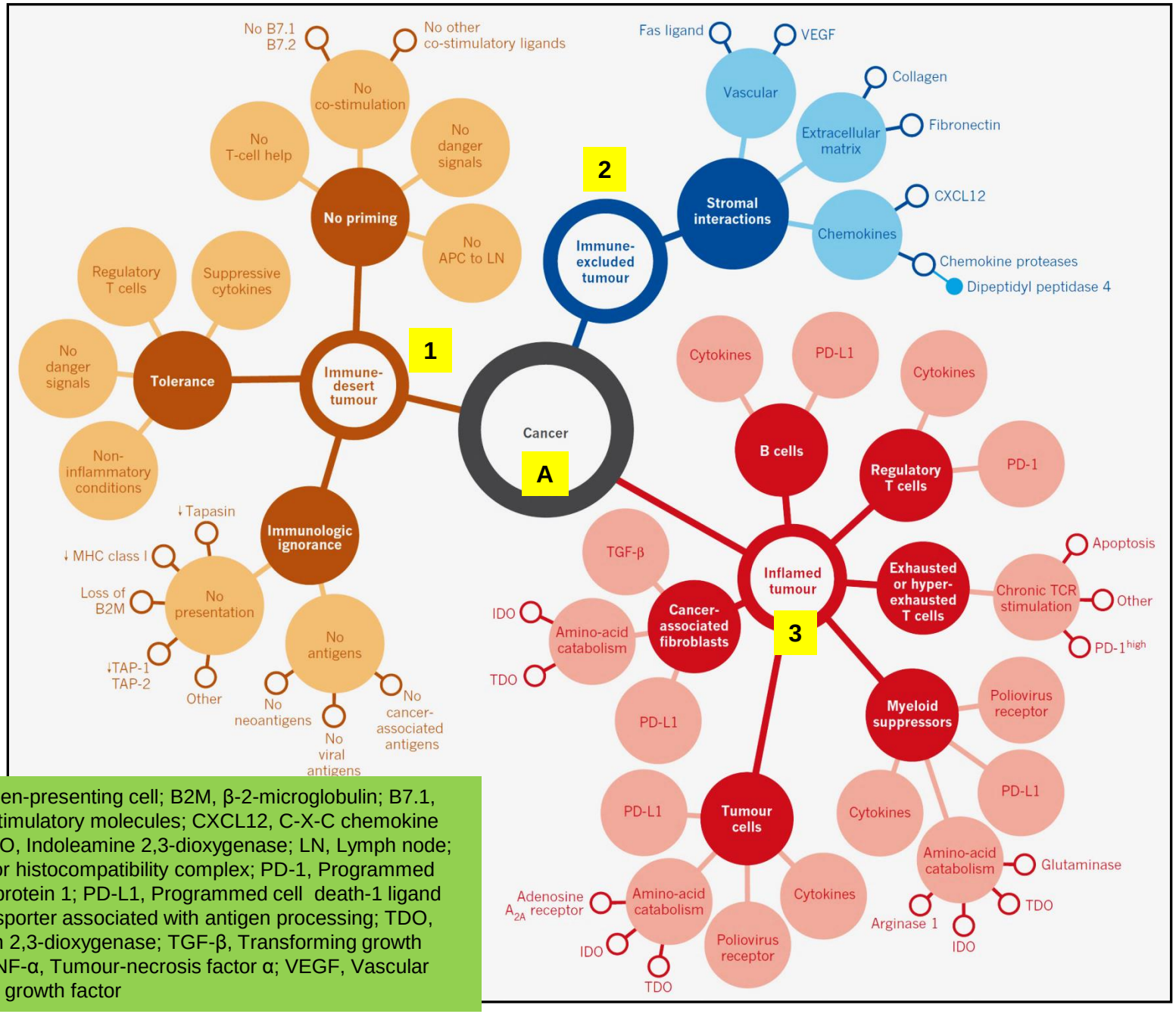
Development and phenotypes CD8+ T-cells



CXCR5, C-X-C chemokine receptor type 5
EOMES, Eomesodermin
IFN-γ, Interferon γ
LAG-3, Lymphocyte activation gene 3
MEKi, MAP kinase pathway inhibitor
PD-1, Programmed cell death protein 1
PD-L1, Programmed cell death-1 ligand
T-bet, T-box transcription factor
TIM-3, T-cell immunoglobulin and mucin-domain containing protein 3
TNF-α, Tumor-necrosis factor α

Chen & Mellman, Nature, 2017

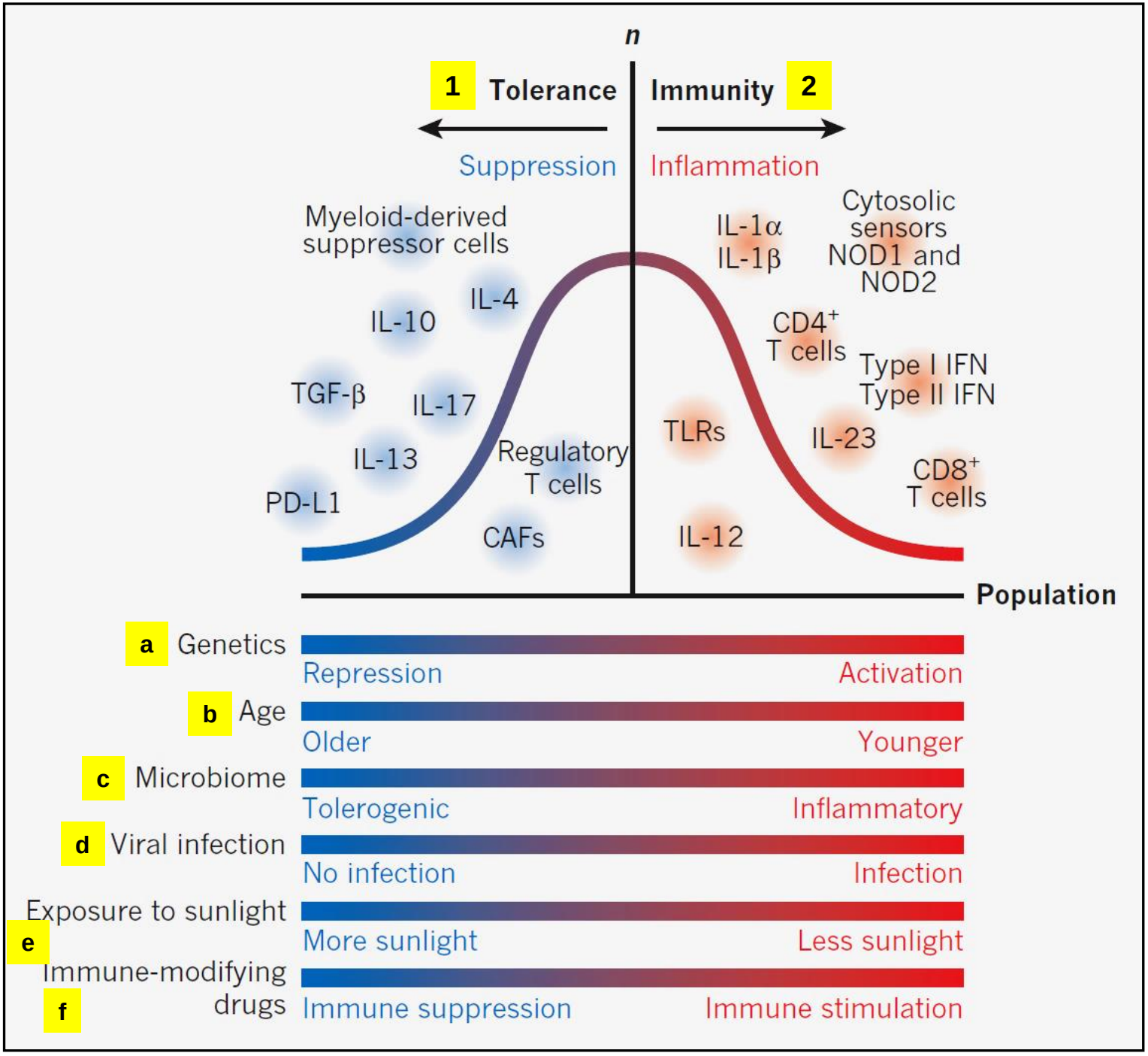
Cancer-immune phenotypes



APC, Antigen-presenting cell; B2M, β-2-microglobulin; B7.1, B7.2, Co-stimulatory molecules; CXCL12, C-X-C chemokine type 12; IDO, Indoleamine 2,3-dioxygenase; LN, Lymph node; MHC, Major histocompatibility complex; PD-1, Programmed cell death protein 1; PD-L1, Programmed cell death-1 ligand TAP, Transporter associated with antigen processing; TDO, Tryptophan 2,3-dioxygenase; TGF-β, Transforming growth factor β; TNF-α, Tumour-necrosis factor α; VEGF, Vascular endothelial growth factor

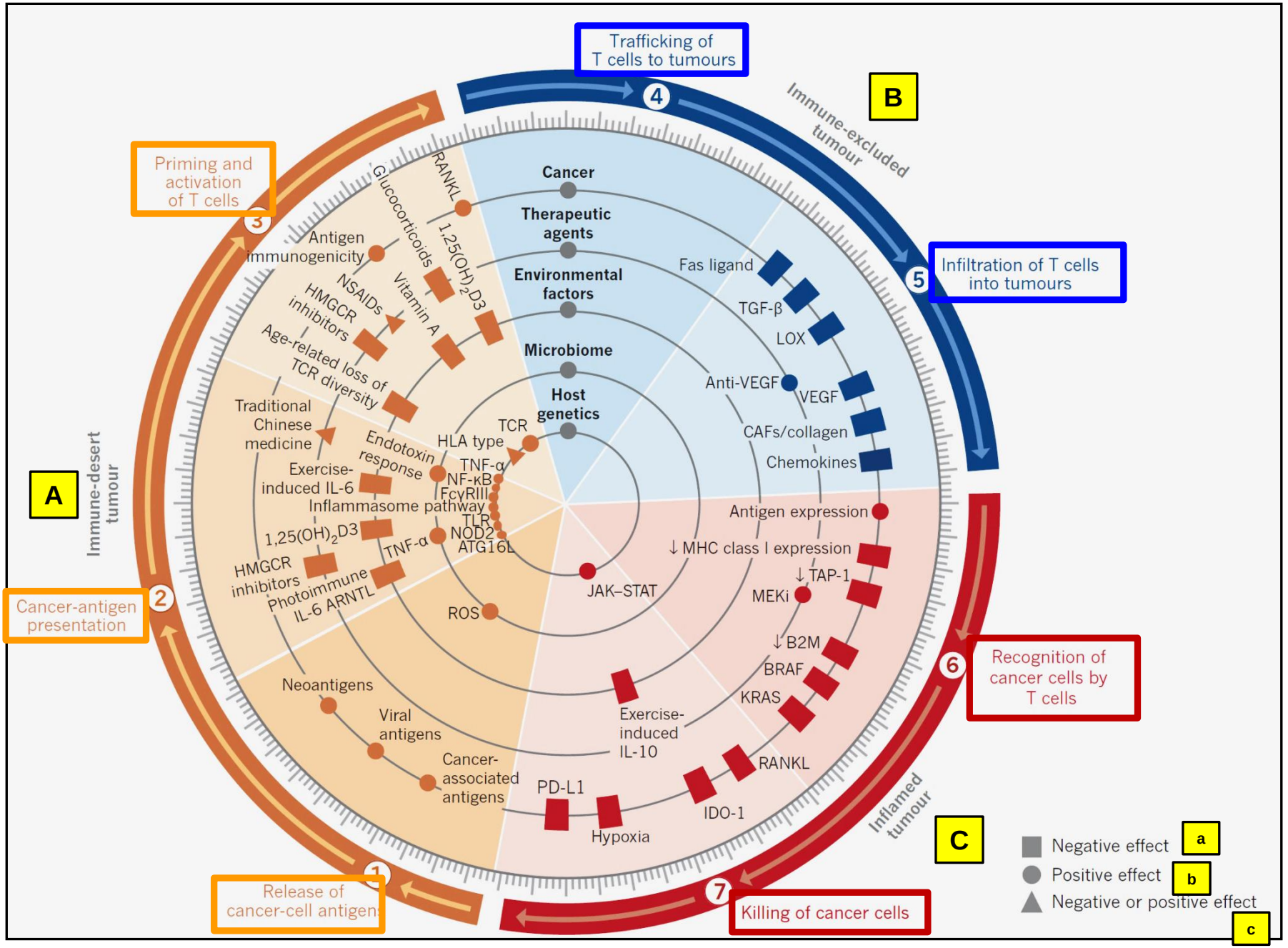
Chen & Mellman, Nature, 2017

Multivariate factors influence tolerance and immunity



Chen & Mellman, Nature, 2017

Factors that influence the cancer-immune set point



Chen & Mellman, Nature, 2017

ARNTL, Aryl hydrocarbon receptor nuclear translocator–like protein 1
ATG16L, Autophagy-related protein 16
B2M, β-2-microglobulin
BRAF, Proto-oncogene B-Raf
CAF, Cancer-associated fibroblast
FcγRIII, Fc γ receptor III
HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase
IDO, Indoleamine 2,3-dioxygenase
IL-6, Interleukin 6
JAK/STAT, Janus kinase–signal transducers and activators of transcription
KRAS, Proto-oncogene
LOX, Lysyl oxidase
MHC, Major histocompatibility complex
NOD, Nucleotide-binding oligomerization domain-containing protein
NSAIDs, Non-steroidal anti-inflammatory drugs
PD-L1, Programmed death ligand 1
RANKL, Receptor activator of NF-κB ligand
ROS, Reactive oxygen species
TAP-1, Transporter associated with antigen processing 1
TCR, T cell receptor
TLR, Toll-like receptor
VEGF, Vascular endothelial growth factor
$1,25(OH)_2D3$, 1,25-dihydroxyvitamin D_3

Chen & Mellman, Nature, 2017

Mathematical expression of cancer-immune set point

$$\int (F_{stim}) - \int (F_{inhib}) \geq 1/\sum_{n=1,y} (TCR_{affinity} \times frequency)$$

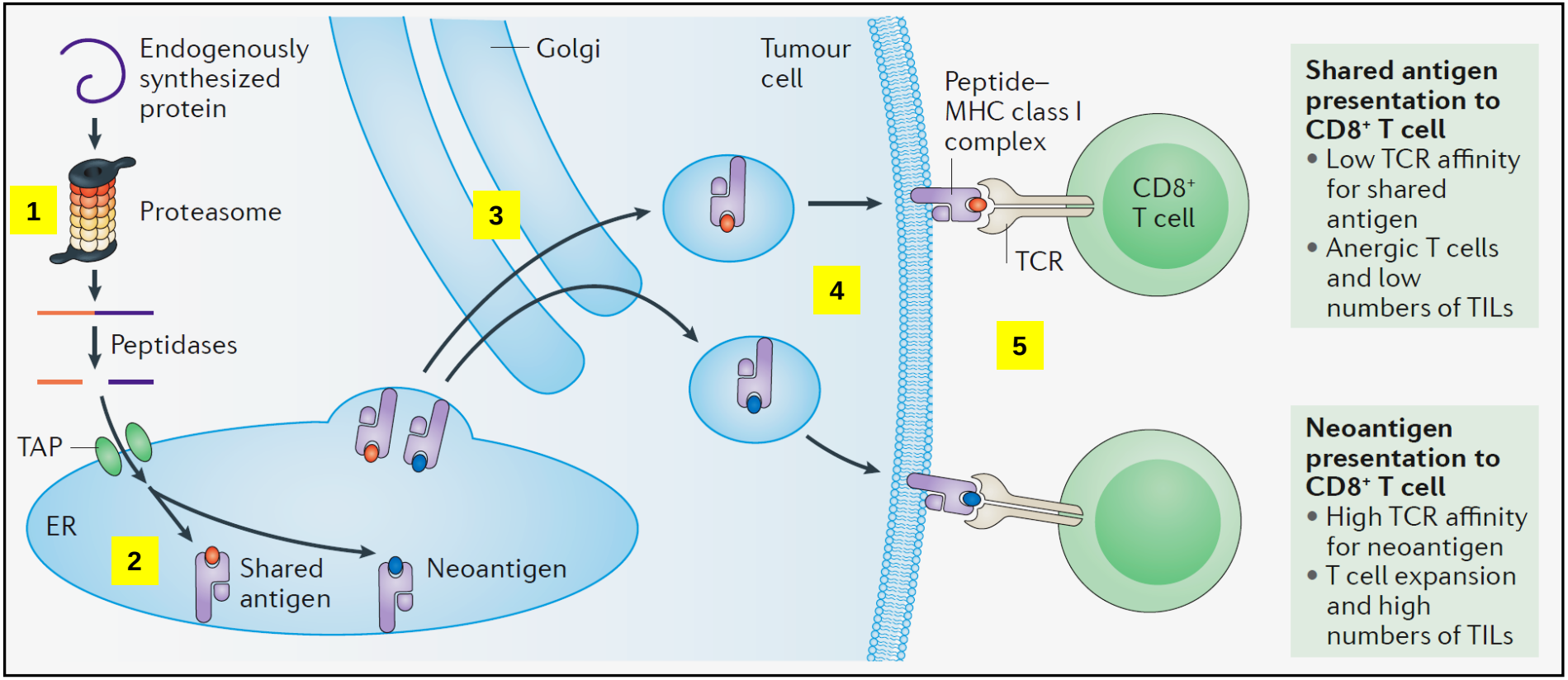
- The set point is defined by the summation of the frequency of peptide–MHC–TCR interactions and TCR signaling in all anticancer CD8+ T-cell clones (mainly, the TCR affinity for the antigen–MHC class I complex) against antigens present in the cancer cells, including neoantigens and cancer-associated antigens, and the endogenous balance of the positive and negative immune regulators that are inherent to each host or patient.

- The aim of immunotherapy is to increase F_{stim}, decrease F_{inhib} or increase TCR signaling to drive progression of the cancer-immunity cycle.

Chen & Mellman, Nature, 2017

II. Targeting of neoantigens

Tumor antigen processing and presentation on MHC class I



ER, Endoplasmic reticulum
TAP, Transporter associated with antigen processing
TCR, T cell receptor
TIL, Tumor-infiltrating lymphocyte

Yarchoan M et al, Nature Rev Cancer, 2017

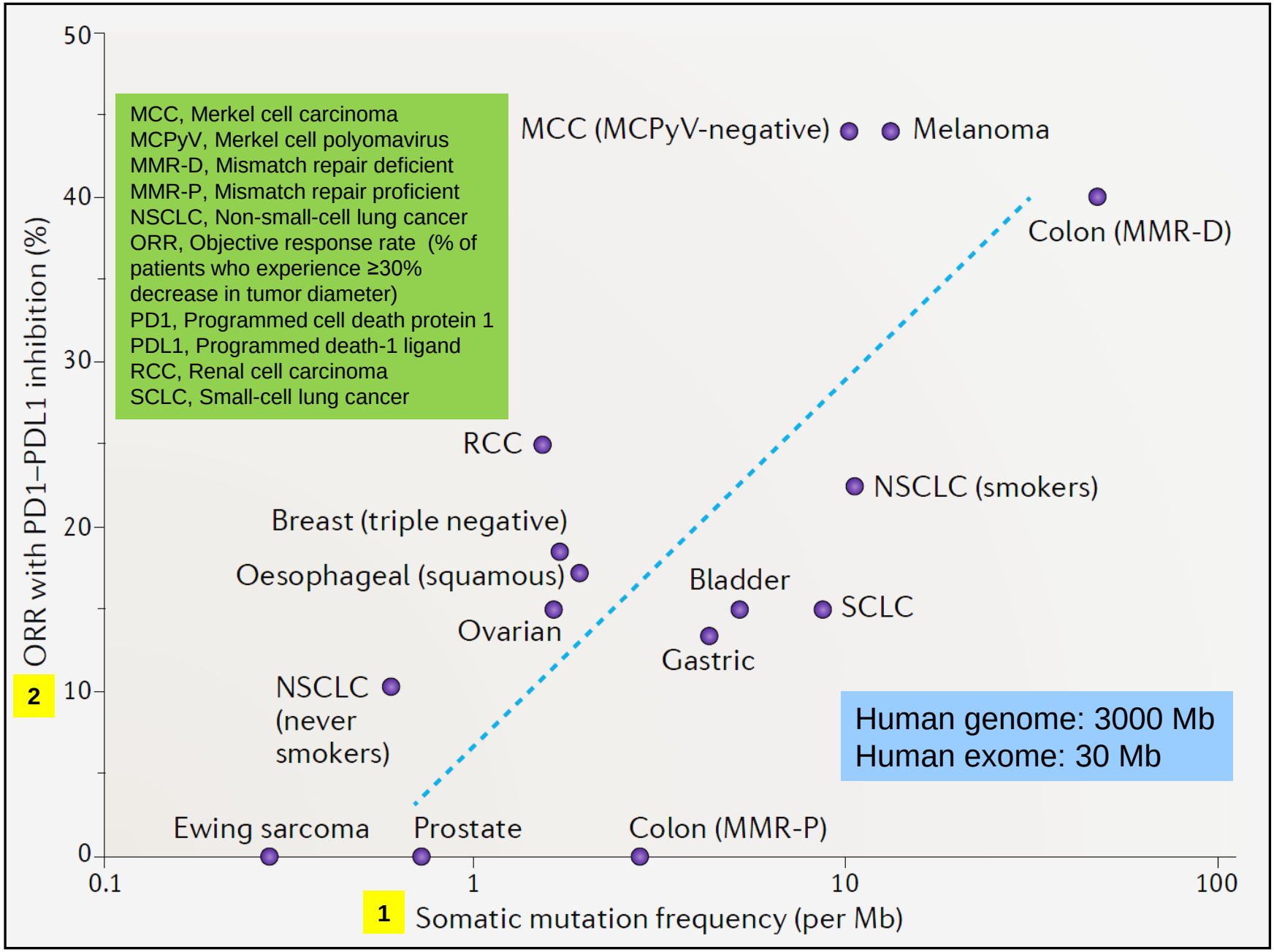
Types of tumor antigens

Antigen type	Description	Examples of antigen type	Examples of approved immunotherapies for target antigen
Tumour-specific antigens[8,9] 1	• Completely absent from normal host cells • Arise in cancer cells from oncogenic viral proteins or nonsynonymous somatic mutations	• HPV oncoproteins E6 and E7 (HPV-associated cancers of the cervix, anus and oropharynx)[11,12] • Individual KRAS mutations (pancreatic, colon, lung and various other cancers)[18,19]	None approved, multiple in clinical development
Tumour-associated antigens[9] 2	• Low levels of expression on normal host cells • Disproportionately expressed on tumour cells • Often result from genetic amplification or post-translational modifications • Can be selectively expressed by the cell lineage from which the cancer evolved	• ERBB2 (some breast cancers and various other cancers)[158] • Mesothelin (pancreatic cancer and mesothelioma)[159–161] • CD19 on B cell malignancies[27,28]	• Sipuleucel-T (anti-PAP vaccine, prostate cancer)[135] • Blinatumomab (CD19–CD3 bispecific antibody, ALL)[130]
Cancer/testis antigens[13,14] 3	• Absent on normal adult cells, except in reproductive tissues (e.g. testes, fetal ovaries and trophoblasts) • Selectively expressed by various tumour types	• MAGE (various cancers)[162] • NY-ESO-1 antigen (various cancers)[163]	None approved, multiple in clinical development

ALL, acute lymphoblastic leukaemia; HPV, human papillomavirus; MAGE, melanoma-associated antigen; PAP, prostatic acid phosphatase.

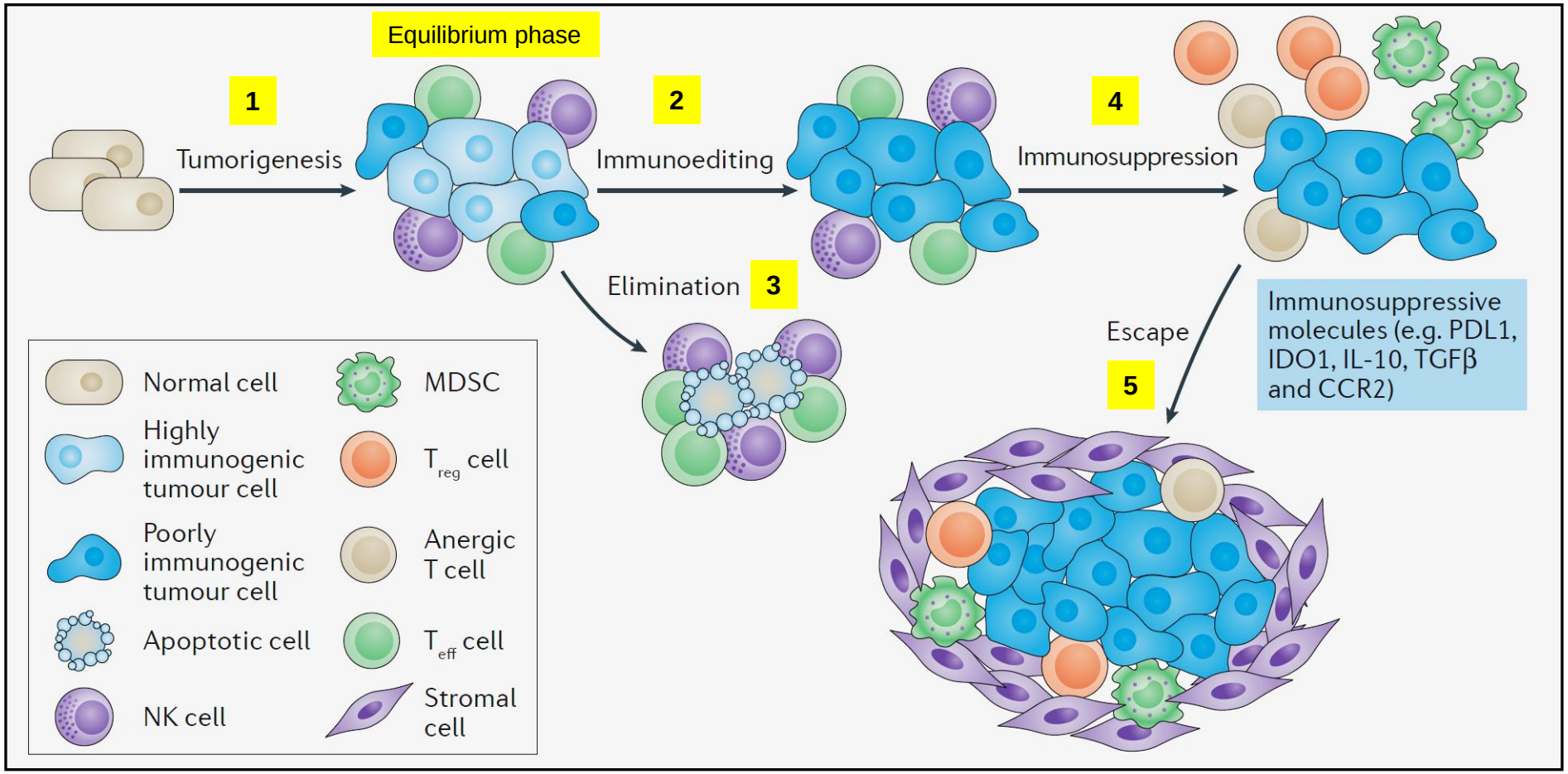
Yarchoan M et al, Nature Rev Cancer, 2017

Correlation of tumor somatic mutation frequency with objective response rates to immune checkpoint blockade



Yarchoan M et al, Nature Rev Cancer, 2017

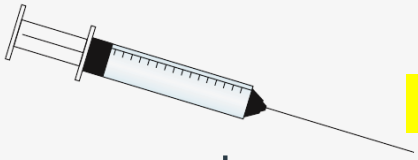
Cancers acquire immune tolerance



CCR2, C-C chemokine receptor type 2
IDO, Indoleamine 2,3-dioxygenase
MDSC, Myeloid-derived suppressor cell
NK, Natural killer cell
PDL1, Programmed death-1 ligand
T_{eff}, Effector T cell
T_{reg}, Regulatory T cell
TGF-β, Transforming growth factor β

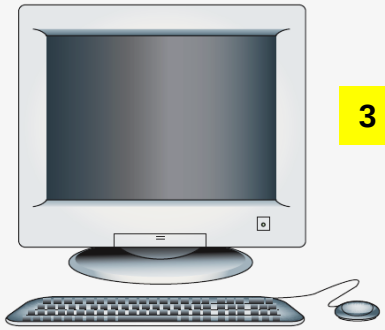
Yarchoan M et al, Nature Rev Cancer, 2017

Identification and targeting of tumor neoantigens

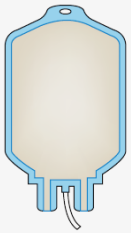


1 **Obtain tumour biopsy**
Intratumour heterogeneity may be underestimated in a single core tumour biopsy

2 **Identify expressed somatic mutations**
Whole-exome sequencing of tumour and normal cells



3 **Neoantigen prediction and prioritization**
- Mutation leads to altered amino acid sequence
- Somatic mutation in an expressed gene
- Abnormal amino acid sequence expressed on MHC
- Abnormal amino acid sequence confers increased MHC binding
- Antigen sufficiently different from non-mutated counterpart



4 **Personalized therapy for predicted neoantigens**
- ACT using neoantigen-specific cell products
- Personalized vaccines encoding predicted neoantigens

5a
- PDL1
- CTLA4
- IDO1
- LAG3
- TIM3
- A2AR
- B7H3
- MDSCs

5b
- CD40L
- GITR
- CD137
- OX40
- ICOS
- CD28

5 **Combine with other immune modulators**
Combination therapy with immune adjuvants and immune checkpoint inhibitors can maximize response and prevent immune escape

Suppressive | Activating

A2AR, Adenosine A2A receptor
ACT, Adoptive cell therapy
B7H3, also known as CD276
CD137, also known as 4-1BB
CD40L, CD40 ligand
CTLA4, Cytotoxic T lymphocyte-associated antigen 4
GITR, Glucocorticoid-induced tumor necrosis factor receptor family related protein
ICOS, Inducible T cell co-stimulator
IDO1, Indoleamine-2,3-dioxygenase 1
LAG3, Lymphocyte activation gene 3
MDSCs, Myeloid-derived suppressor cells
PDL1, Programmed cell death 1 ligand 1
TIM3, T cell immunoglobulin mucin receptor 3.

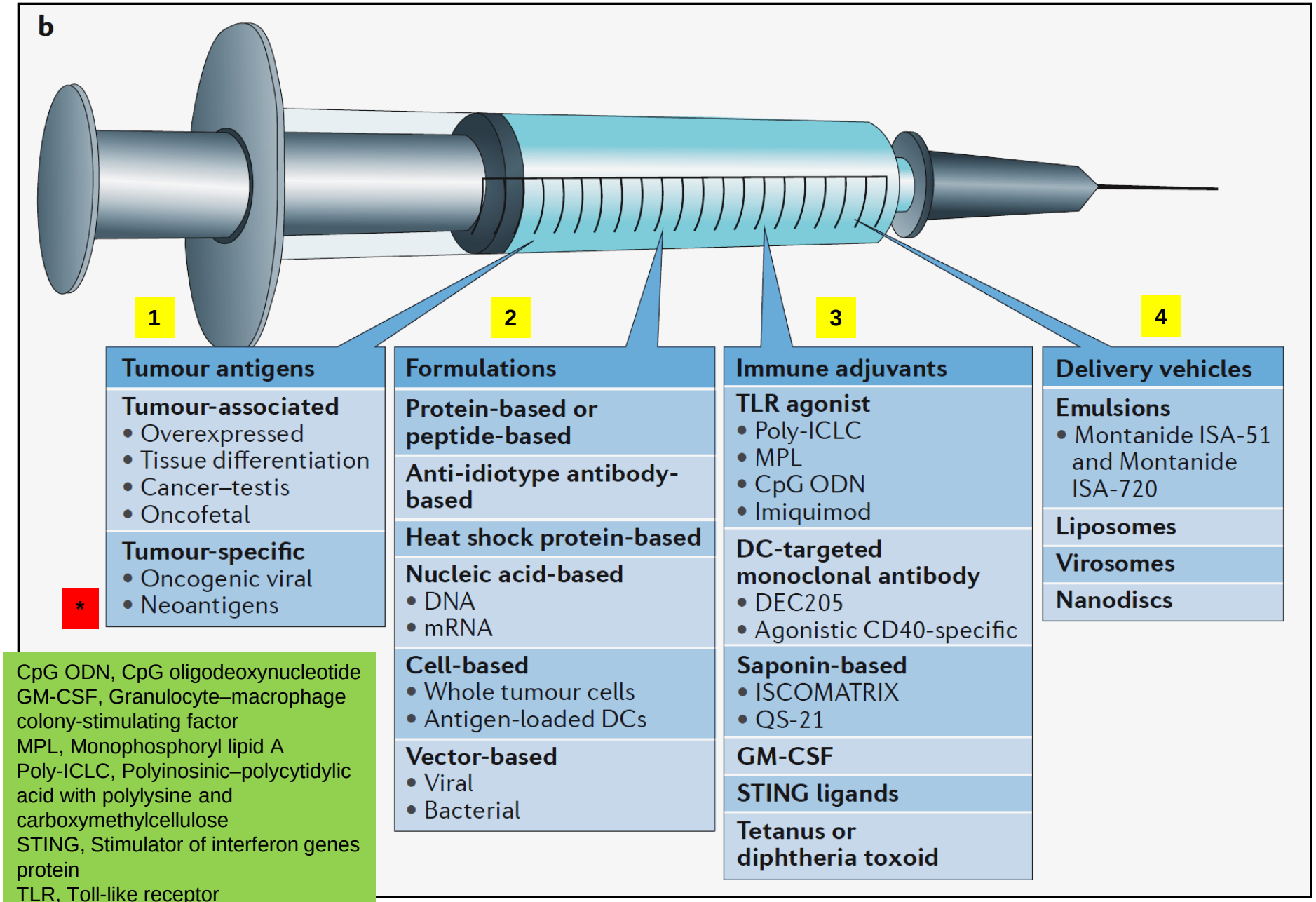
Neoantigens

Antigens arising from mutation of the tumor genome that causes tumor cells to express specific proteins that are not expressed on normal cells.

Yarchoan M et al, Nature Rev Cancer, 2017

III. Personalized immunotherapy

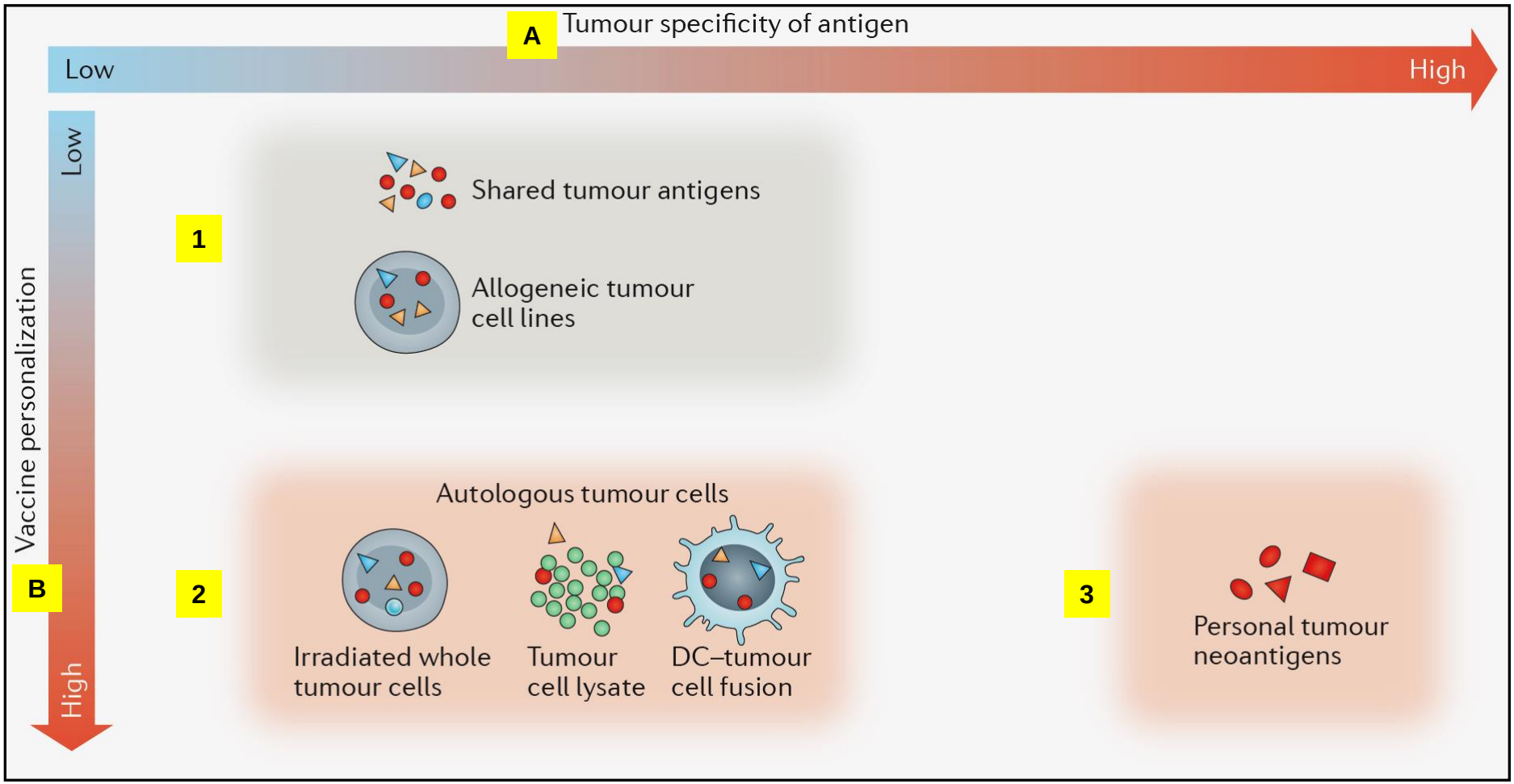
Components of cancer vaccines



CpG ODN, CpG oligodeoxynucleotide
GM-CSF, Granulocyte–macrophage colony-stimulating factor
MPL, Monophosphoryl lipid A
Poly-ICLC, Polyinosinic–polycytidylic acid with polylysine and carboxymethylcellulose
STING, Stimulator of interferon genes protein
TLR, Toll-like receptor

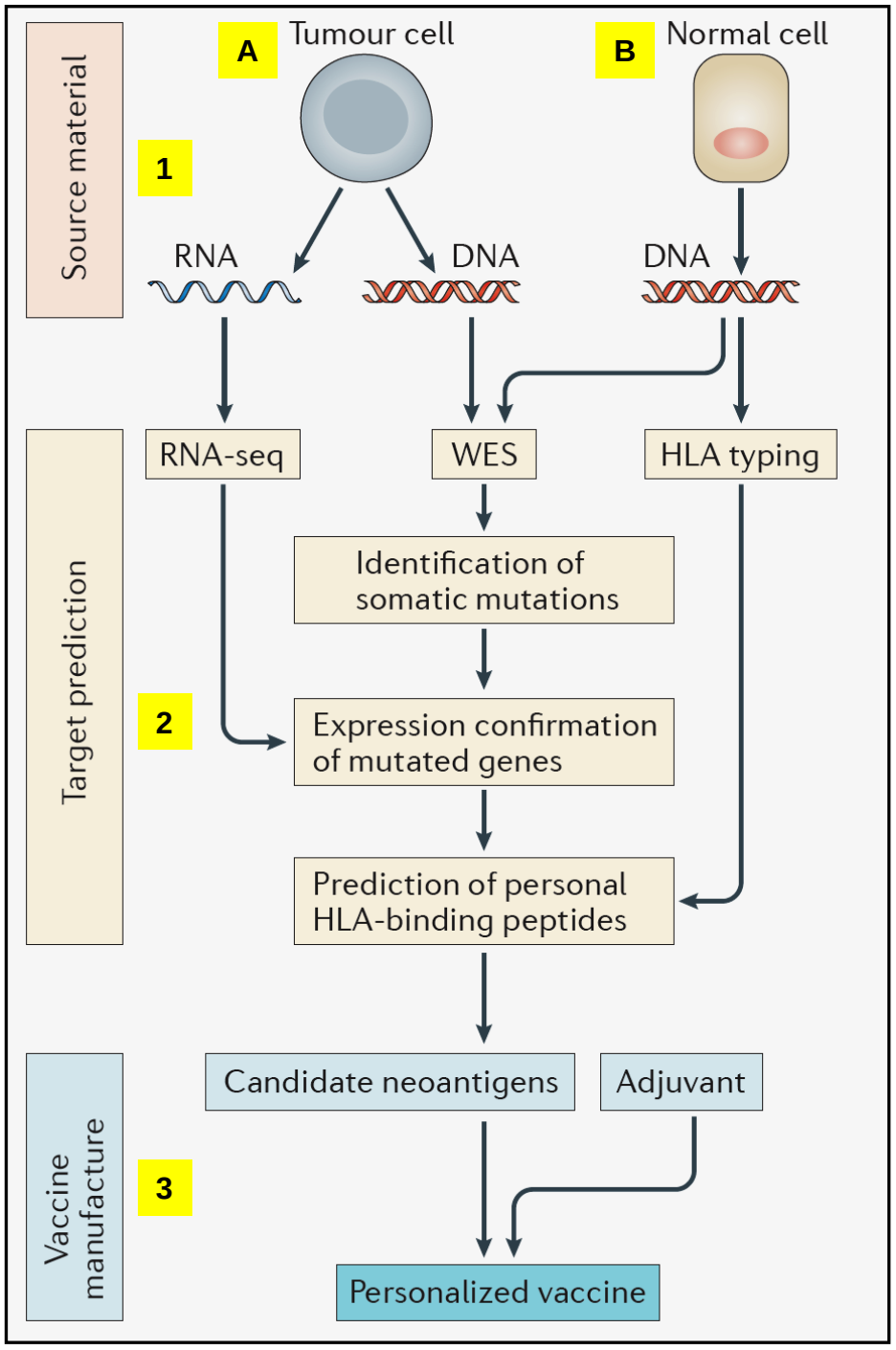
Hu Z et al, Nature Rev Immuol, 2017

Neoantigens: targets of cancer vaccines



Potential antigens for use in cancer vaccines differ in terms of tumor specificity and vaccine personalization. Neoantigens are optimal targets for personalized, tumor-specific cancer vaccines.

Hu Z et al, Nature Rev Immuol, 2017



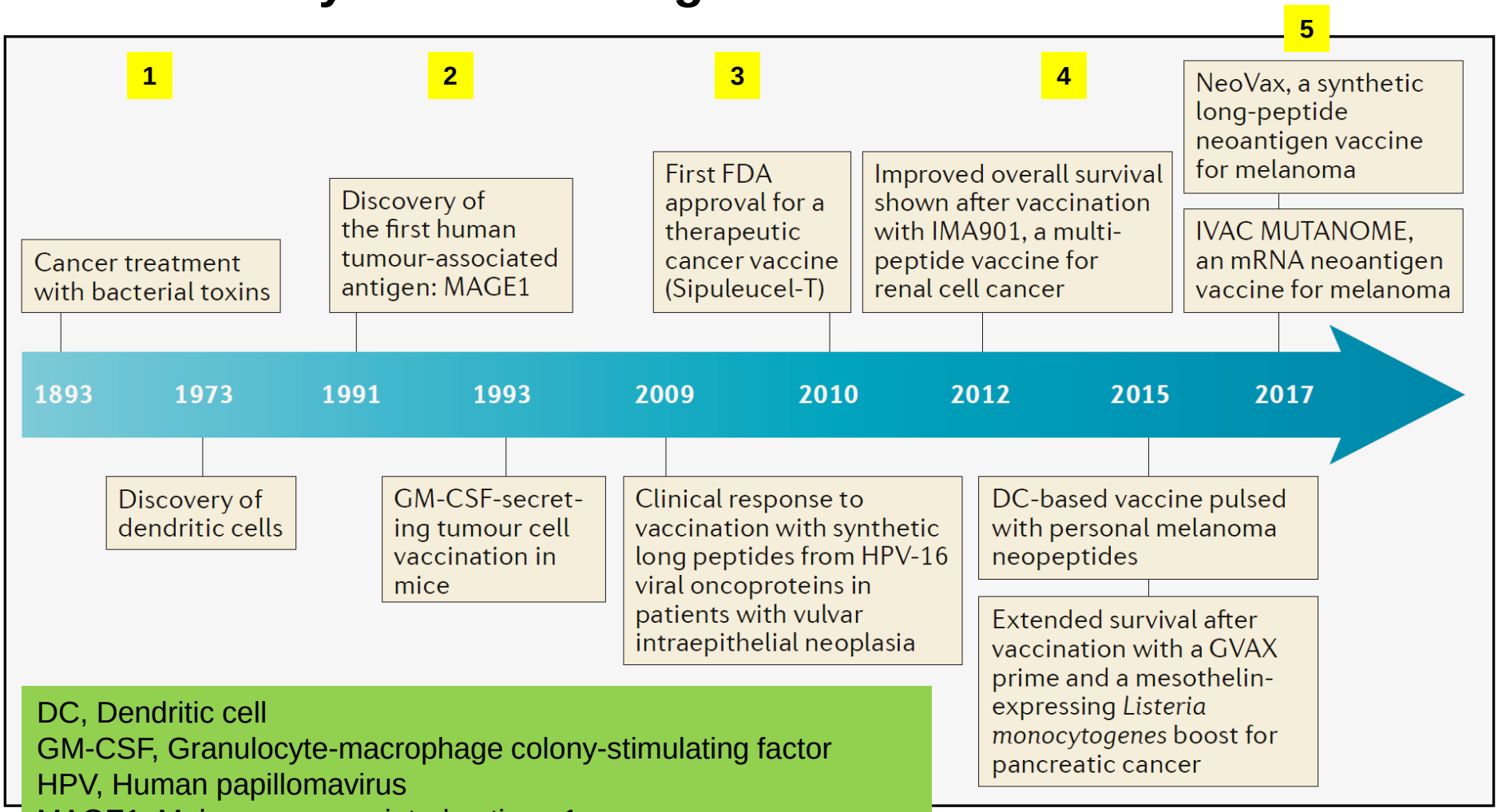
Neoantigen-based therapeutic cancer vaccines

Typical workflow for neoepitope selection and vaccine manufacture.

- DNA and RNA are extracted from single-cell suspensions of tumor cells and matched normal tissue cells. Somatic mutations of tumor cells are discovered by whole-exome sequencing (WES).
- RNA sequencing (RNA-seq) narrows the focus to mutations of expressed genes. HLA typing is carried out on DNA from normal tissue. The potential antigenicity of neoepitopes identified by WES and RNA-seq is assessed by predicting the affinity of the neoepitopes for binding to the HLA type of that individual (using NetMHCpan), thereby generating candidate vaccine epitopes.
- Validated epitopes are selected for incorporation into the personalized cancer vaccine, which is administered to patients in combination with an immune adjuvant.

Hu Z et al, Nature Rev Immuol, 2017

History of tumor antigens and cancer vaccines

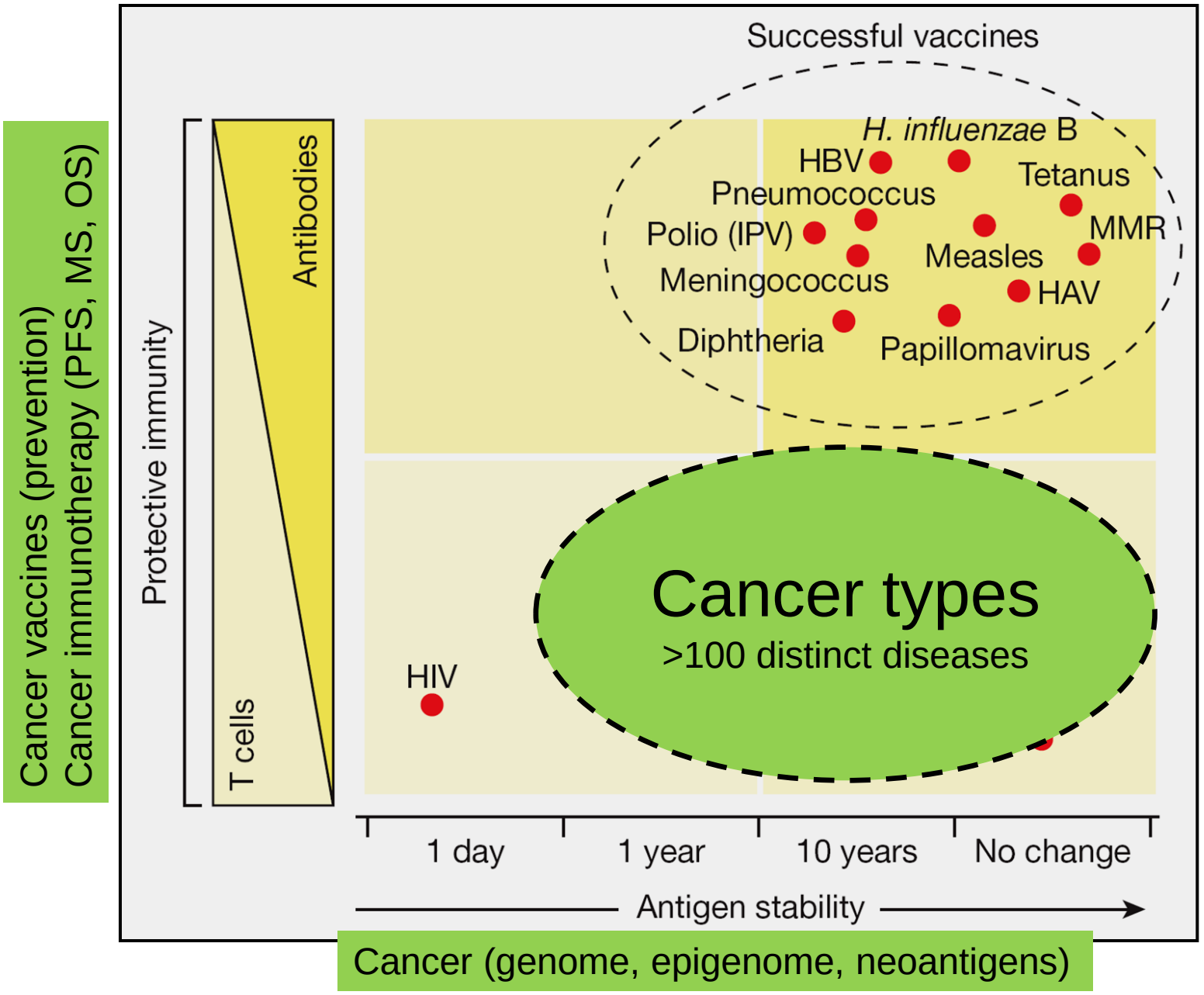


DC, Dendritic cell
GM-CSF, Granulocyte-macrophage colony-stimulating factor
HPV, Human papillomavirus
MAGE1, Melanoma-associated antigen 1

GVAX, Cancer vaccine composed of whole tumor cells genetically modified to secrete (GM-CSF), and then irradiated.
IVAC mutanome vaccine, Individualized, poly-neo-epitopic encoding, ribonucleic acid (RNA)-based cancer vaccine that targets a variety of patient-specific, immunogenic mutant epitopes, with potential immunostimulatory and antineoplastic activities.

Hu Z et al, Nature Rev Immuol, 2017

Will cancer vaccines be successful?



Rappuoli & Aderem, Nature, 2011
Modifed: Najbauer J, 2018