Immunogenicity Beginner Training: Mechanisms of Immunogenicity; Overview of Non-Clinical Risk Assessment Tools

EIP Open Symposium

16th March 2026

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Immunogenicity Beginner Training: **Mechanisms of Immunogenicity**; Overview of Non-Clinical Risk Assessment Tools

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16th- 19th March 2026

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Outline

Part 1: Introduction to Immunology and Immunogenicity

- Introduction immune system and immunogenicity
- Factors influencing unwanted immunogenicity
- Consequences of unwanted immunogenicity
- Biologics and unwanted immunogenicity

Part 2: Immunogenicity Potential Assessment

- Pre-clinical immunogenicity potential assessment
- Assessment tools – in silico, *in vitro* tools (*in vivo*)
- Rationale use/application

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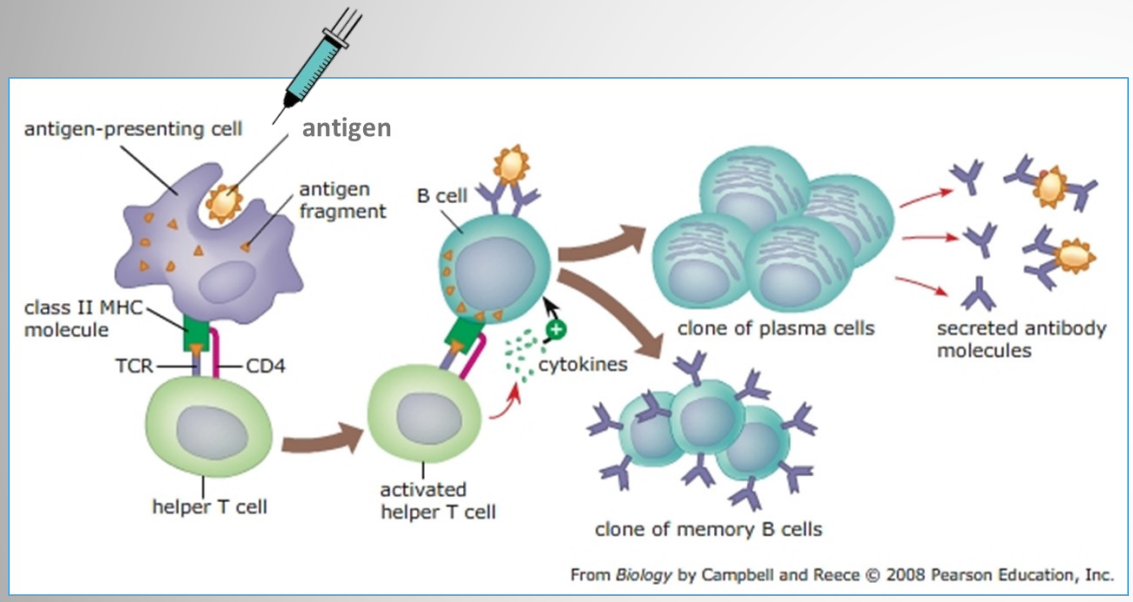
Immunogenicity

"The ability of a particular substance, such as an antigen or epitope, to induce an immune response"



WANTED	UNWANTED	
Vaccines	**Therapeutic protein**	**Stem Cells and Gene Therapy products**
Immune response against the pathogen/epitope (virus, bacteria, cancer) aiming at protecting the organism	Production of antidrug- antibodies (ADAs) possibly neutralising the therapeutic effects of the treatment and, in rare cases, inducing adverse effects	Cellular and humoral responses Anti HLA antibodies Immune rejections Potential safety effect

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Part 1: Introduction to Immunology and Immunogenicity

INTRODUCTION TO IMMUNOGENICITY PART 1

- **Introduction immune system and immunogenicity**
- Factors influencing unwanted immunogenicity
- Consequences of unwanted immunogenicity
- Biologics and unwanted immunogenicity

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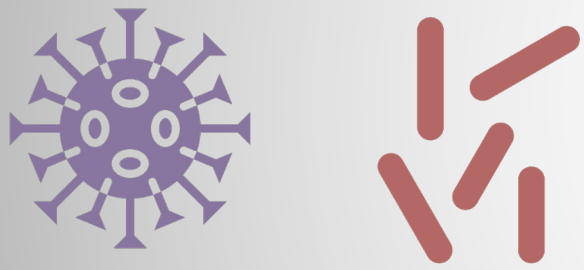
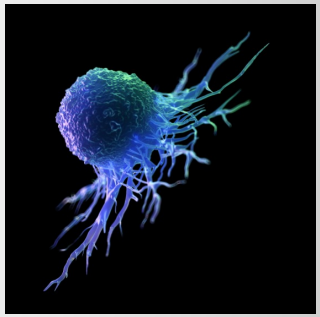
Our Immune System

Virus, bacteria



Over-reactive
Auto immune diseases

Tumor cells



Immune deficiencies
Infections, malignancies

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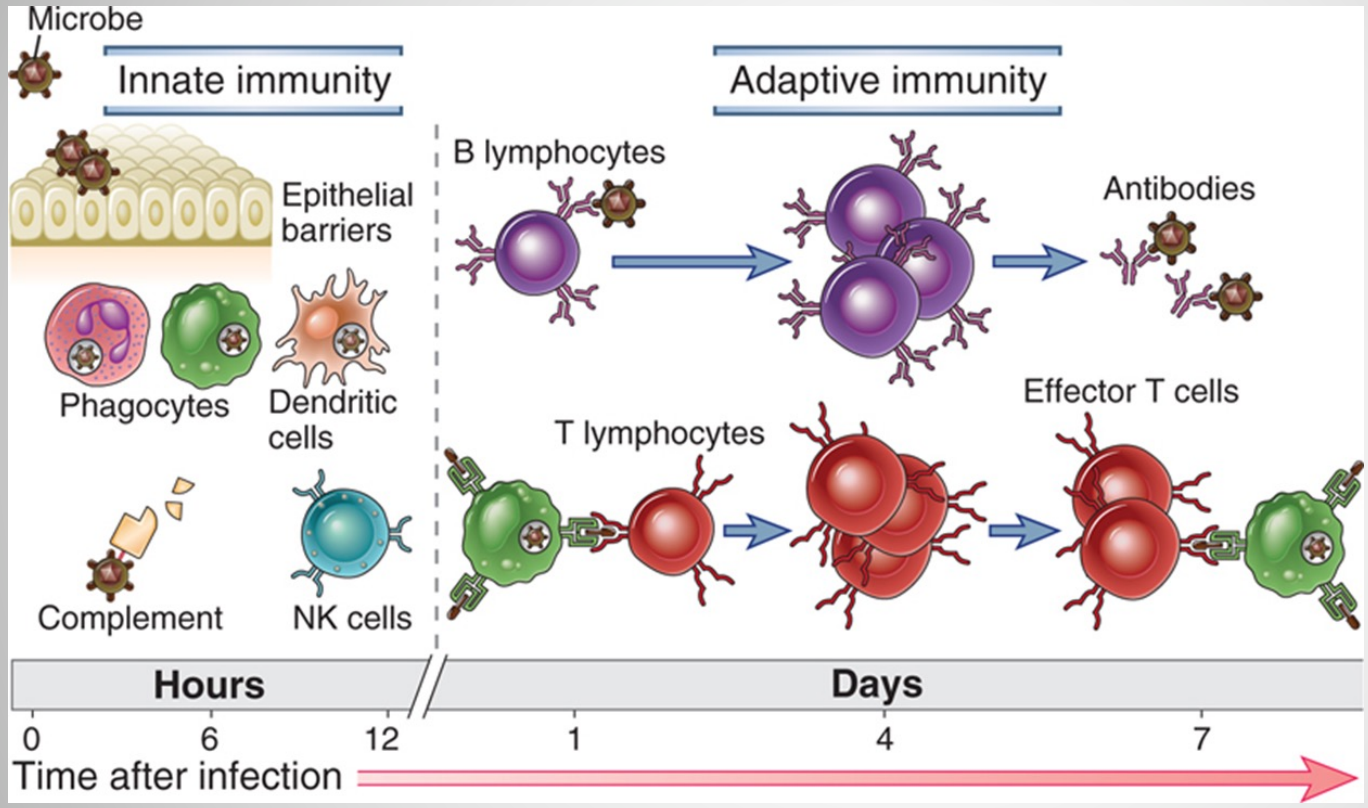
Our Immune System: Key Principles



Source: Cellular and molecular immunology - Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai

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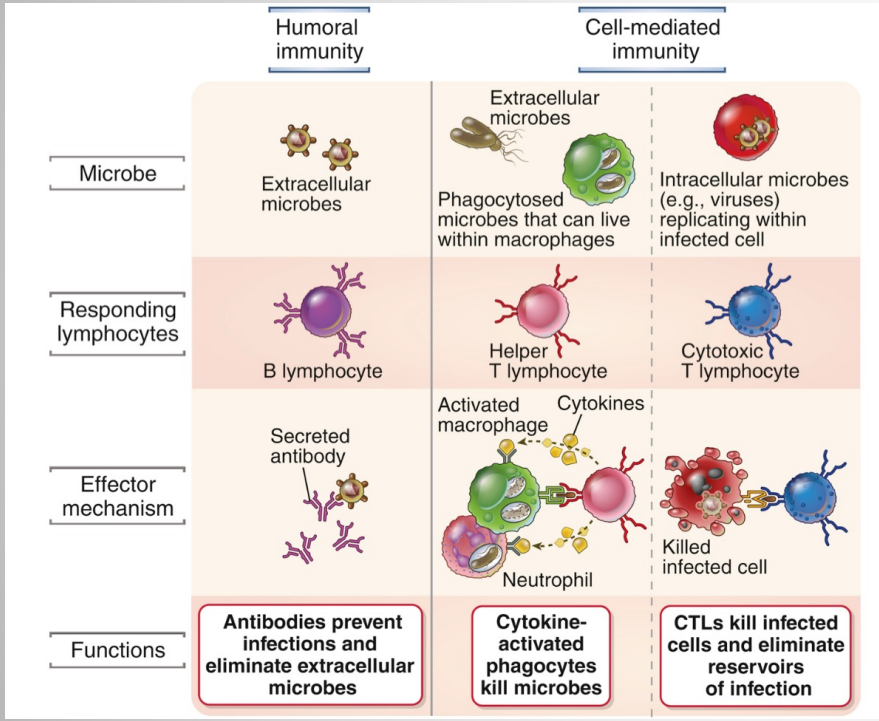
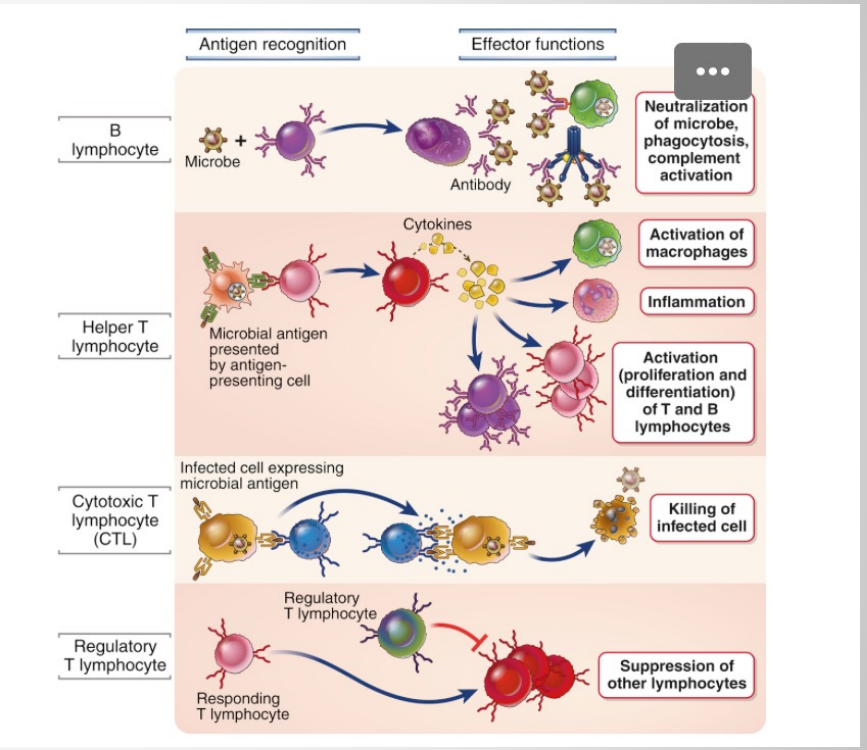
The Adaptive Immune System: Principles





Source: Cellular and molecular immunology - Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai

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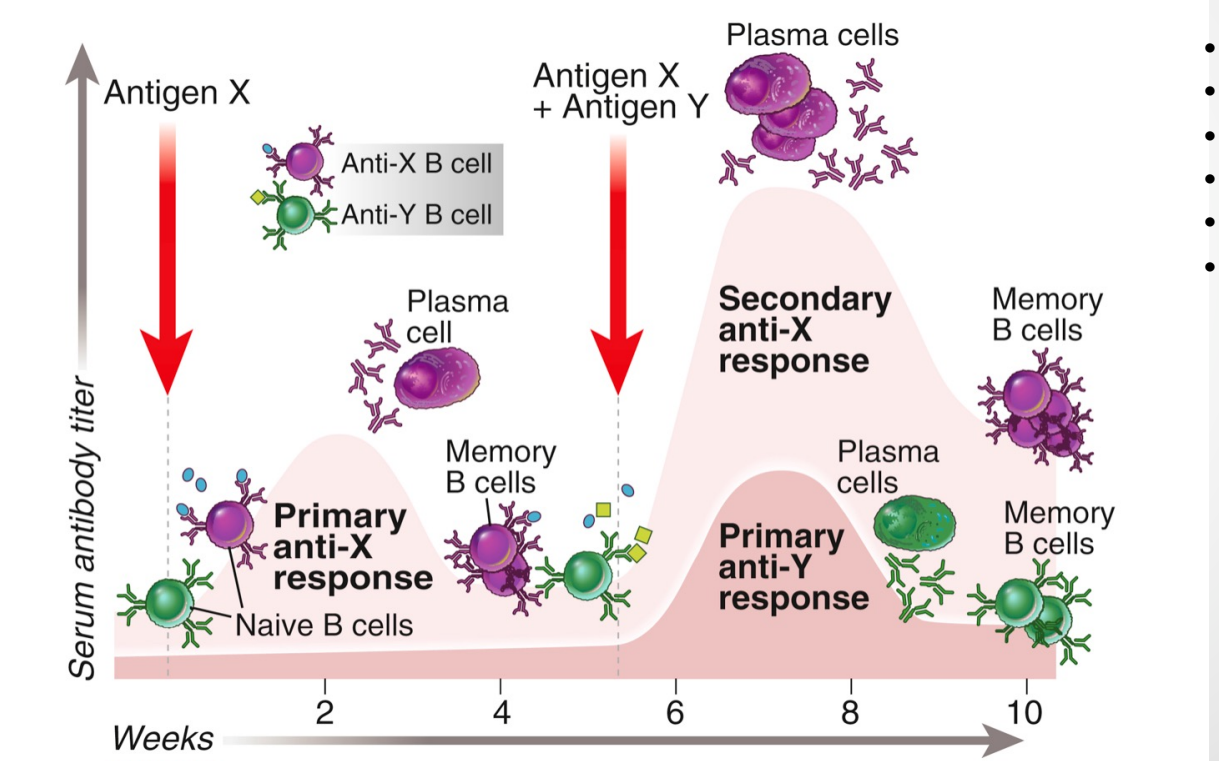
The Adaptive Immune System: Principles



- specificity
- diversity
- memory
- self-tolerance
- systemic
- feedback loop and control mechanism

Source: Cellular and molecular immunology - Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai

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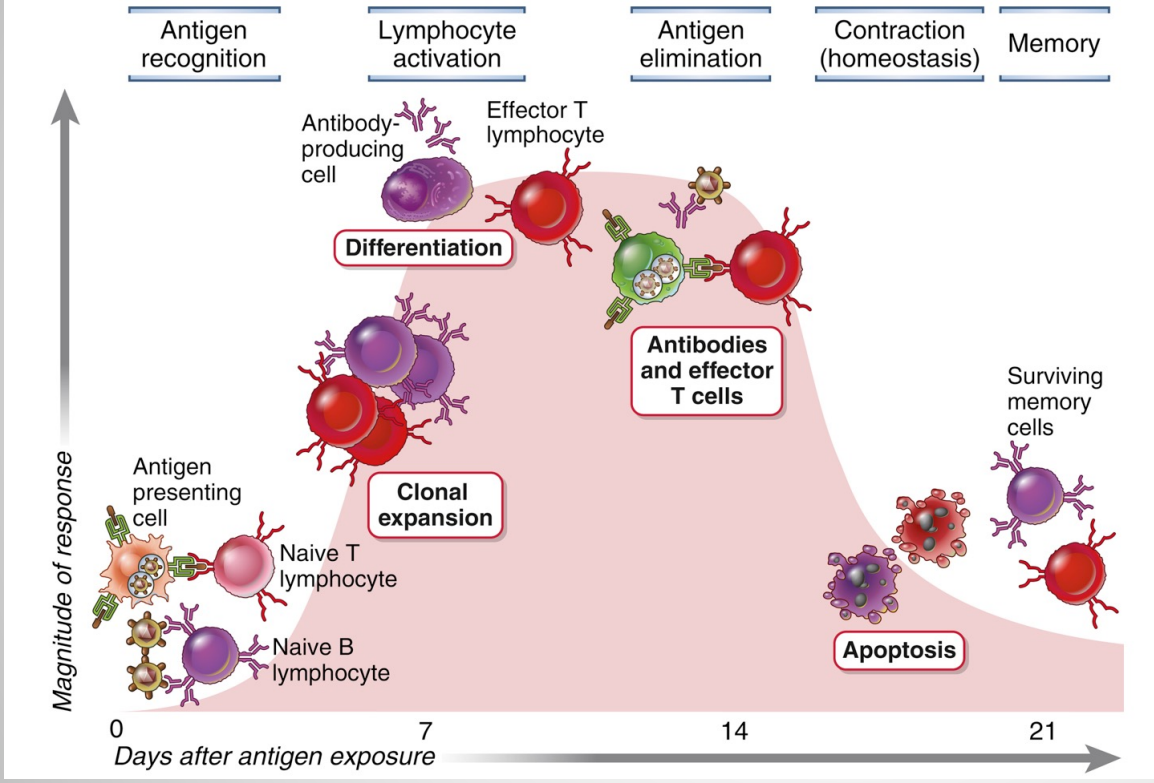
The Adaptive Immune System: Principles (2)



Source: Cellular and molecular immunology - Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai

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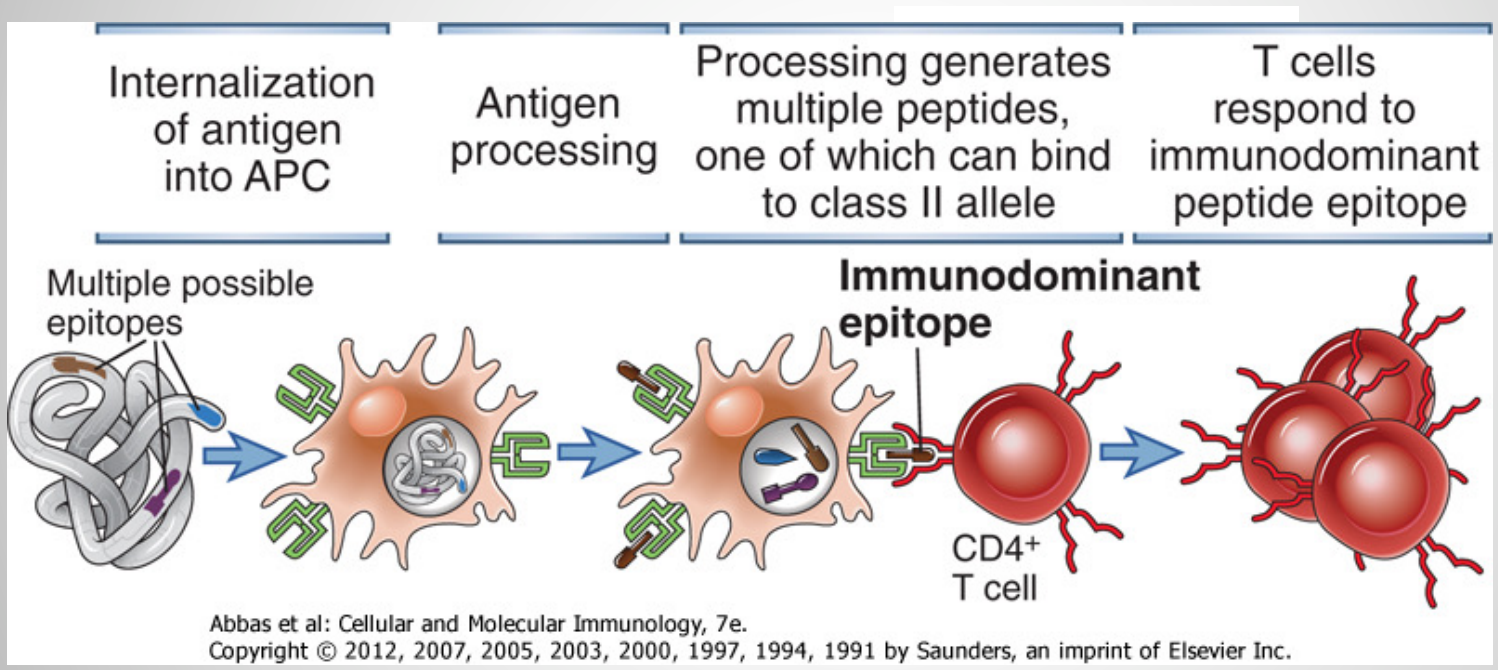
The Key Players: Dendritic and T cells



Abbas et al: Cellular and Molecular Immunology, 7e.

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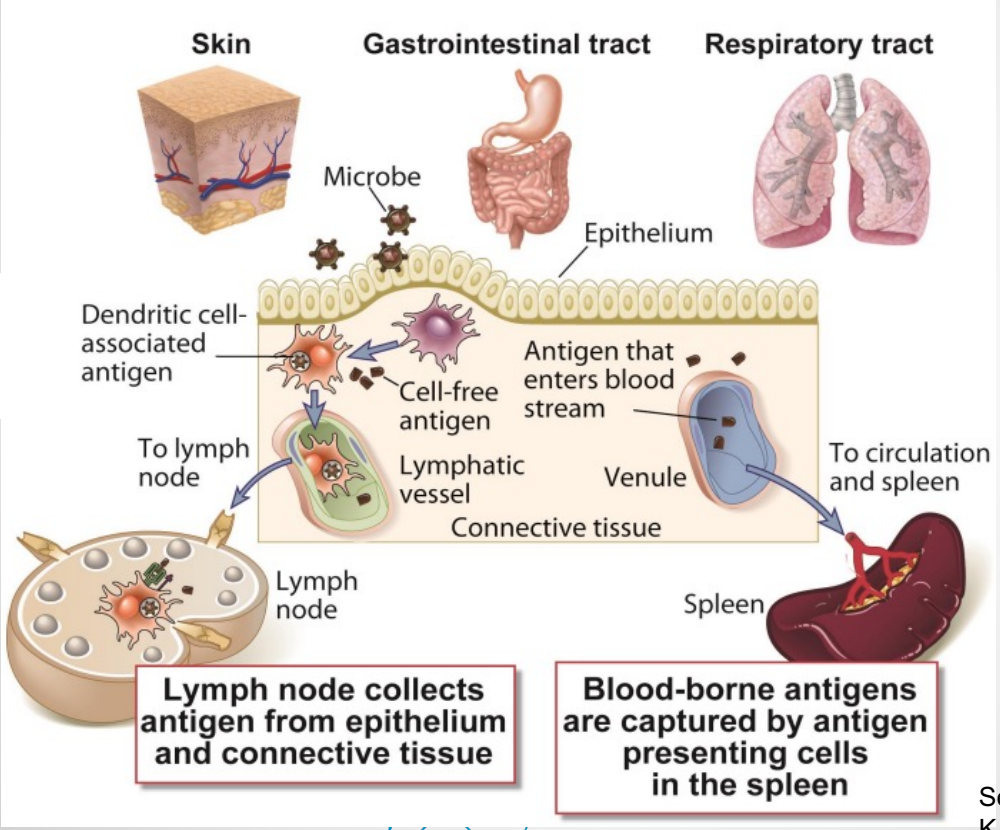
The Key Players: Dendritic and T cells

Entry of antigen

Capture of antigen

Collection and capture of antigen



Source: Cellular and molecular immunology - Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai

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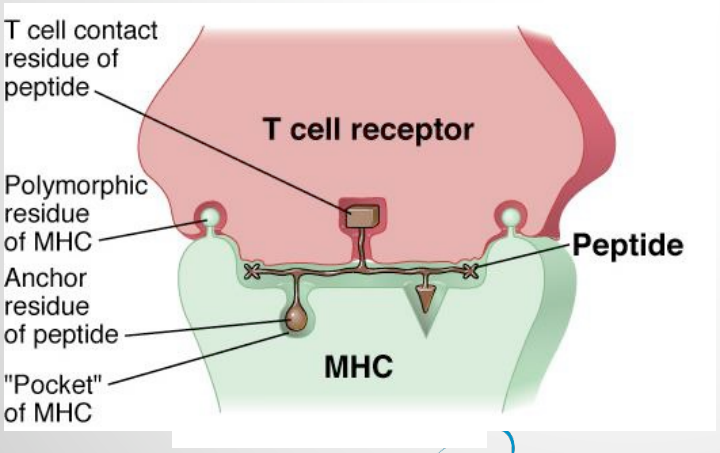
The Key Players: Dendritic and T cells

What do T cells see?

To ensure cellular communications, T cells see antigens NOT in the circulation but only when displayed by molecules on the surface of other cells

-> These molecules are HLA (generic name: MHC) and the cells displaying the antigen are APCs



Because MHC molecules are on cells and can display only peptides, **T lymphocytes can recognize only cell-associated protein antigens**

Source : Abbas, Lichtman and Pillai. *Cellular and Molecular Immunology, 7th edition, 2011* Elsevier

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The Key Players: Dendritic and T cells

What antigens do CD4+ and CD8+ T cells recognize?

Lymphocytes must respond to each microbe in ways that are able to eradicate that microbe

-> Extracellular microbes: antibodies; destruction in phagocytes (**need helper T cells**)
-> Intracellular microbes: killing of infected cells (**need CTLs**)

=> How do T cells distinguish antigens in different cellular locations?

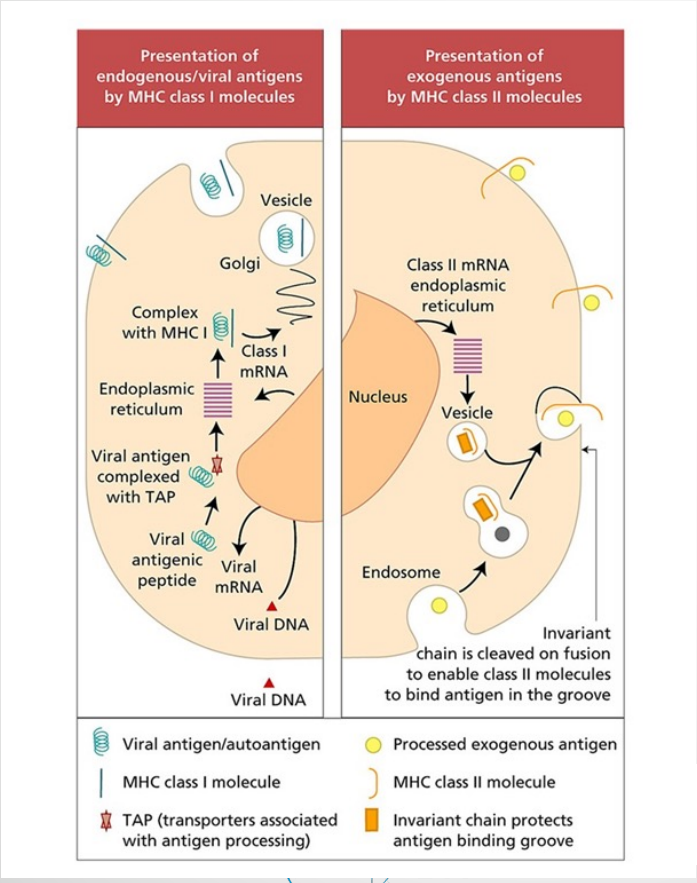
The Key Players: Dendritic cells

MHC I

- Constitutively expressed
- On virtual all nucleated cells
- $CD8^+$ CTLs

⬇

Kill cells infected with intracellular microbes.



MHC II

- Expressed (increased) upon activation
- Only on DCs, B cells, macrophages and a few other cell types
- $CD4^+$ T cells

⬇

Help

- macrophages to eliminate extracellular microbes
- B cells to make Abs.

Essentials of Clinical Immunology, Sixth Edition. Helen Chapel, Mansel Haeney, Siraj Misbah, and Neil Snowden.

The Key Players: Dendritic and T cells

MHC Polymorphism

- Most polymorphic genes in biology
  - Large number of variants (alleles) in the population
  - Each variant presents only some peptides and is recognized by some T cells
- Polymorphism evolved to ensure recognition of any microbial peptide
- Polymorphism means unrelated individuals express different MHC molecules
  - Every person may recognize slightly different peptides
  - T cells from any individual recognize and react against MHC of any other individual
  - 1 in 4 chance of siblings having the same HLA

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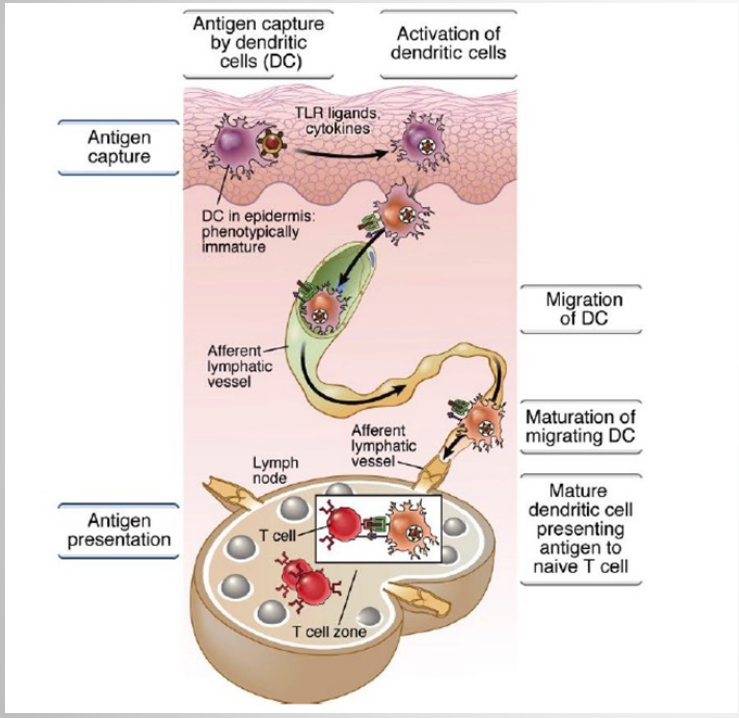
The Key Players: Dendritic cells



	Immature dendritic cell	Mature dendritic cell
Principal function	Antigen capture	Antigen presentation to T cells
Expression of Fc receptors, mannose receptors	++	–
Expression of molecules involved in T cell activation: B7, ICAM-1, IL-12	– or low	++
Class II MHC molecules		
Half-life	~10 hr	>100 hr
Number of surface molecules	~10^6	~7×10^6

Source: Abbas et al.: Cellular and Molecular Immunology, 7e.

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The Key Players: Dendritic Cells

Functions of antigen-presenting cells

- Capture antigens and take them to the “correct” place
  -> Antigens are concentrated in peripheral lymphoid organs, through which naïve lymphocytes circulate

- Display antigens in a form that can be recognized by specific lymphocytes
  -> For T cells: MHC-associated peptides (cytosolic peptides to class I, vesicular peptides to class II)
  -> For B cells: native antigens

- Provide “second signals” for T cell activation
  -> Critical for initiation of responses

The Adaptive Immune System

The challenge of finding antigens:

- **Very few lymphocytes in the body are specific for any one microbe (or antigen)**
  -> Specificity and diversity of antigen receptors: the immune system recognizes and distinguishes between 10^6-10^9 antigens; therefore, few lymphocytes with the same receptors

- **These few lymphocytes must be able to locate microbes that enter and reside anywhere in the body**
  -> The small number of lymphocytes specific for each antigen cannot patrol all epithelia (routes of microbe entry) or tissues where the antigen may be present

- **Therefore, antigens and lymphocytes must be brought together**
  -> The function of lymphoid organs

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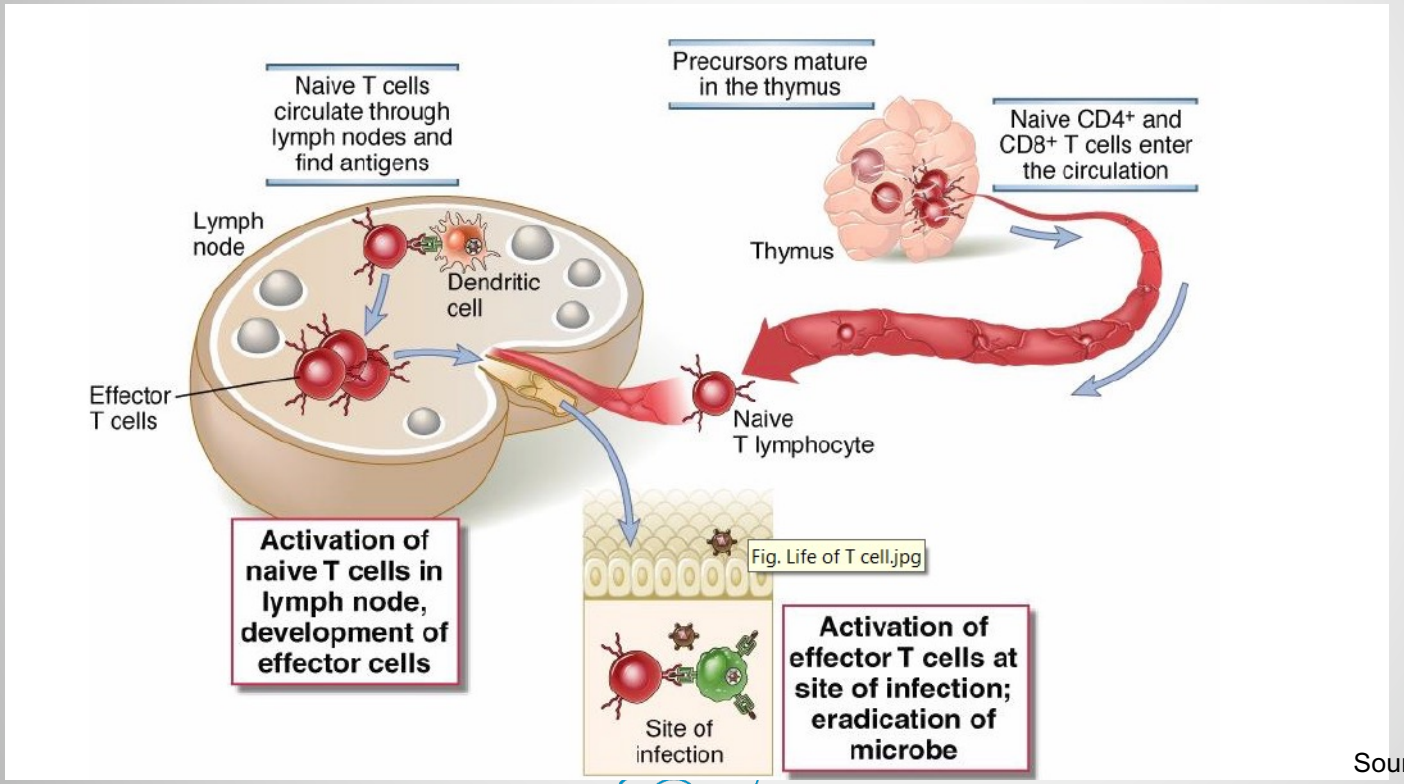
The Lifecycle of T cells



Source: Abbas et al.: Cellular and Molecular Immunology, 7e.

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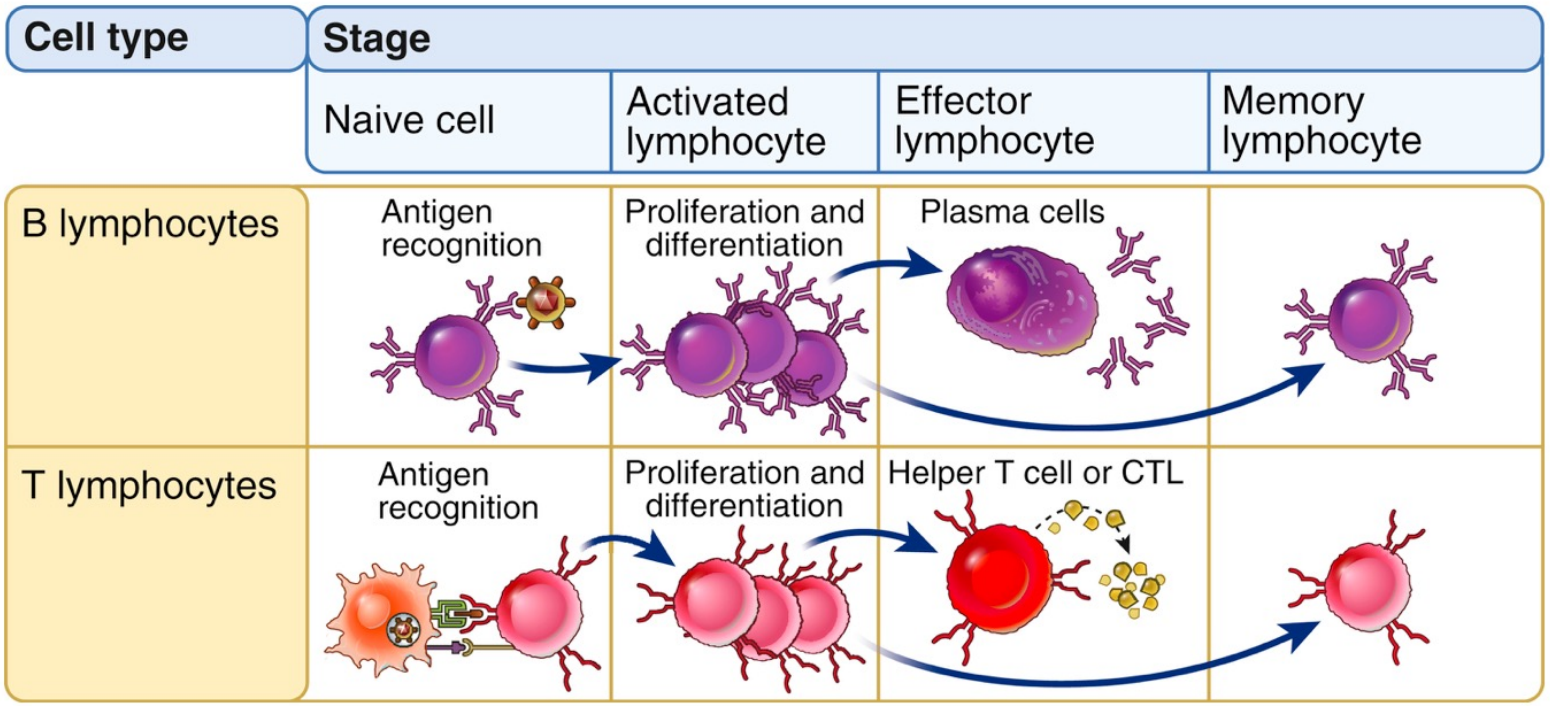
The Lifecycle of B and T cells



Source: Abbas et al.: Cellular and Molecular Immunology, 7e.

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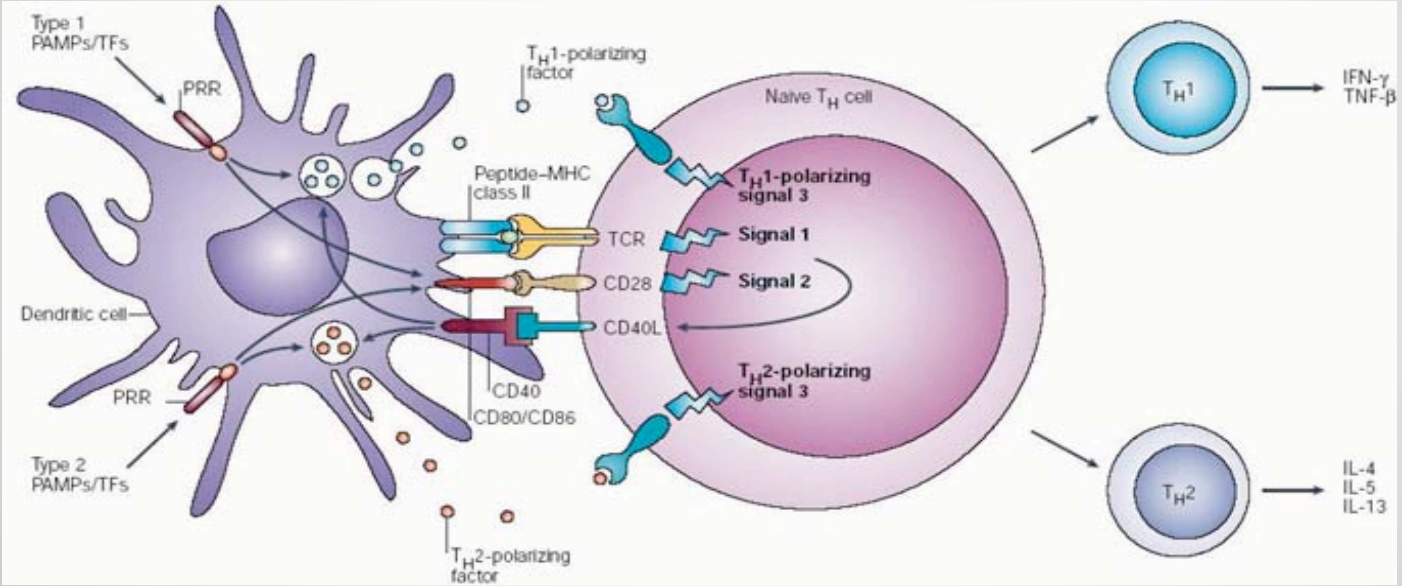
T Cell Activation



Source : Kapsenberg, Nature Reviews Immunology 2003

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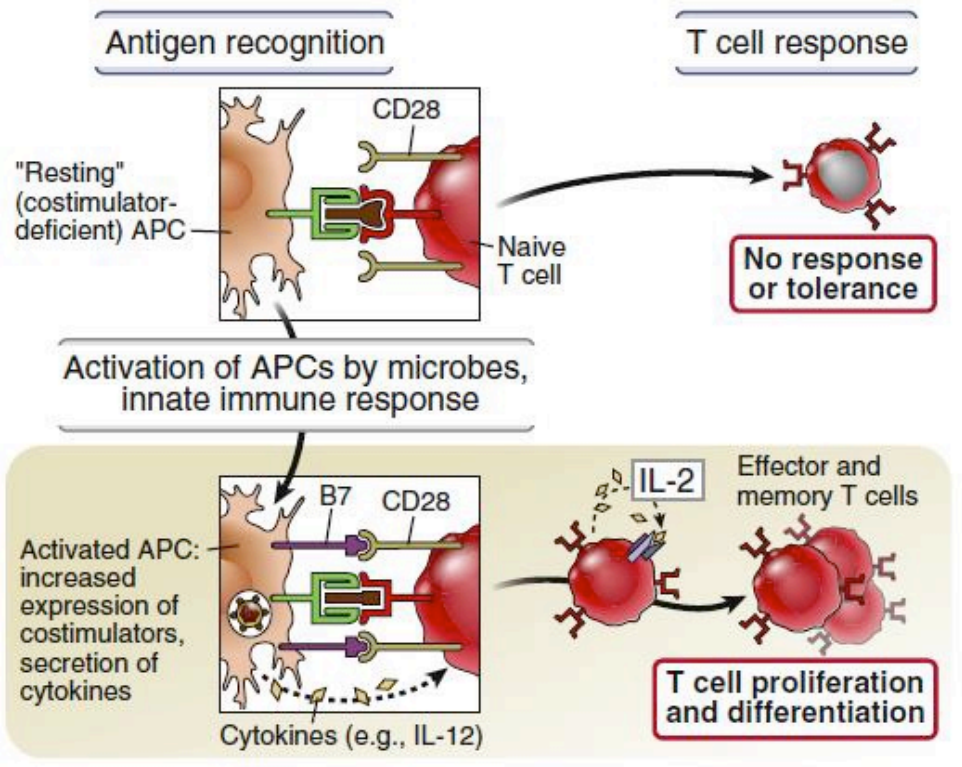
T Cell Activation: Role of Co-stimulation



- Required for initiating T cell responses (activation of naïve T cells)
- Ensures that T cells respond to microbes (the most potent inducers of co-stimulators) and not to harmless antigens
- Memory cells are less dependent on co-stimulation than are naïve T cells

Source : Abbas, Lichtman and Pillai. *Basic Immunology, 5th edition, 2016*

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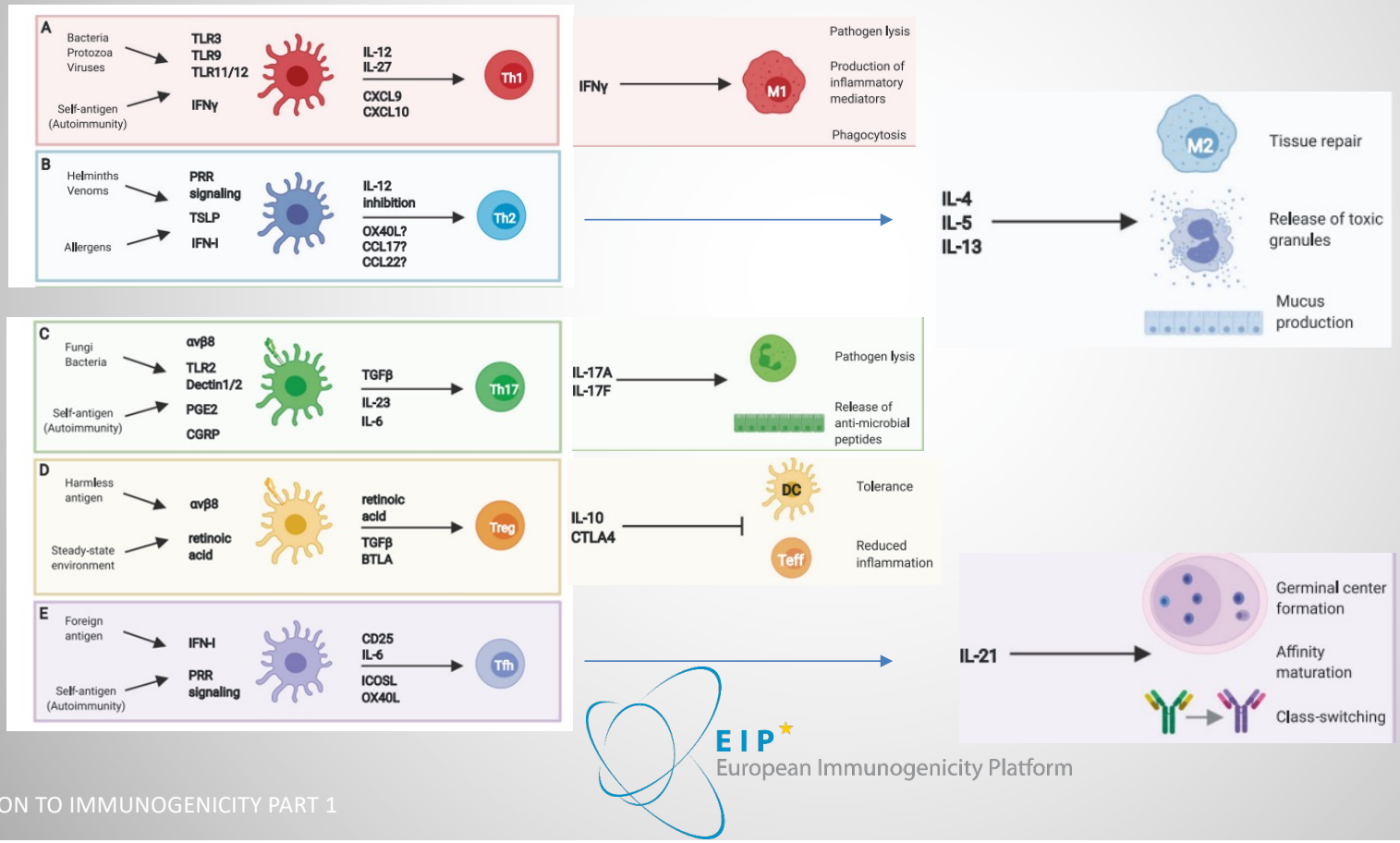
T Cell Differentiation



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Adapted from Hilligan & Ronchese, Cellular & Molecular Immunology 2020

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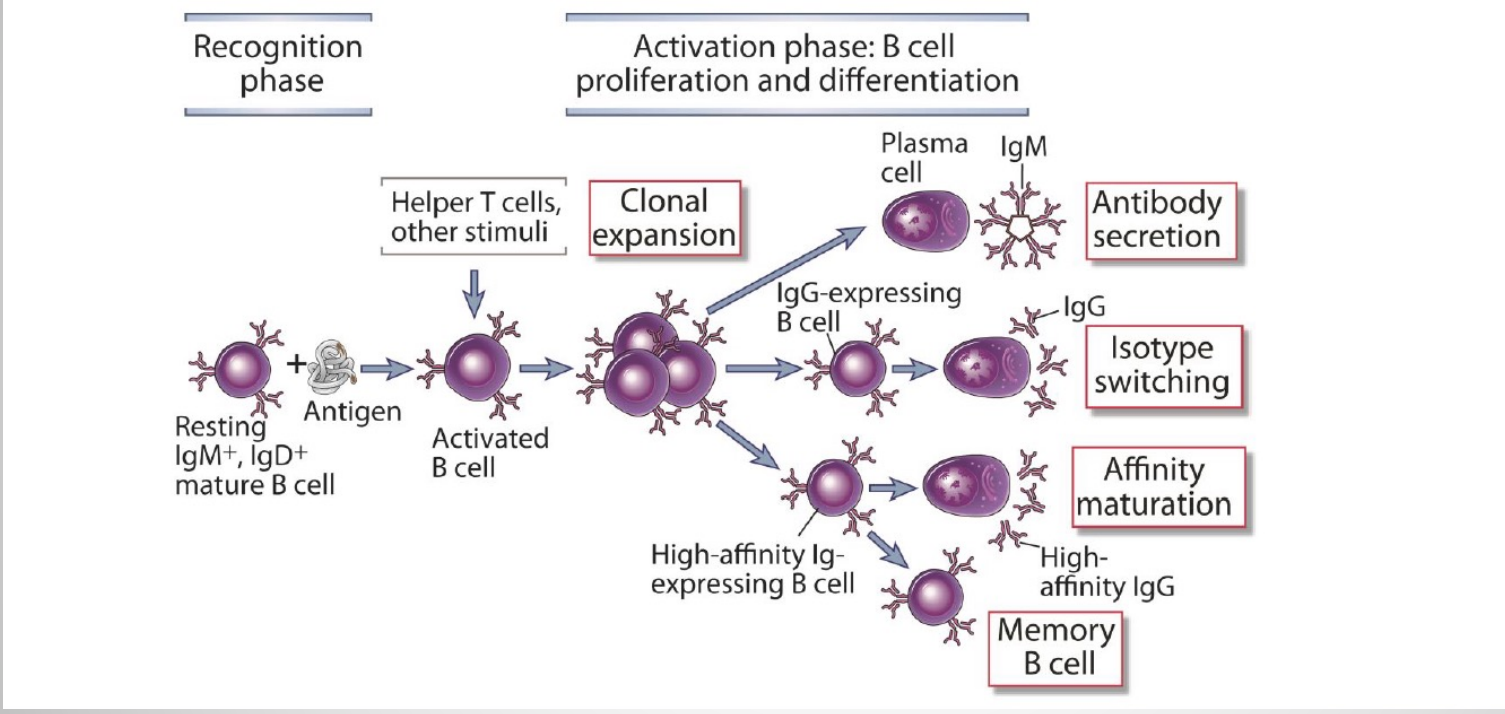
B Cell Development



Source: Cellular and molecular immunology - Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai

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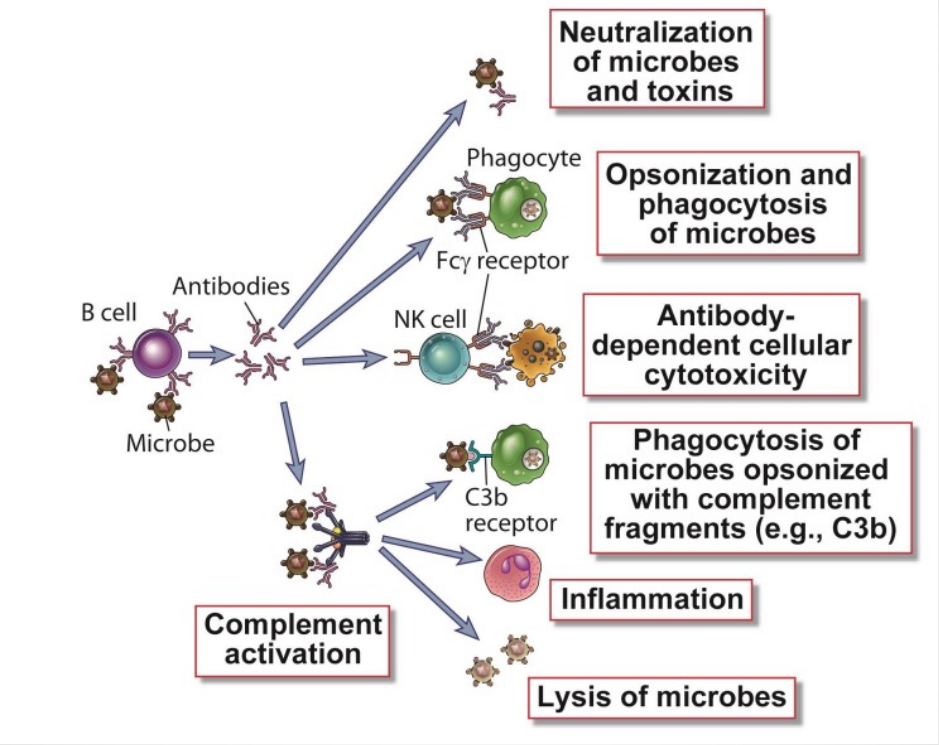
Effector functions of antibodies



Source: Cellular and molecular immunology - Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai

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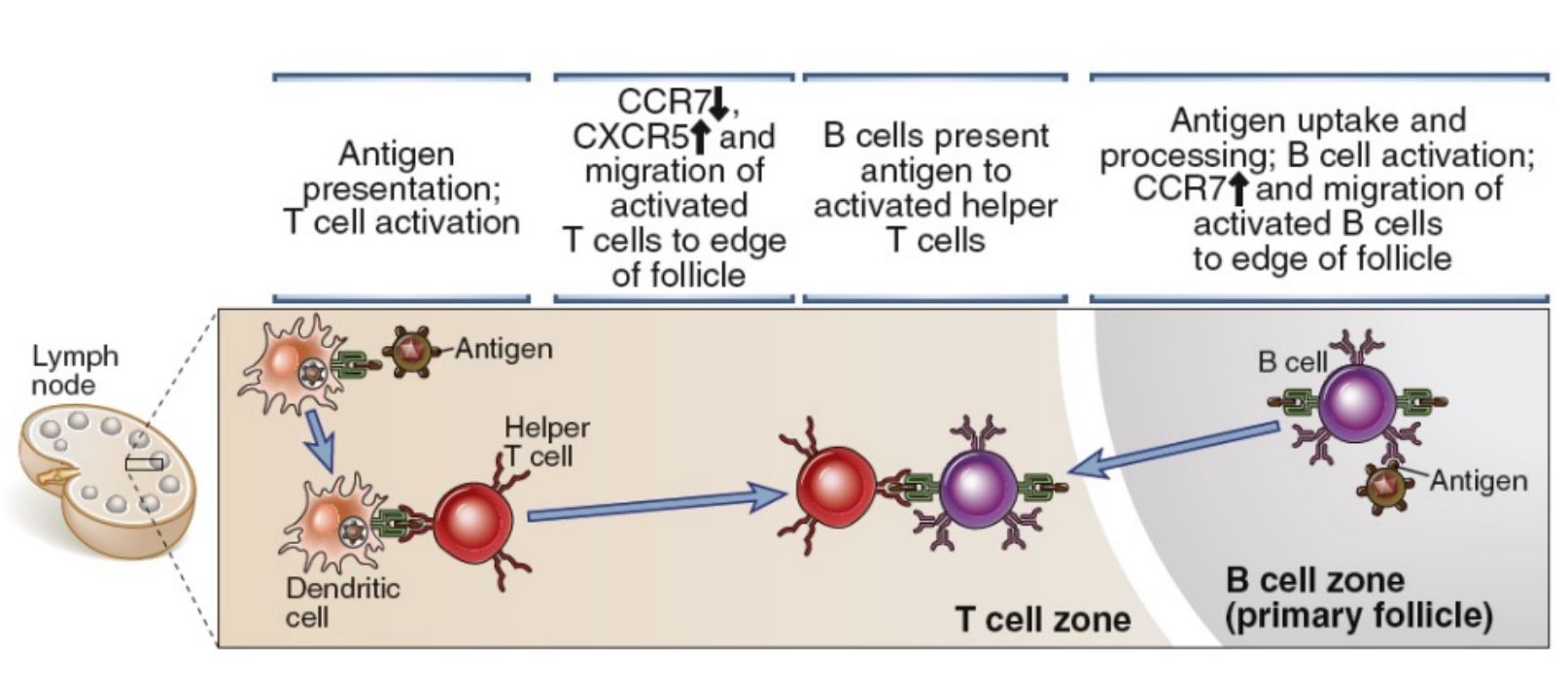
T cell – B cell interaction



Source: Cellular and molecular immunology - Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai

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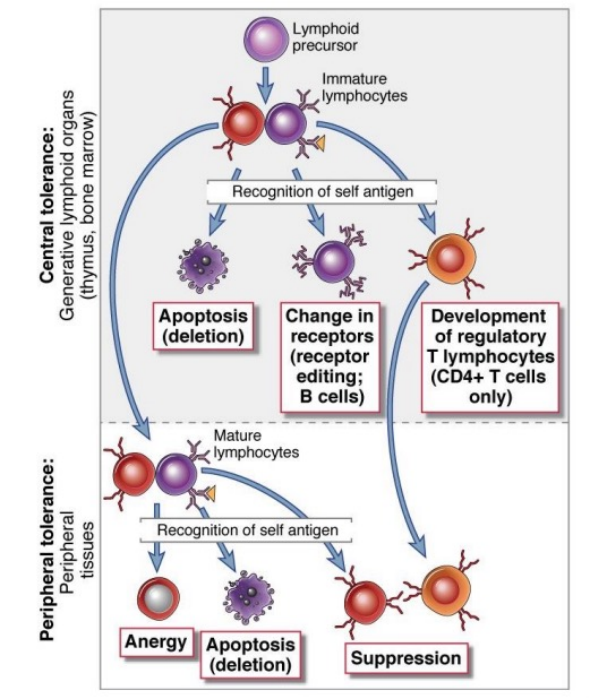
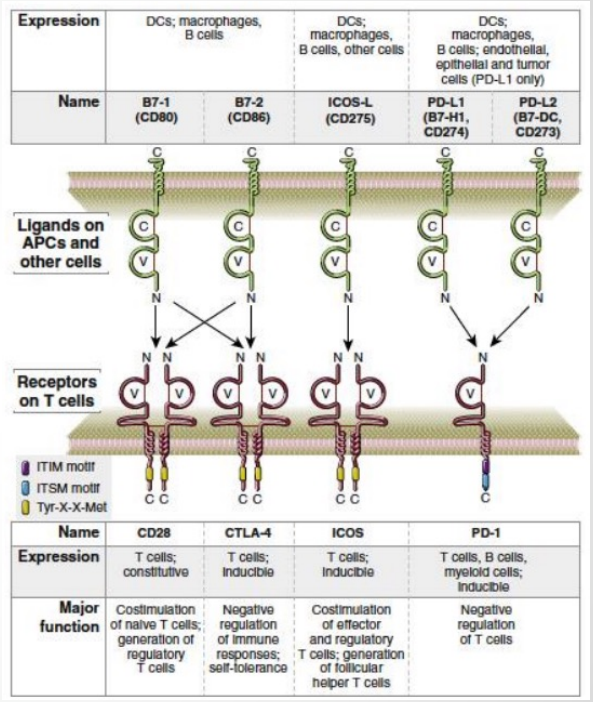
Tolerance & balance between activation and inhibition





Source: Cellular and molecular immunology - Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai

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Part 1: Introduction to Immunology and Immunogenicity

INTRODUCTION TO IMMUNOGENICITY PART 1

- Introduction immune system and immunogenicity
- **Factors influencing unwanted immunogenicity**
- Consequences of unwanted immunogenicity
- Biologics and unwanted immunogenicity

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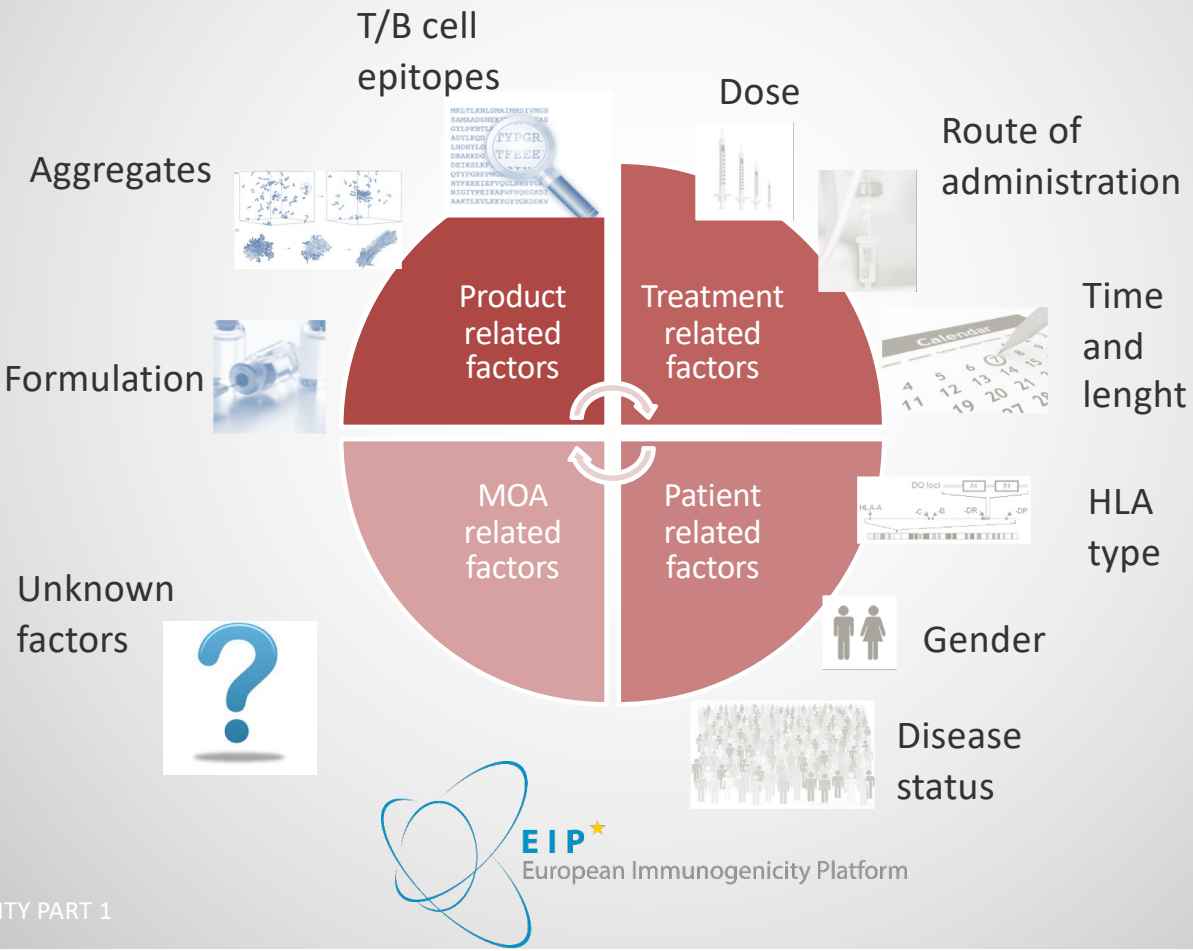
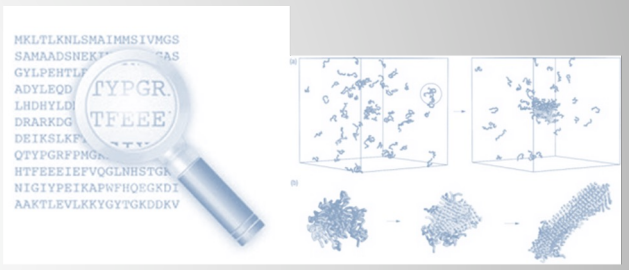
Factors impacting Immunogenicity



Factors Influencing Immunogenicity

Drug-related factors:

- Primary sequence, structure
- Novel epitopes
- Glycosylation, deamidation, oxydation, …
- Product impurities, aggregation and degradation products
- Formulation and storage conditions
- Murine, chimeric, humanized, human mAbs
- Excipients with allergenic potential in biological drugs: mannitol, albumin, polysorbate 80, latex, papain, trometamol
- Aggregates



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Factors Influencing Immunogenicity

Therapy-related factors

- Duration and length
- Dose
- Route and frequency of administration

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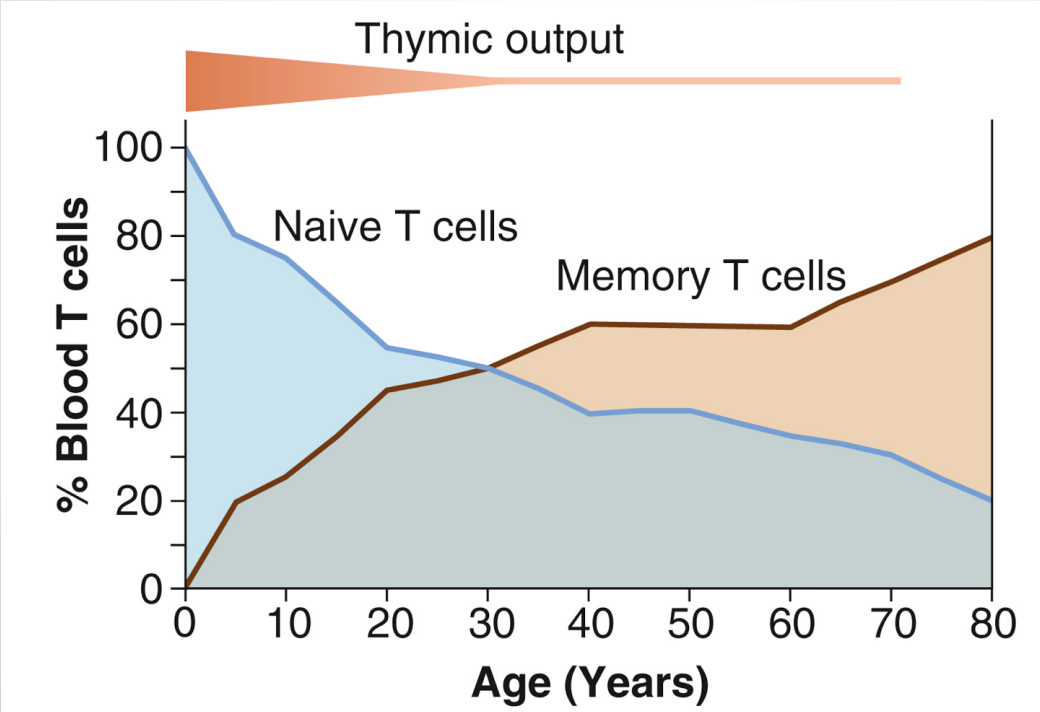
Factors Influencing Immunogenicity

Patient-related factors:

- Age



Source: Cellular and molecular immunology - Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai

Factors Influencing Immunogenicity

Patient-related factors:

- Age
- Gender
- Disease status
- Genetic make up
- Immune status
- Pre-exposure, pre-existing (cross-reactive) antibodies

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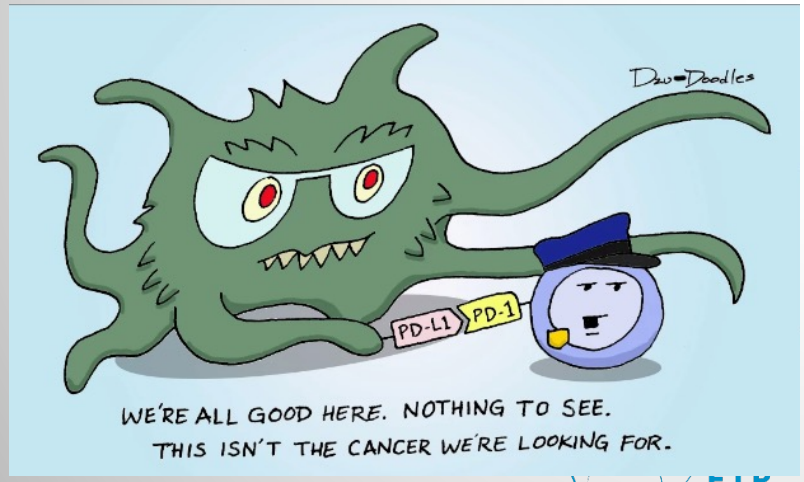
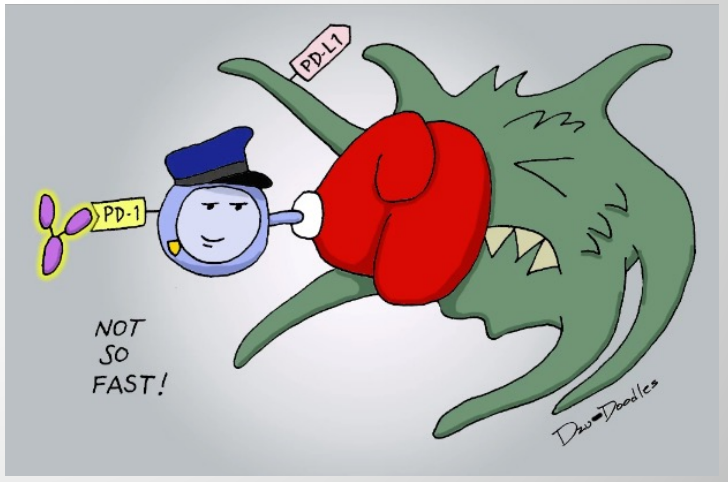
Factors Influencing Immunogenicity

Mechanism of action related factors:

- Immune cell targets
- Immuno-oncology drugs
  - Check-point inhibitors





Source images : dzu doodles

Factors Influencing Immunogenicity

Unknown factors:

Food

Lifestyle

Emotions & thoughts

ROS

Hormones

Neurological system

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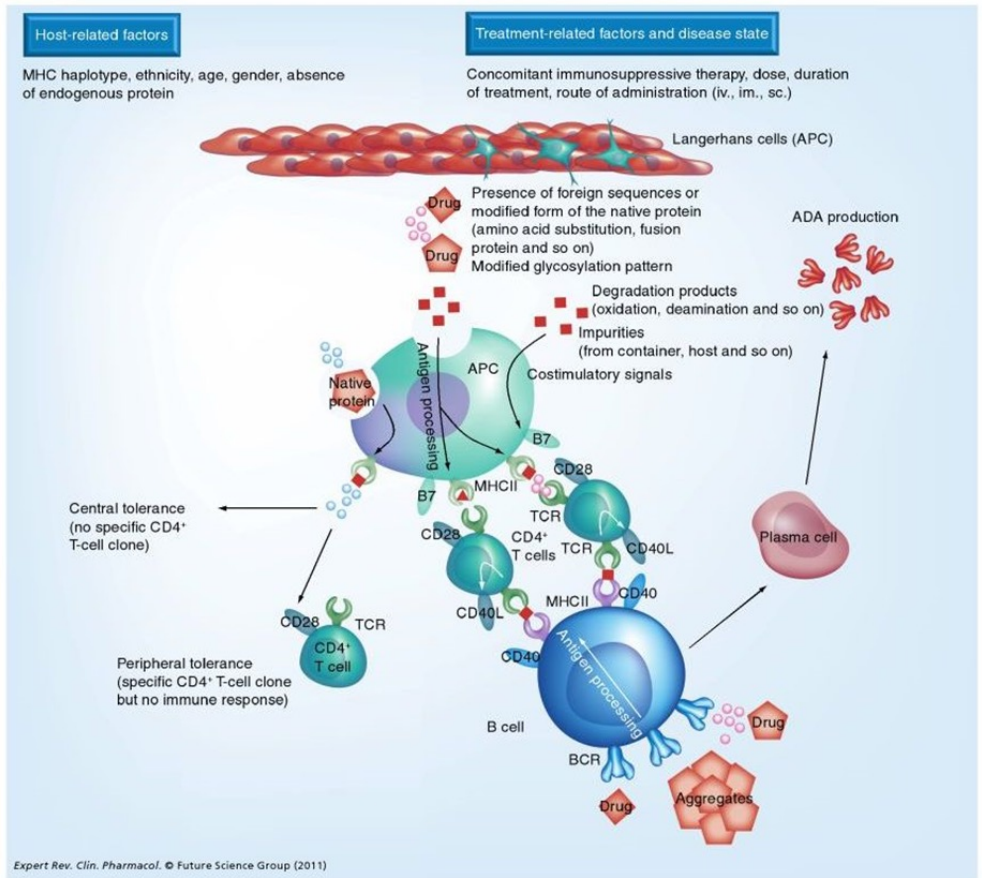
Interplay Between Different Factors



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Consequences of Unwanted Immunogenicity

- Almost **all therapeutics** induce an immune response that can lead to the formation of antibodies with varying characteristics (binding, neutralizing or a mixture of the above).

- The induction of anti-drug antibodies can be related to **adverse effects** like **hypersensitivity** and **anaphylactic** reactions or **cross-reactions** with the **endogenous** counterpart of the drug.

- The presence of anti-drug antibodies can also lead to **decreased efficacy and potency**

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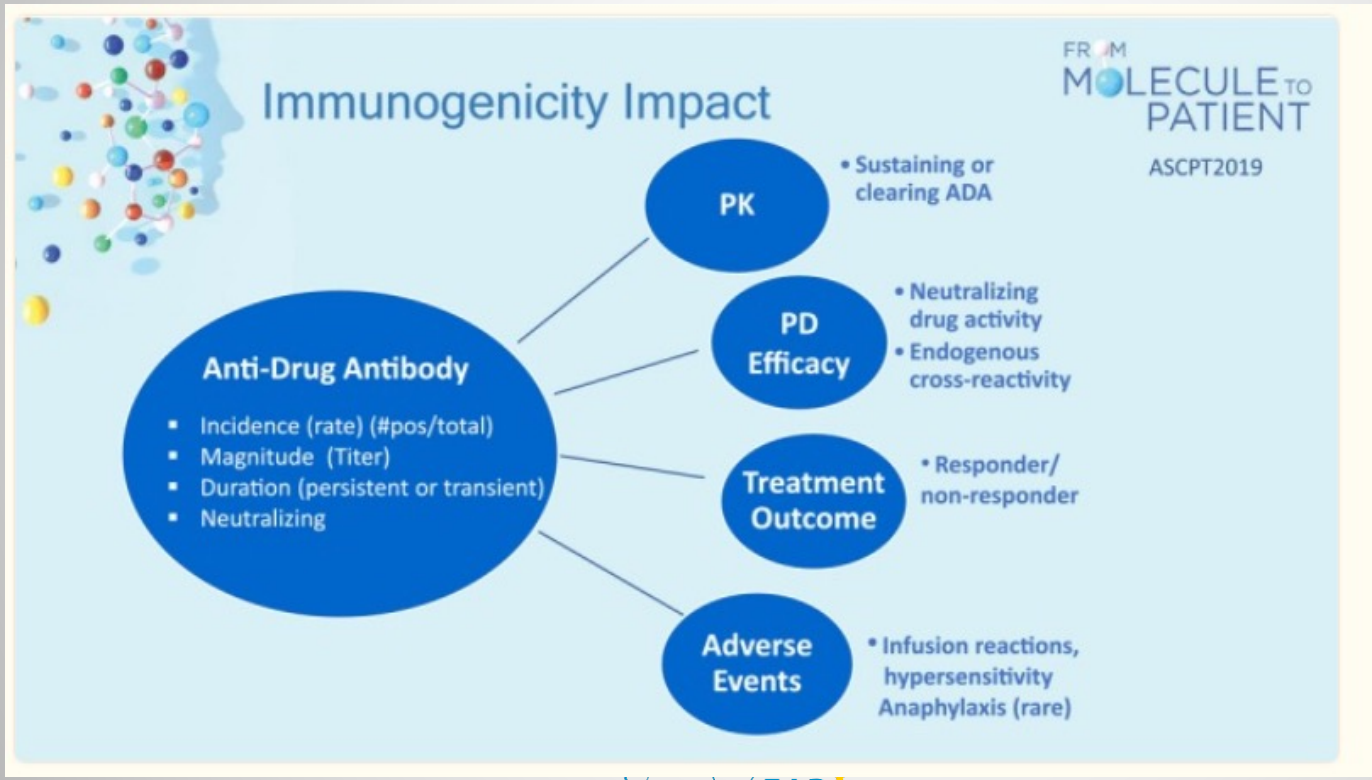
Consequences of Unwanted Immunogenicity



Source : Shakhnovich et al. Clin Transl Sci. 2020

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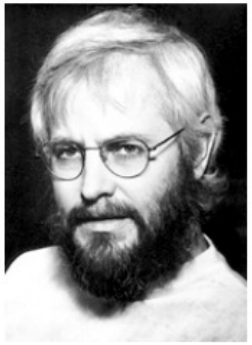
History Monoclonal Antibodies

The Nobel Prize in Physiology or Medicine 1984

Niels K. Jerne
Prize share: 1/3



Georges J.F. Köhler
Prize share: 1/3

César Milstein
Prize share: 1/3

The Nobel Prize in Physiology or Medicine 1984 was awarded jointly to Niels K. Jerne, Georges J.F. Köhler and César Milstein *"for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies"*.

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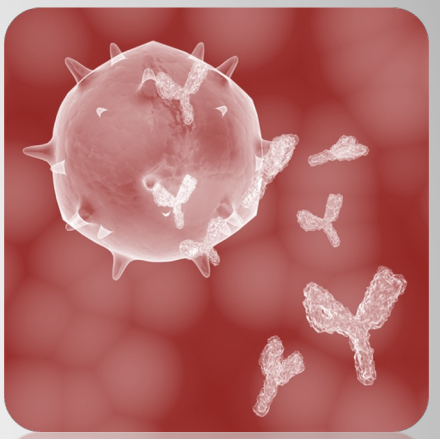
First Applications of Therapeutic Monoclonal Antibodies

- The first FDA approved monoclonal antibody was a murine IgG2a, muromonab (1986)
- Target: anti-CD3
- Indication: prevent graft-transplant rejection

- **New products new issues: unwanted immunogenicity**
- Immunogenic(ity) first used in the Lancet (1931) and Science (1944) vaccine contex



- Shawler DL, Bartholomew RM, Smith LM, Dillman RO (1985) Human immune response to multiple injections of murine monoclonal IgG. J Immunol 135:1530–1535
- Schroff, R.W. et al. (1985) Human anti-murine responses in patients receiving monoclonal antibody therapy. Cancer Res. 45, 879–885

Source: https://labs.selfdecode.com/blog/immunoglobulin-m/

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From Murine to Fully Human Therapeutics

Immunogenicity

Source: www.crohnsite.be/

Source: www.nahdionline.com

Source: www.cancerhealth.com

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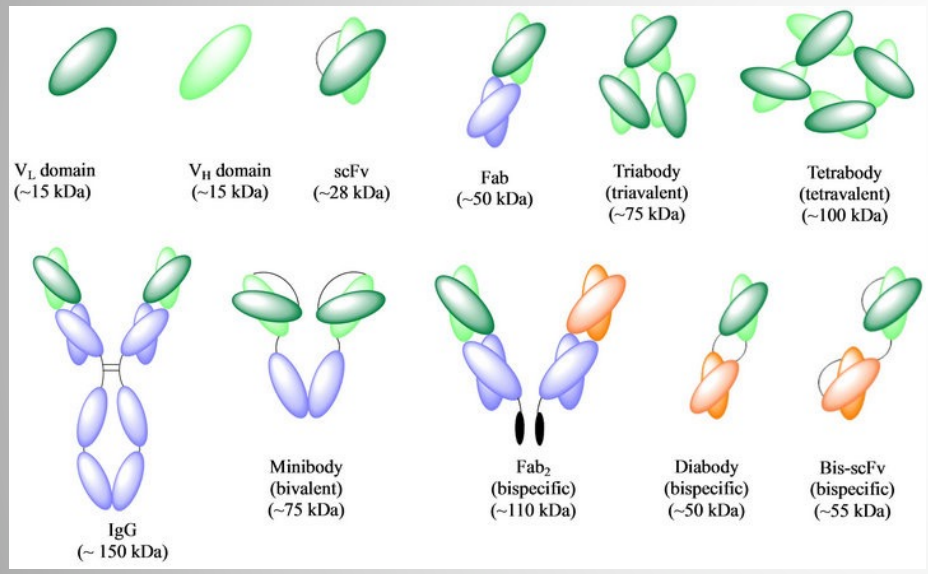
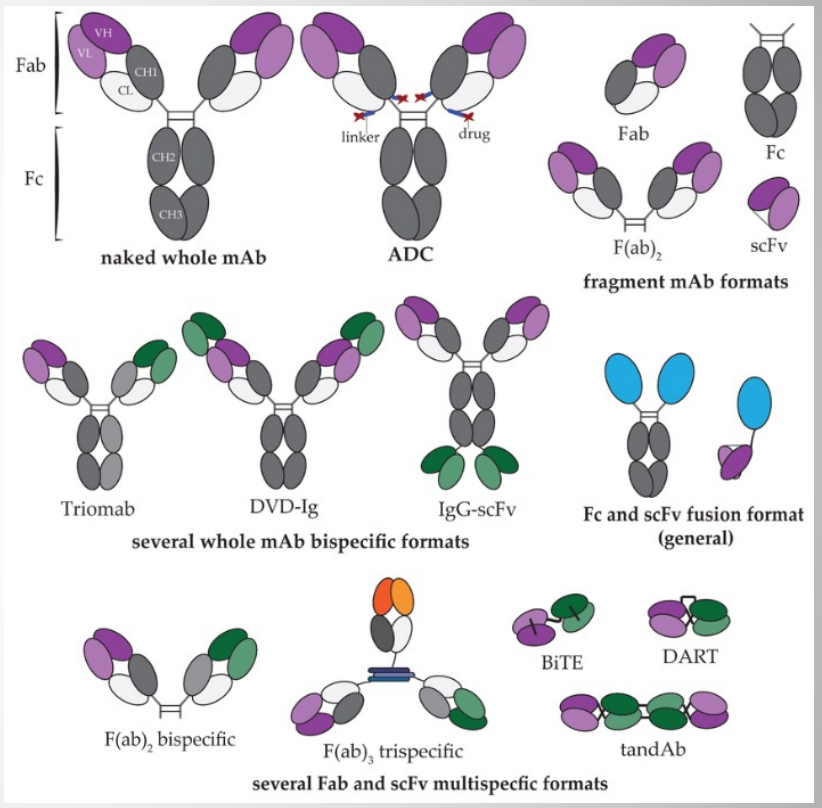
New Antibody Formats and Mimetics



Source : Herrington-Symes et al. Advances in Bioscience and Biotechnology. Vol.4 No.5 (2013)



Source : Sifniotis et al. Antibodies (2019)

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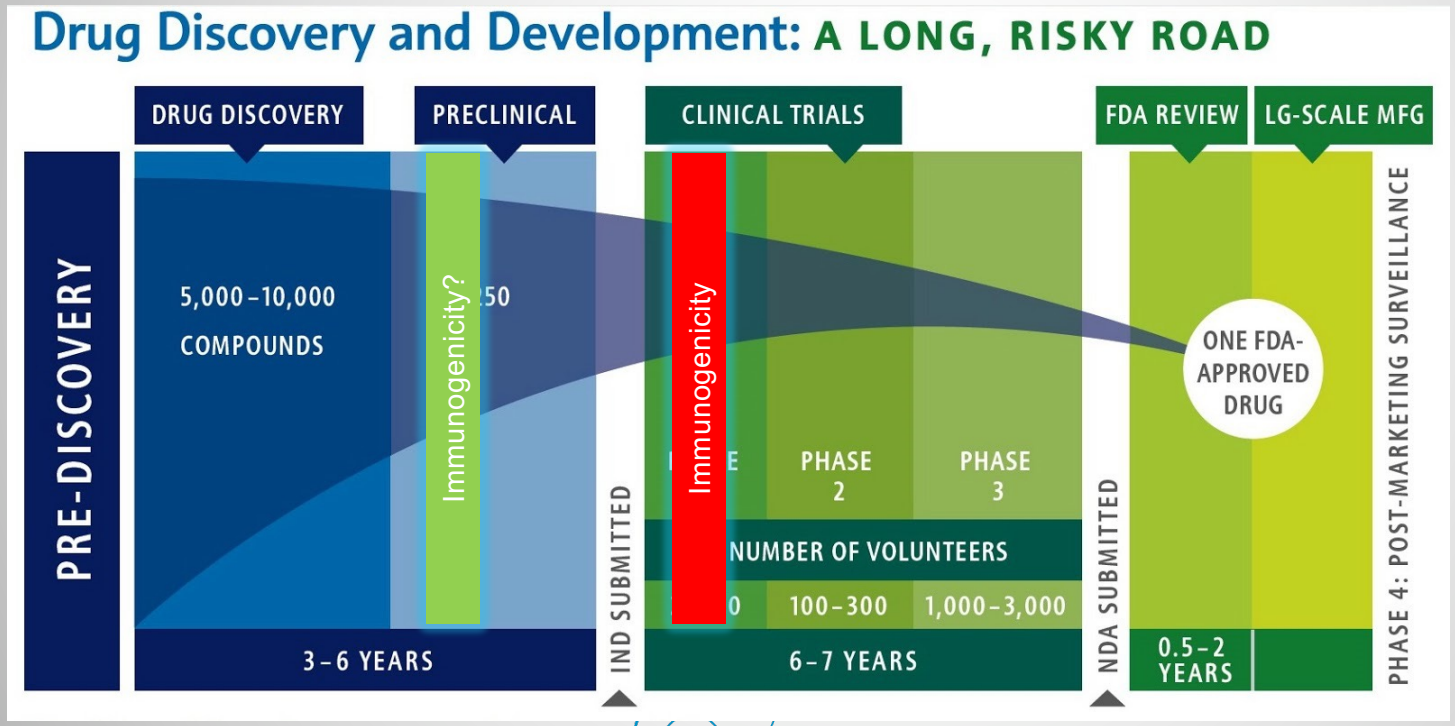
Consequences of Unwanted Immunogenicity



Adapted from Medicines in Development Leukemia & Lymphoma 2013

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Consequences of Unwanted Immunogenicity

Bayer drops hemophilia candidate BAY 86-6150 on safety concerns

06-05-2013 COMMENTS (0)

BAY 86-6150 | Bayer | Pharmaceutical | Research

German drug major Bayer (BAYN: DE) said on Friday (May 3) that it has discontinued a Phase II/III trial evaluating the efficacy and safety of BAY 86-6150 in people with hemophilia A and hemophilia B with inhibitors has been discontinued.

The company said that the hope that BAY 86-6150 might help patients with inhibitors to achieve better control of their disease could not be fulfilled due to the detection of a neutralizing antibody in the trial.

"Patient safety is our primary concern when designing clinical trials and evaluating BAY 86-6150," said Kemal Malik, a member of the Bayer HealthCare executive committee and head of global development, adding: "Due to safety concerns, we are discontinuing the BAY 86-6150 trial as a precautionary measure."

Source: https://www.thepharmaletter.com/

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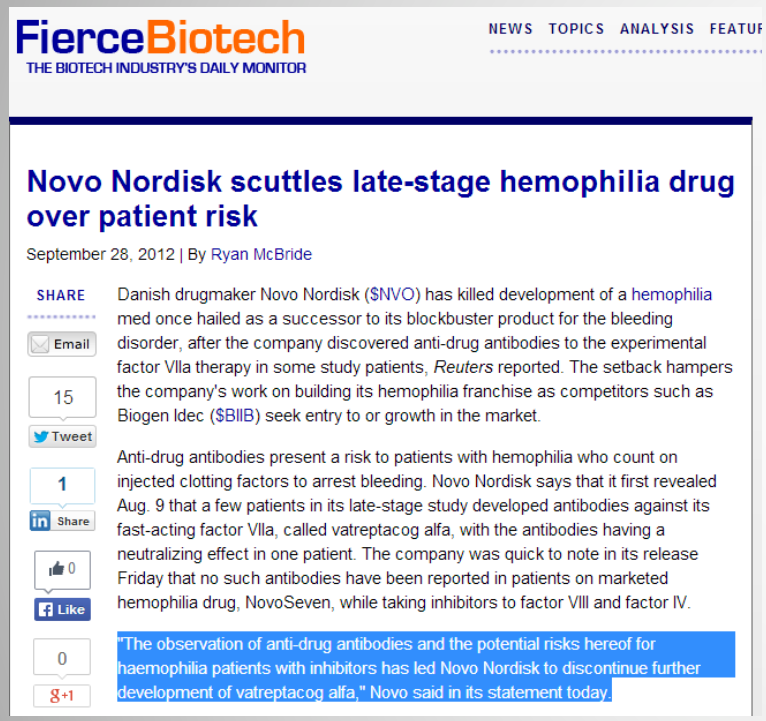

FierceBiotech
THE BIOTECH INDUSTRY'S DAILY MONITOR

NEWS TOPICS ANALYSIS FEATUF

Novo Nordisk scuttles late-stage hemophilia drug over patient risk

September 28, 2012 | By Ryan McBride

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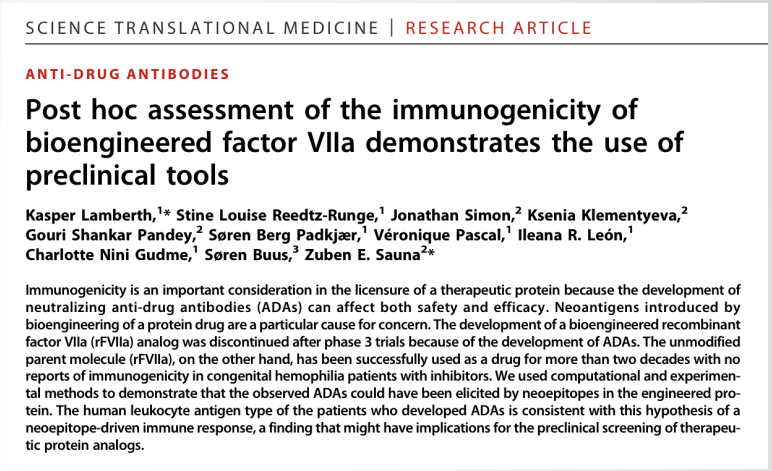
8+1

Danish drugmaker Novo Nordisk ($NVO) has killed development of a hemophilia med once hailed as a successor to its blockbuster product for the bleeding disorder, after the company discovered anti-drug antibodies to the experimental factor VIIa therapy in some study patients, *Reuters* reported. The setback hampers the company's work on building its hemophilia franchise as competitors such as Biogen Idec ($BIIB) seek entry to or growth in the market.

Anti-drug antibodies present a risk to patients with hemophilia who count on injected clotting factors to arrest bleeding. Novo Nordisk says that it first revealed Aug. 9 that a few patients in its late-stage study developed antibodies against its fast-acting factor VIIa, called vatreptacog alfa, with the antibodies having a neutralizing effect in one patient. The company was quick to note in its release Friday that no such antibodies have been reported in patients on marketed hemophilia drug, NovoSeven, while taking inhibitors to factor VIII and factor IV.

"The observation of anti-drug antibodies and the potential risks hereof for haemophilia patients with inhibitors has led Novo Nordisk to discontinue further development of vatreptacog alfa," Novo said in its statement today.

Source: www.fiercebiotech.com



SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

ANTI-DRUG ANTIBODIES

Post hoc assessment of the immunogenicity of bioengineered factor VIIa demonstrates the use of preclinical tools

Kasper Lamberth,[1]* Stine Louise Reedtz-Runge,[1] Jonathan Simon,[2] Ksenia Klementyeva,[2] Gouri Shankar Pandey,[2] Søren Berg Padkjær,[1] Véronique Pascal,[1] Ileana R. León,[1] Charlotte Nini Gudme,[1] Søren Buus,[3] Zuben E. Sauna[2]*

Immunogenicity is an important consideration in the licensure of a therapeutic protein because the development of neutralizing anti-drug antibodies (ADAs) can affect both safety and efficacy. Neoantigens introduced by bioengineering of a protein drug are a particular cause for concern. The development of a bioengineered recombinant factor VIIa (rFVIIa) analog was discontinued after phase 3 trials because of the development of ADAs. The unmodified parent molecule (rFVIIa), on the other hand, has been successfully used as a drug for more than two decades with no reports of immunogenicity in congenital hemophilia patients with inhibitors. We used computational and experimental methods to demonstrate that the observed ADAs could have been elicited by neoepitopes in the engineered protein. The human leukocyte antigen type of the patients who developed ADAs is consistent with this hypothesis of a neoepitope-driven immune response, a finding that might have implications for the preclinical screening of therapeutic protein analogs.

Transl Med. 2017 Jan 11;9(372)

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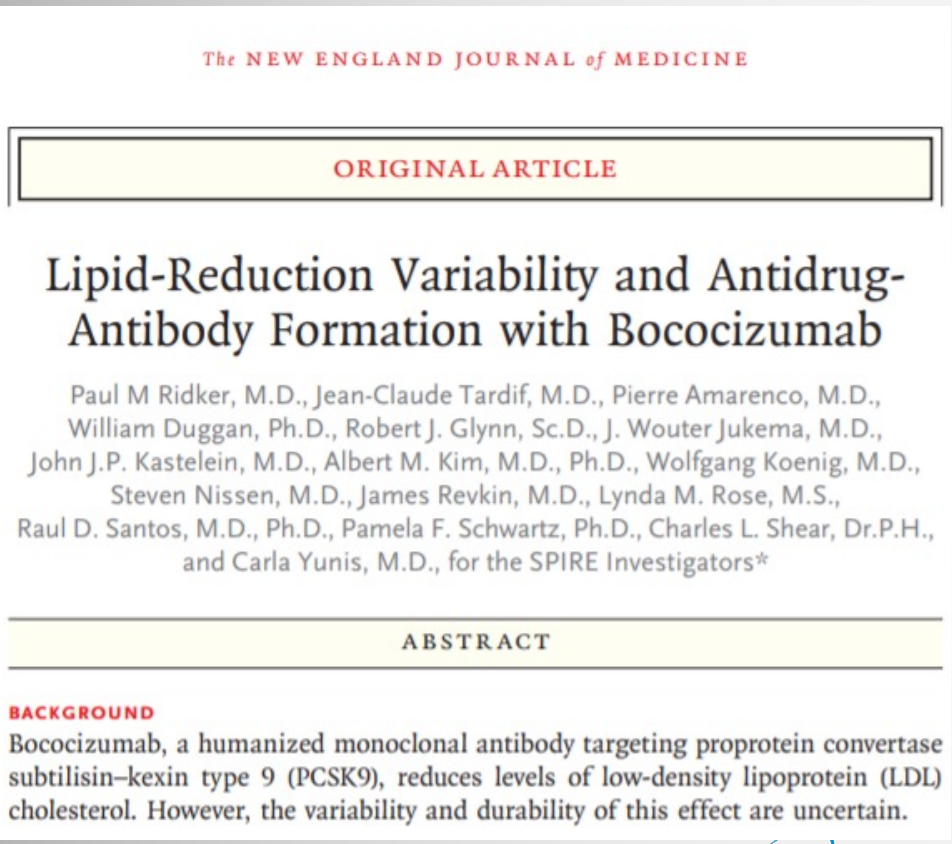
Unwanted Immunogenicity: One Hurdle of the Drug Development Cycle



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Lipid-Reduction Variability and Antidrug-Antibody Formation with Bococizumab

Paul M Ridker, M.D., Jean-Claude Tardif, M.D., Pierre Amarenco, M.D., William Duggan, Ph.D., Robert J. Glynn, Sc.D., J. Wouter Jukema, M.D., John J.P. Kastelein, M.D., Albert M. Kim, M.D., Ph.D., Wolfgang Koenig, M.D., Steven Nissen, M.D., James Revkin, M.D., Lynda M. Rose, M.S., Raul D. Santos, M.D., Ph.D., Pamela F. Schwartz, Ph.D., Charles L. Shear, Dr.P.H., and Carla Yunis, M.D., for the SPIRE Investigators*

ABSTRACT

BACKGROUND

Bococizumab, a humanized monoclonal antibody targeting proprotein convertase subtilisin–kexin type 9 (PCSK9), reduces levels of low-density lipoprotein (LDL) cholesterol. However, the variability and durability of this effect are uncertain.

Source: N Engl J Med 2017; 376:1517-1526

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Unwanted Immunogenicity: One Hurdle of the Drug Development Cycle

Clinical Trial > J Clin Pharmacol. 2014 Jan;54(1):14-22. doi: 10.1002/jcph.158. Epub 2013 Sep 17.

Anti-IL21 receptor monoclonal antibody (ATR-107): Safety, pharmacokinetics, and pharmacodynamic evaluation in healthy volunteers: a phase I, first-in-human study

Fei Hua [1], Gail M Comer, Lori Stockert, Bo Jin, John Nowak, Susan Pleasic-Williams, David Wunderlich, John Cheng, Jean S Beebe

Source: J Clin Pharmacol 2014; 54(1):14-22

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Immunogenicity Beginner Training: Mechanisms of Immunogenicity; **Overview of Non-Clinical Risk Assessment Tools**

EIP Open Symposium

16th- 19th March 2026

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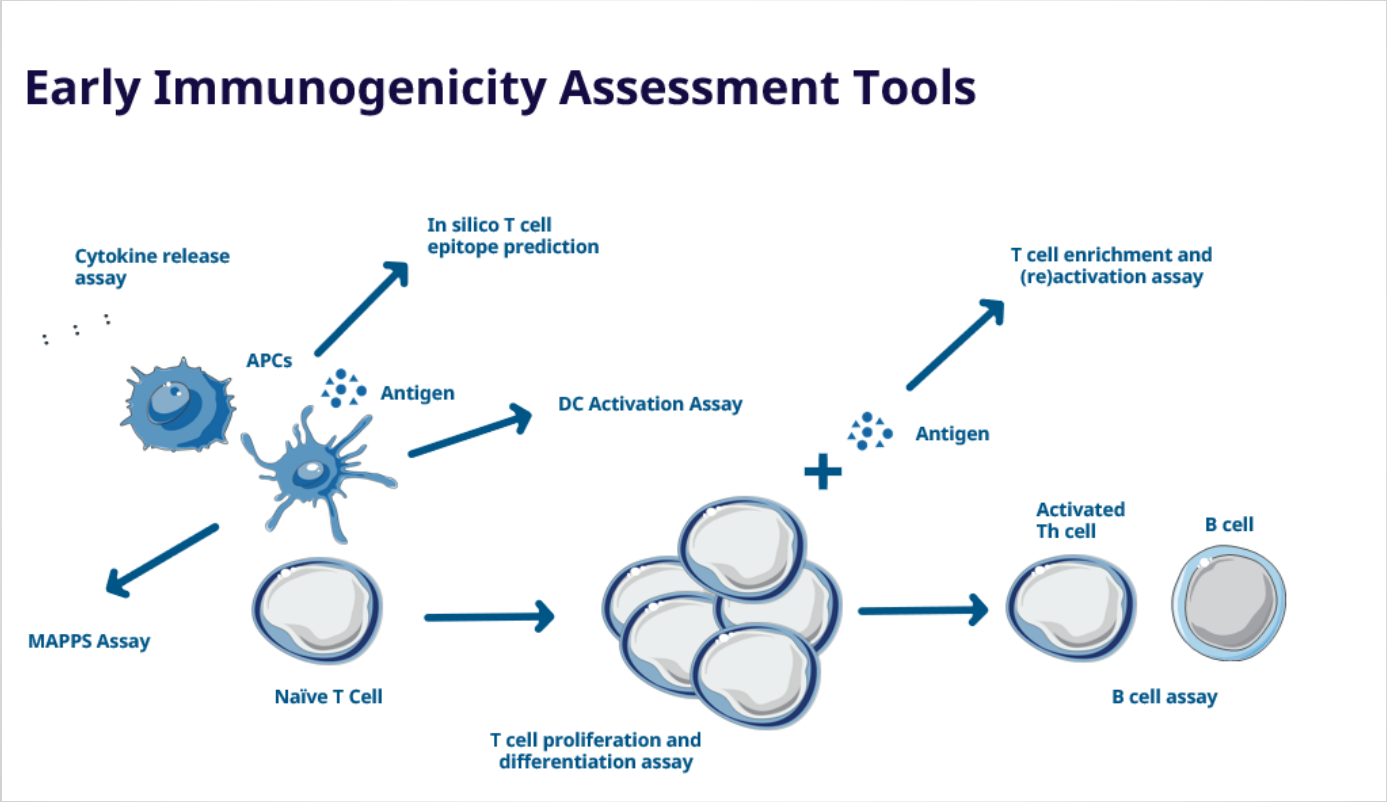
Early immunogenicity assessment tools



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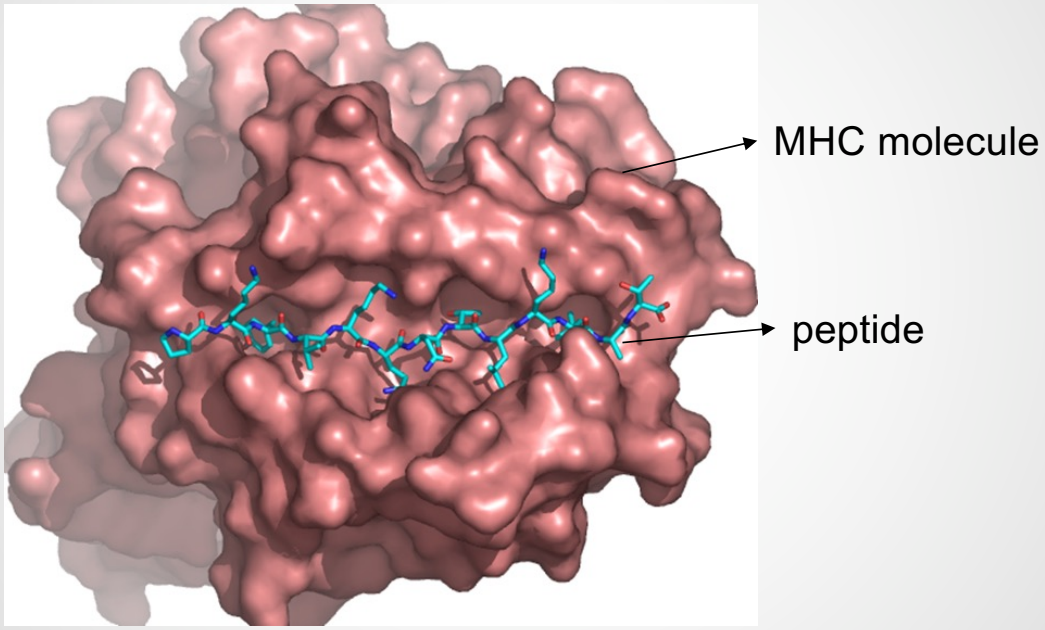
In silico T cell epitope prediction



Source: EIR Sciences, Denmark

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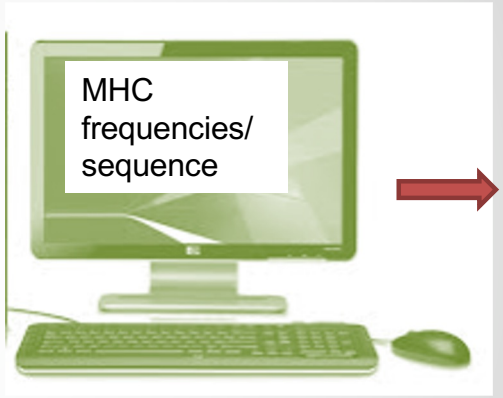
In silico T cell epitope prediction



Identify potential epitopes

Test sequence → Test population → Algorithm → Immunogenicity potential assessment

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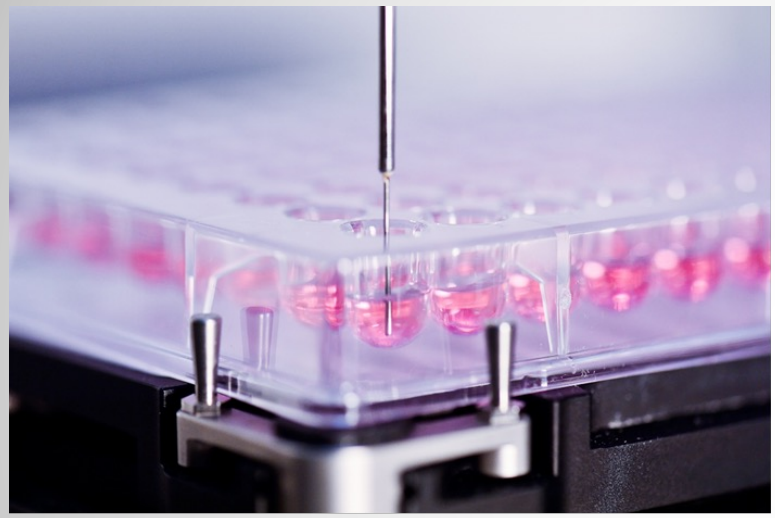
In vitro tools: cellular assays



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In vitro tools: cellular assays

Innate Response /Early

Incubation of whole blood/PBMC/ Dendritic cells, reporter cell lines with test products for 24-48 hours

Analysis of cytokine signature or maturation markers

Adaptive Response/ Late

Incubation of PBMC with test products for 7 days, DC-T cell assay

Analysis of proliferation/cytokine production

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In vitro assays using primary cells

- **Quality of the primary cells**:
  - Variability and reproducibility of the results highly depends on the initial quality
  - Quality = viability and **<u>functionality</u>**
  - Most critical reagent
  - Standardized procedures for sampling, shipping, isolation, cryopreservation, thawing, handling, …
  - Need for a large number of HLA-typed donors in order to represent the wide range of responders (strong-responders versus medium-low responders)

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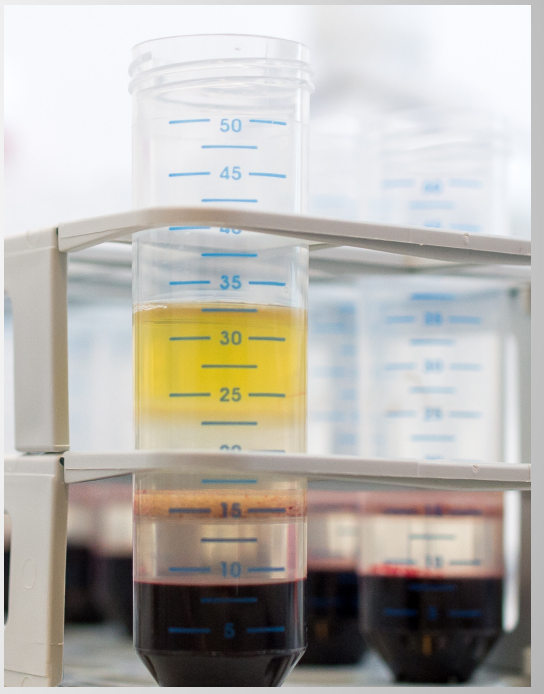
Primary cells: preserving quality

Preserving quality of the primary cells:

- Standardized procedures for sampling, shipping, isolation, cryopreservation, thawing, handling, …
- Start isolation of PBMC within 8 hours after blood draw
- Alternatively, use CPT™ tubes for blood sampling
- Perform an extended quality control on all cell preparations



Source: https://www.hce-uk.com/

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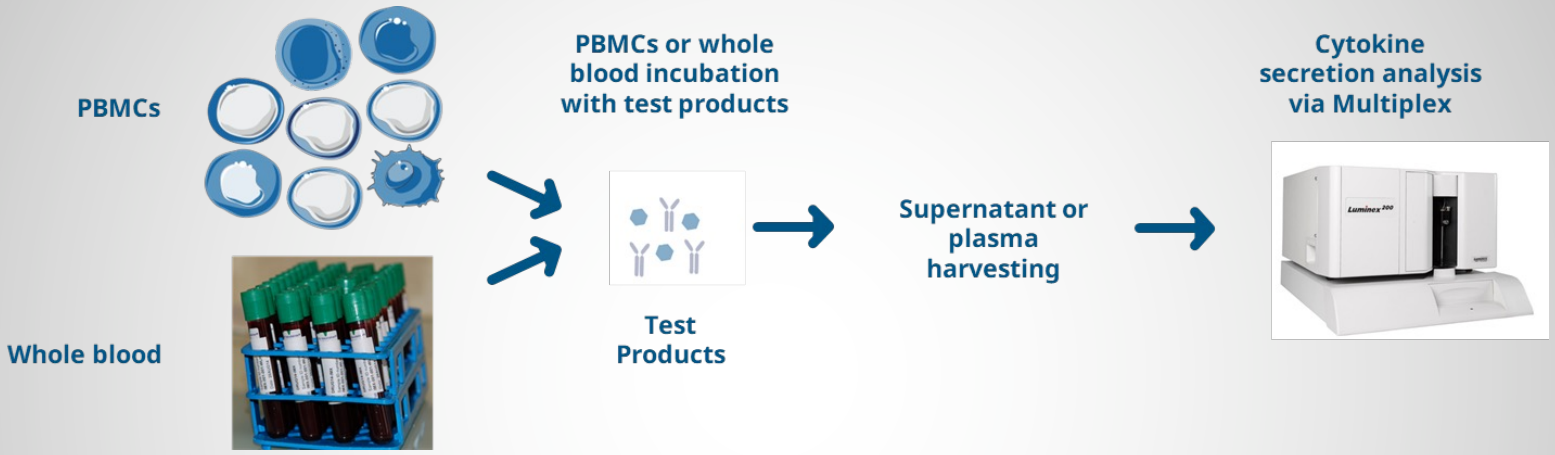
Innate Response /Early

In vitro whole blood/PBMC cytokine release assay



- Test molecules' potential to induce a cytokine release response assessment using:
  - Whole Blood Cytokine Release Assay
  - PBMC Cytokine Release Assay
- RO: Measurement of cytokines/chemokines in supernatant or plasma (Multiplex) and viability (Flow cytometry)
  - Early phase cytokines: TNF-α, IL-2, IL-8
  - Late phase cytokines: IFN-γ, IL-6, IL- 10
  - Additional extended cytokine panels

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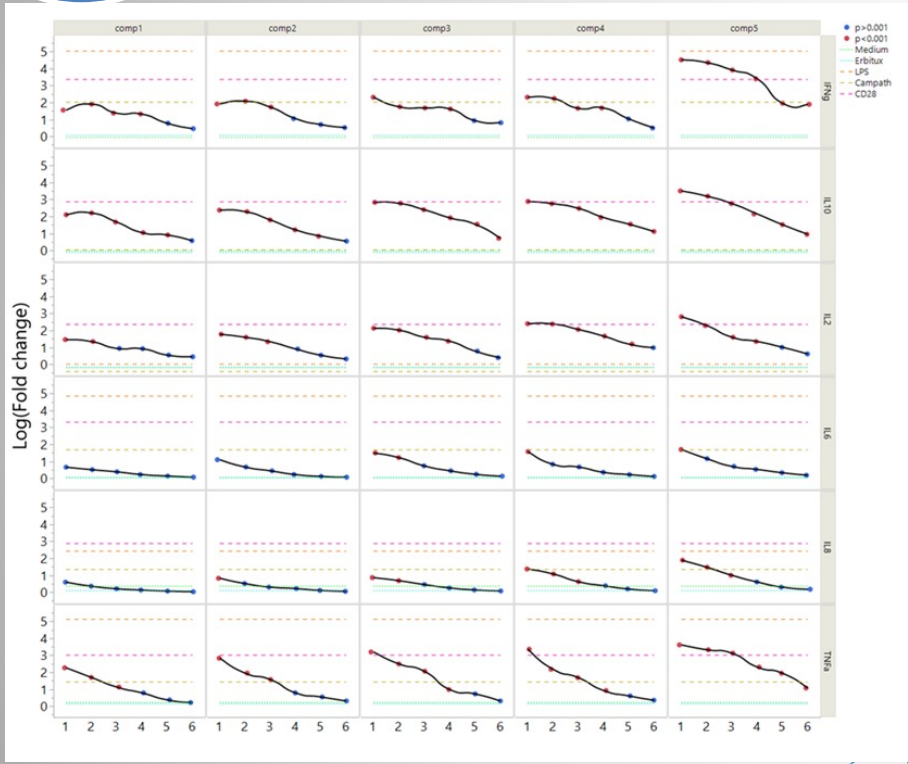
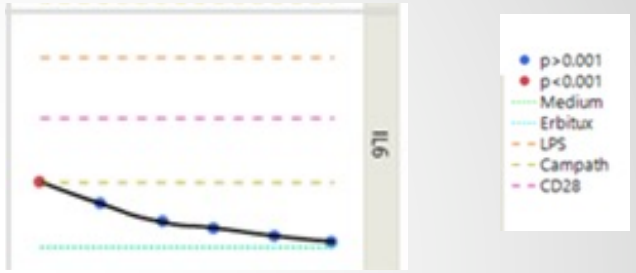
Innate Response /Early

In vitro whole blood cytokine release assay





Fresh blood from 10 healthy donors was incubated with test molecules (6 dilutions) and benchmark molecules for 24 hours, plasma was harvested, and cytokine analysis performed via Luminex.

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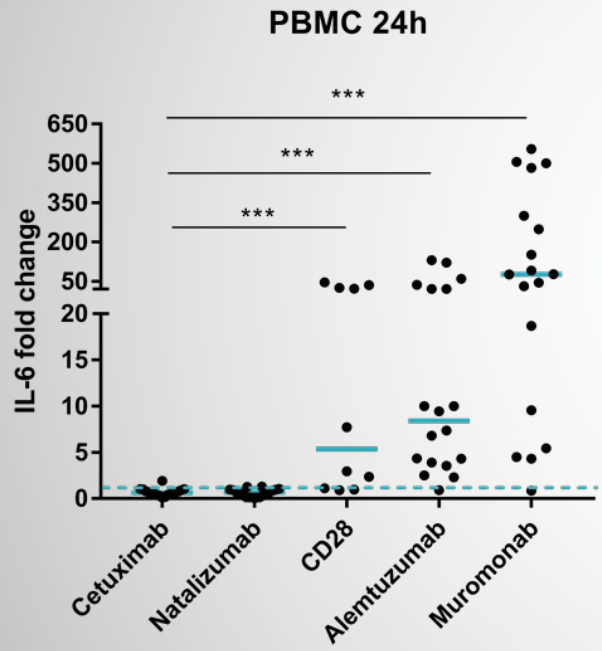
Innate Response /Early

In vitro PBMC cytokine release assay



Cryopreserved PBMC from 10 healthy donors were thawed and seeded in the presence of the test compound (in solution). After 24h, supernatant was harvested, and cytokine production analyzed via Luminex

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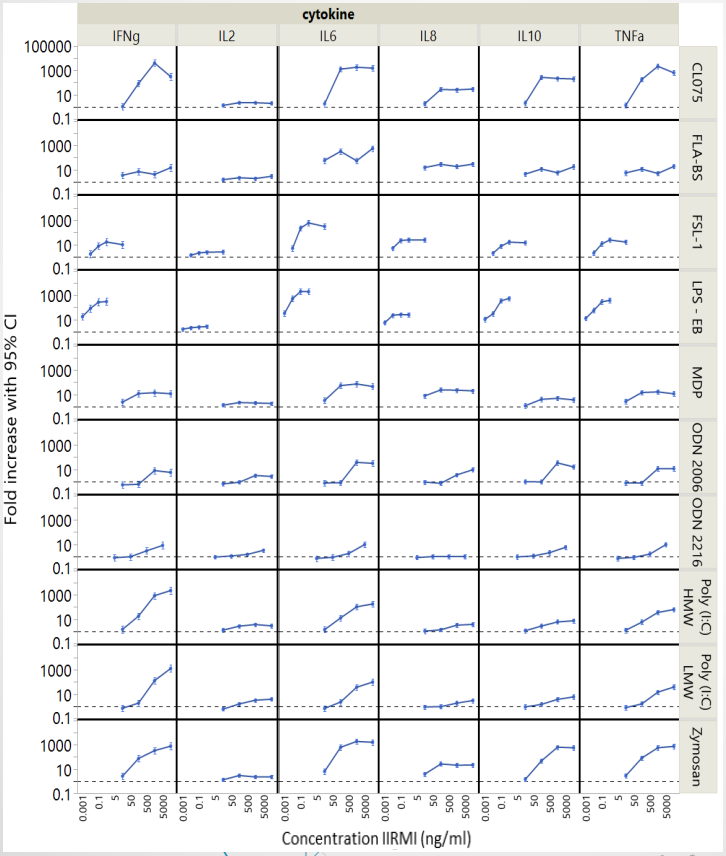
Innate Response /Early

In vitro IIRMI PBMC cytokine release assay

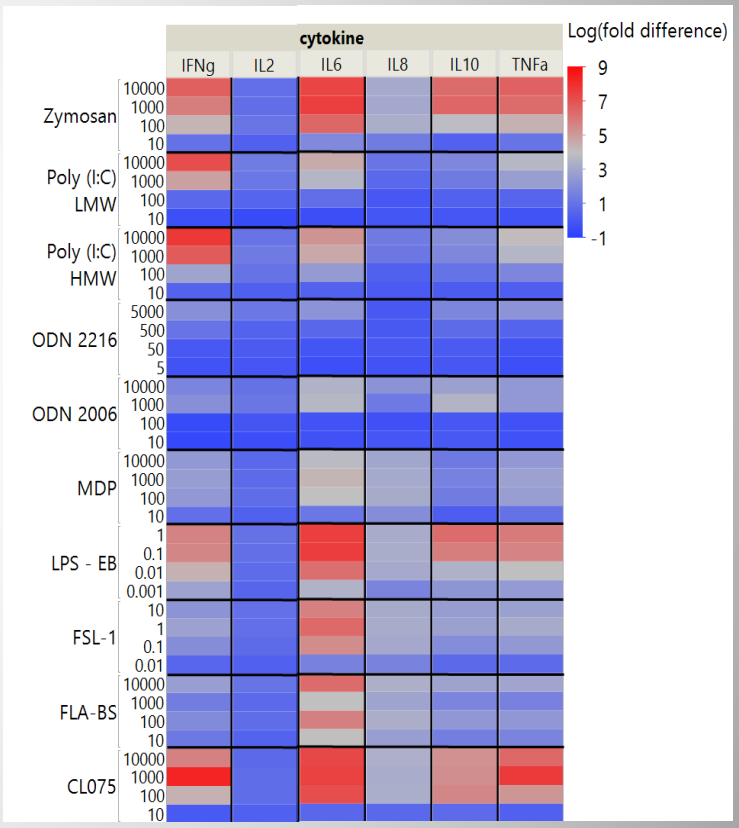
Cytokine Release Assay (CRA) for detection of IIRMIs in therapeutic proteins

Ten IIRMIs were evaluated in a PBMC CRA on their capacity to induce an innate immune response in PBMCs of 10 donors

PBMCs are sensitive to the presence of IIRMIs and can elicit a quantifiable cytokine response



Average fold changes with 95% confidence intervals

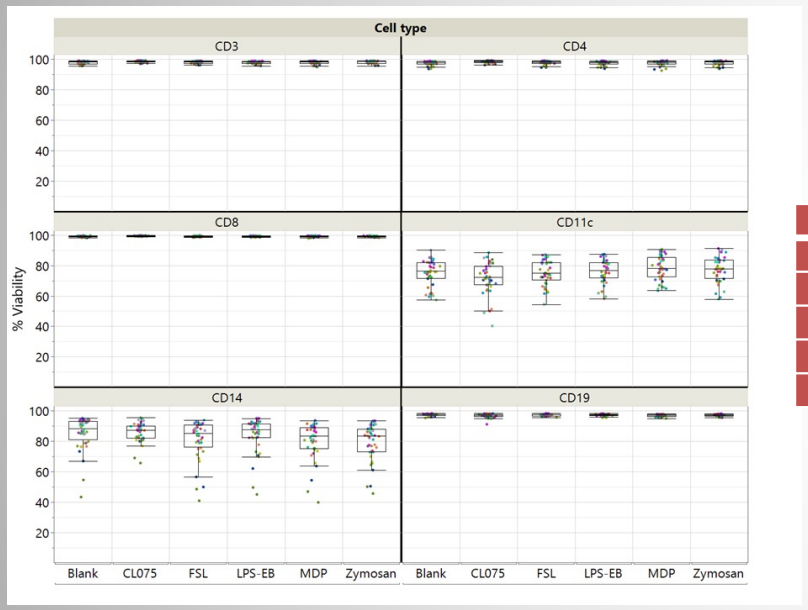


Heatmap with as color code fold change compared to unstimulated cells (on log scale).

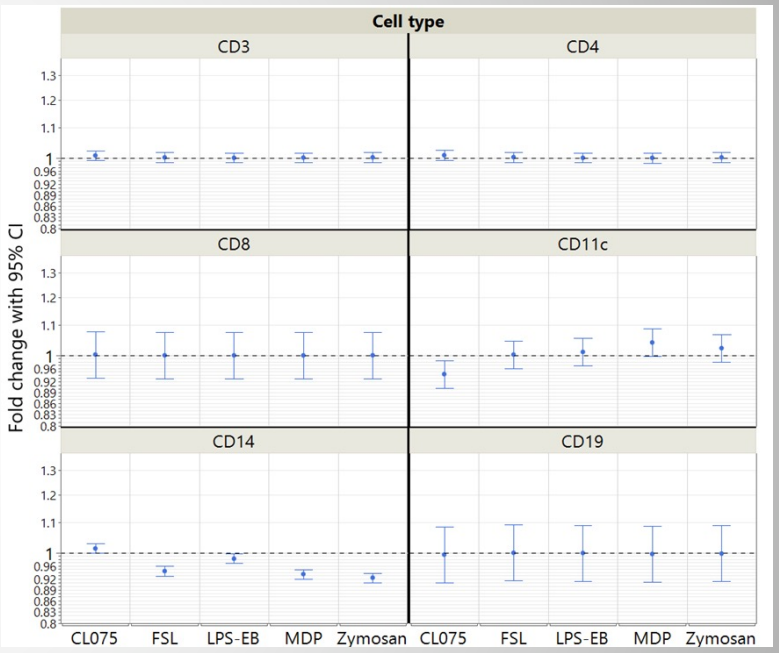
Innate Response /Early

In vitro IIRMI PBMC cytokine release assay

Cell viability – Percentage – Fold change



IIRMI	Concentration
CL075	500 ng/ml
FSL-1	0.01 ng/ml
LPS - EB	0.01 ng/ml
MDP	100 ng/ml
Zymosan	100 ng/ml



Viability is high across conditions for CD3, CD4, CD8 and CD19

For CD11c and CD14 viability is lower, but in most donors, viability is still higher than 70%

All conditions show % viability close to untreated cells, with fold changes and their entire 95% CI close to 1

A slight decrease in % viability is observed for CD11c with CL075 and for CD14 with all IIRMIs, except CL075

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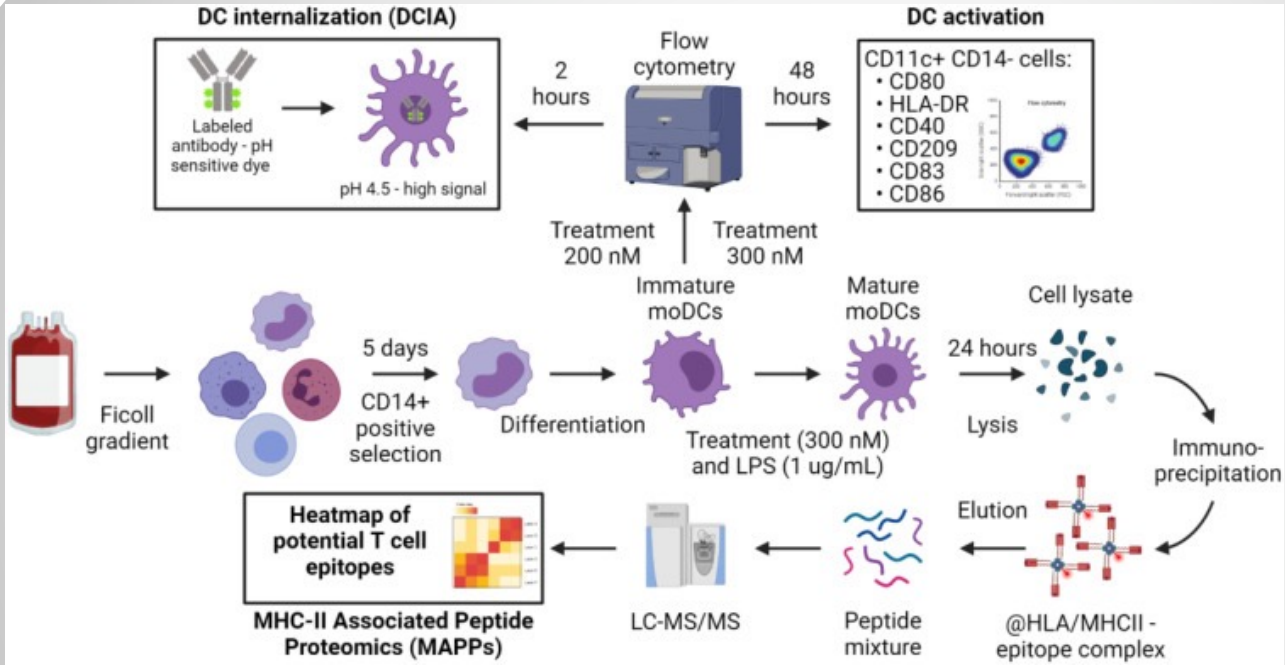
Innate Response /Early

DC activation/internalisation/uptake assay



Siegel M, Padamsey A, Bolender AL, Hargreaves P, Fraidling J, Ducret A, Hartman K, Looney CM, Bertinetti-Lapatki C, Rohr O, Hickling TP, Kraft TE, Marban-Doran C. **Development and characterization of dendritic cell internalization and activation assays contributing to the immunogenicity risk evaluation of biotherapeutics**. Front Immunol. 2024 Aug 20;15:1406804. doi: 10.3389/fimmu.2024.1406804. PMID: 39229274; PMCID: PMC11368763.

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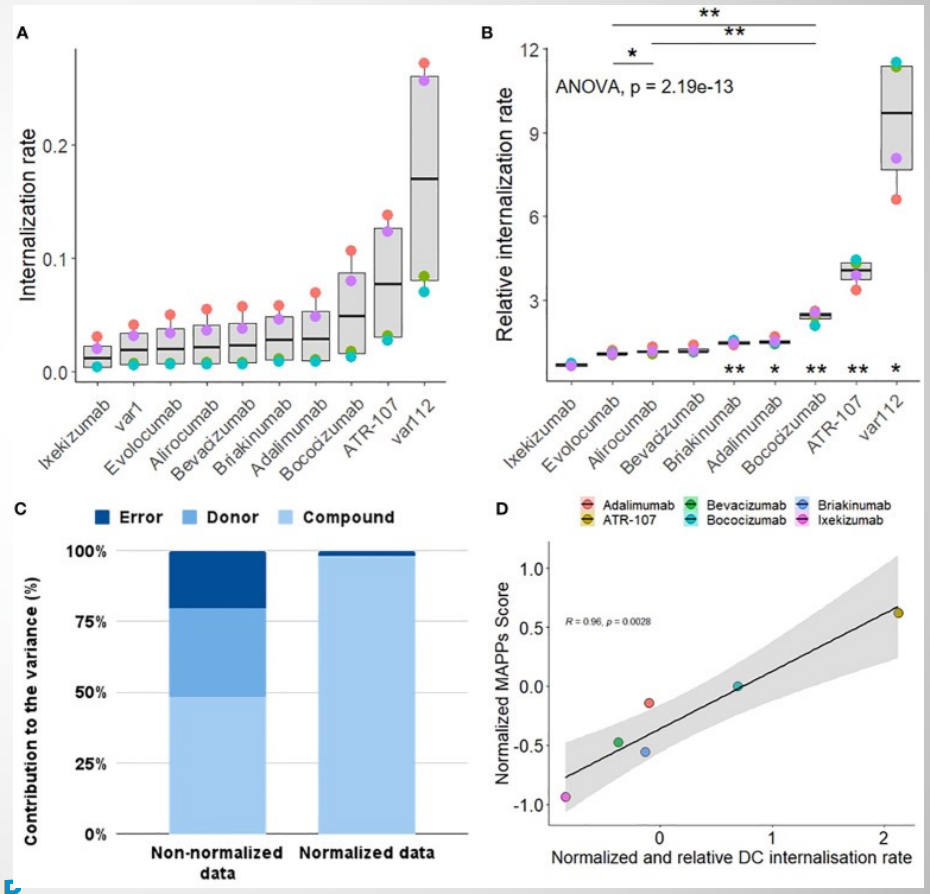
Innate Response /Early

DC internalisation/uptake assay



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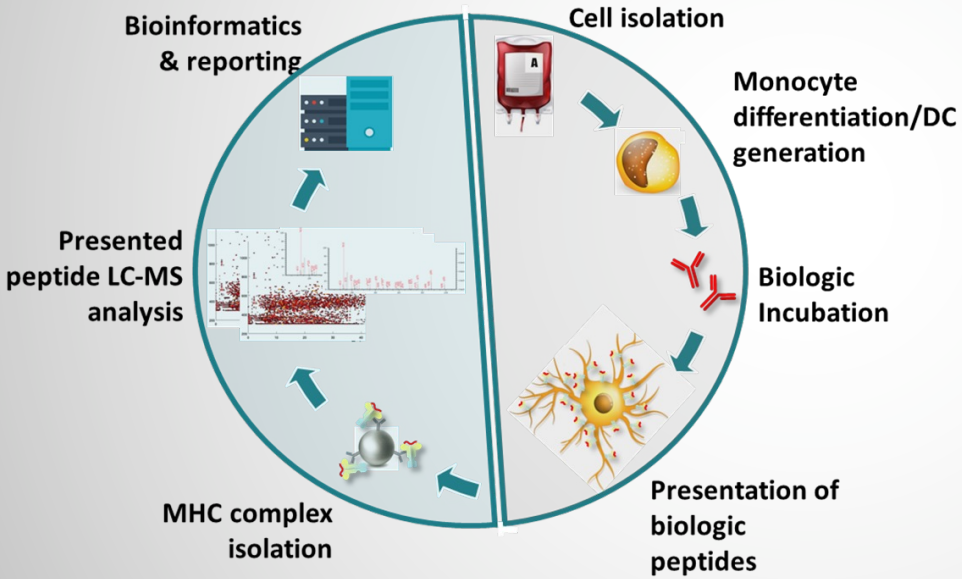
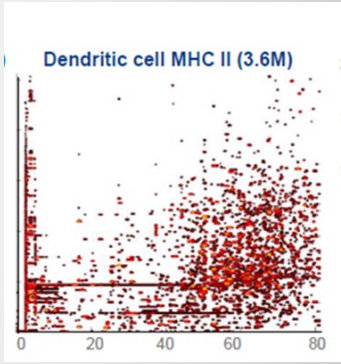
Innate Response /Early

MAPPs assay

Identification of processed and presented epitopes using MHC associated peptide proteomics





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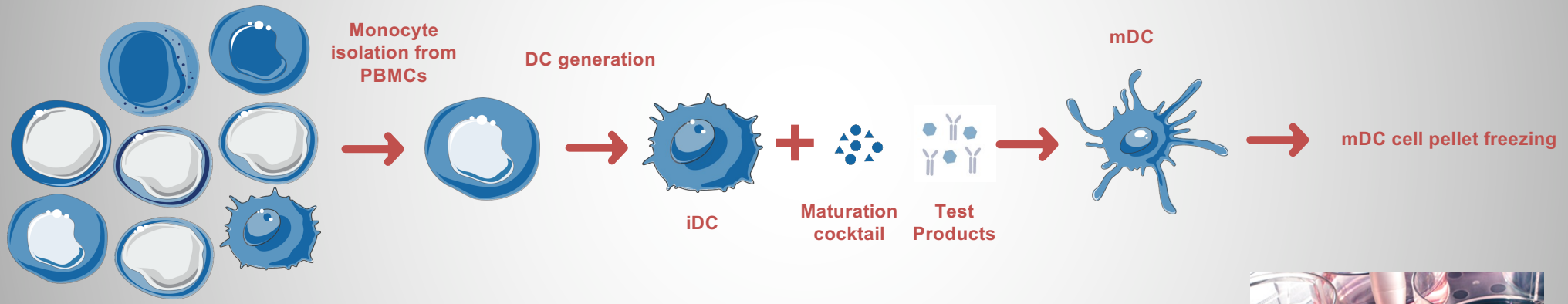
Innate Response /Early

MAPPs assay



Generation of immature dendritic cells (iDC)
Incubation with test products and maturation agent
Mature (peptide presenting) dendritic cell (mDC) cell pellet freezing

MHC-I and MHC-II setting
Multiplex MAPPS

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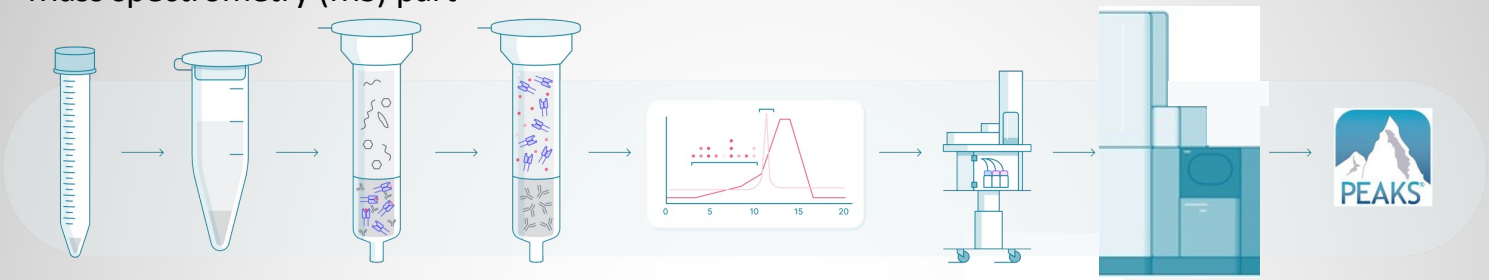
Innate Response /Early

MAPPs assay

Mass spectrometry (MS) part



1 High-outcome purification

State-of-the-art protocol for optimized solubilization • purification • extraction

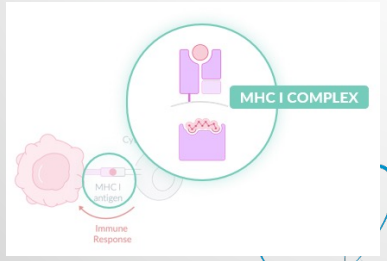
Semi-automated workflow • HT

2 Sensitive mass spectrometry

Ultraprecise mass spectrometry analysis using a TimsTOF-SCP

3 Extensive data analysis

Optimized data analysis for complete immunogenic reports.



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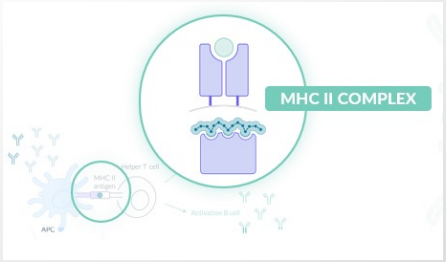

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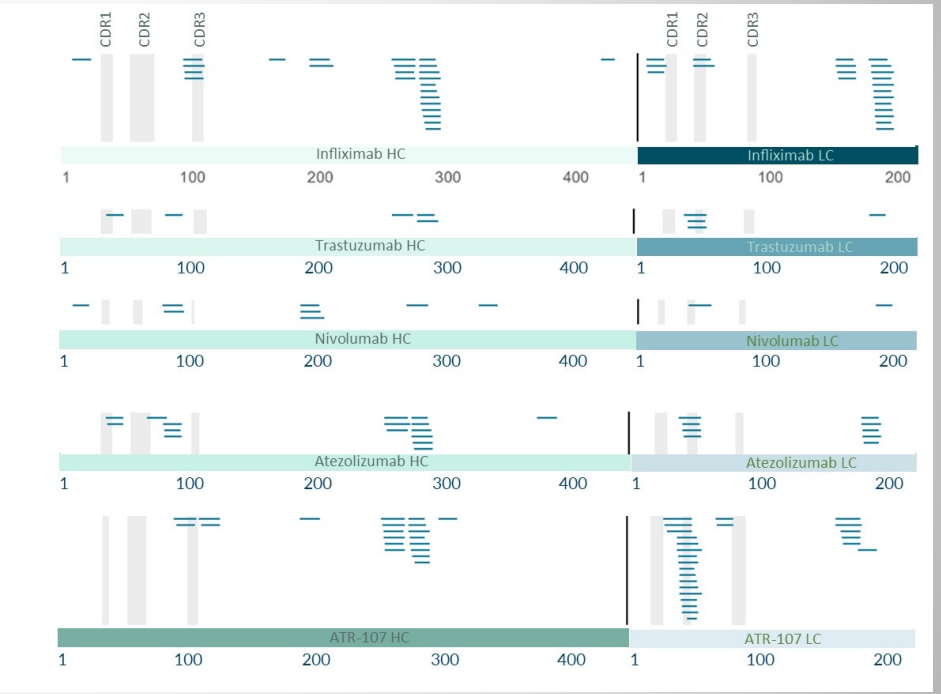
Innate Response /Early

MAPPs assay

Case Study: Identification of MHC II presented peptides from moDCs

Comparison MAPPS analysis of different biologics loaded on moDCs

	Total peptides identified (PSM)	Total different peptides	# Heavy Chain peptides	# Light Chain peptides	% Biologic peptides
Infliximab	34904	15797	25	21	0.29%
Trastuzumab	33790	15251	5	4	0.06%
Nivolumab	32250	15290	8	2	0.08%
Atezolizumab	34844	15644	16	9	0.16%
ATR107	34330	15666	20	25	0.29%
Unloaded	34846	15753	0	0	0%

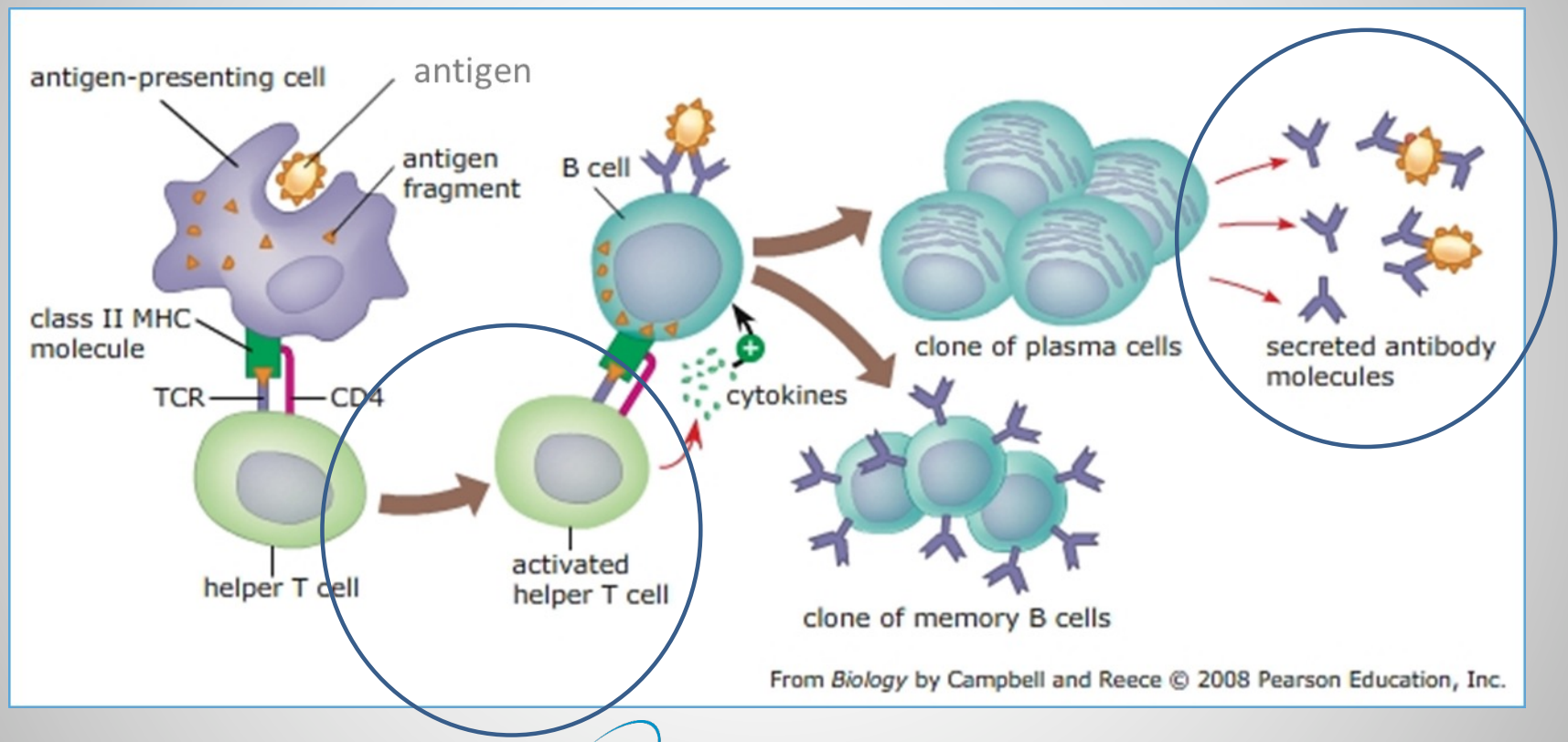


Correlation between # identified peptides and #identified clusters in MAPPS assay and immunogenicity incidence & immunogenicity risk

High-sensitivity of MAPPS assay: high-confidence immunogenic profile of test article

Adaptive response/late

T cell assays



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Adaptive response/late

Importance of donor selection

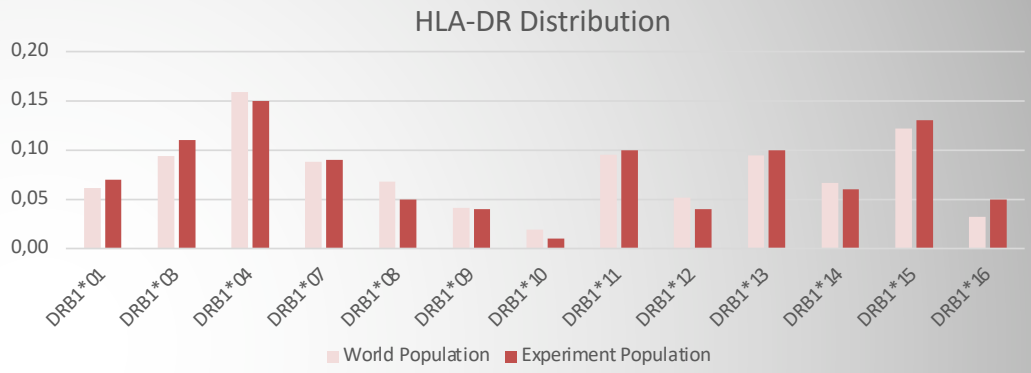
Test population should be

Representative of the population of interest

HLA coverage and distribution

Sufficiently large to ensure sensitivity

Can ensure sufficient sensitivity to detect responses in population with similar HLA distribution, also considering non-HLA related factors in healthy individuals, on in vitro response



Response rate population

n test sample	10%	20%	30%	40%
10	43%	17%	6%	2%
20	19%	3%	<1%	<1%
30	8%	<1%	<1%	<1%
40	4%	<1%	<1%	<1%
50	2%	<1%	<1%	<1%

Probability of 0 responses in test sample in function of sample size and response rate in the population, assuming 80% assay sensitivity

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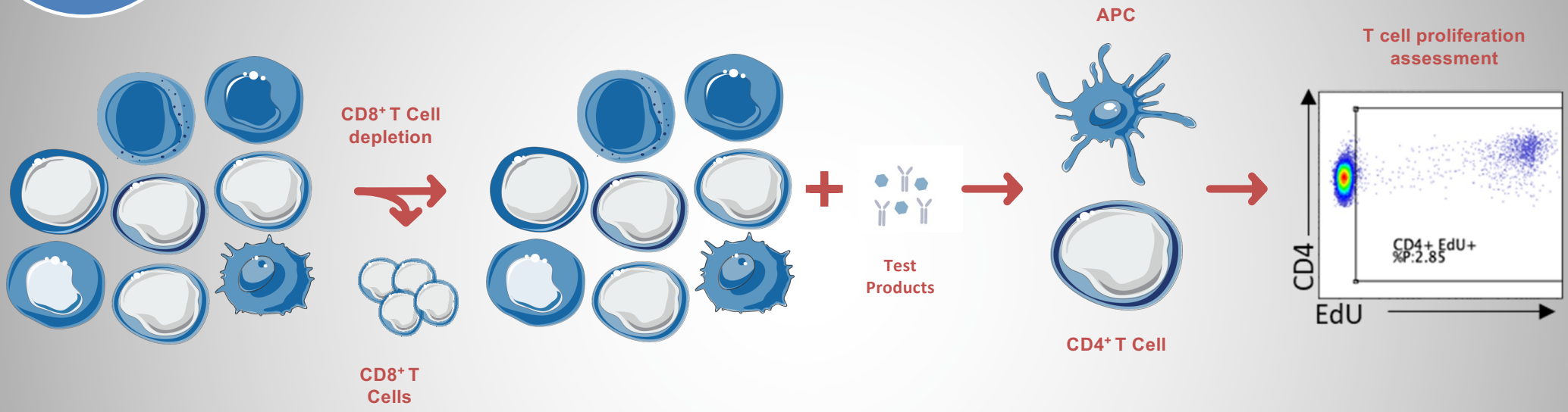
Adaptive response/late

CD8-depleted PBMC assay



T cell activation and proliferation assays to assess and compare the immunogenicity potential of test molecules

Format depends on the nature and function of the test products:

The CD8-depleted PBMC format is used for test products with **non-immuno-modulatory functions**

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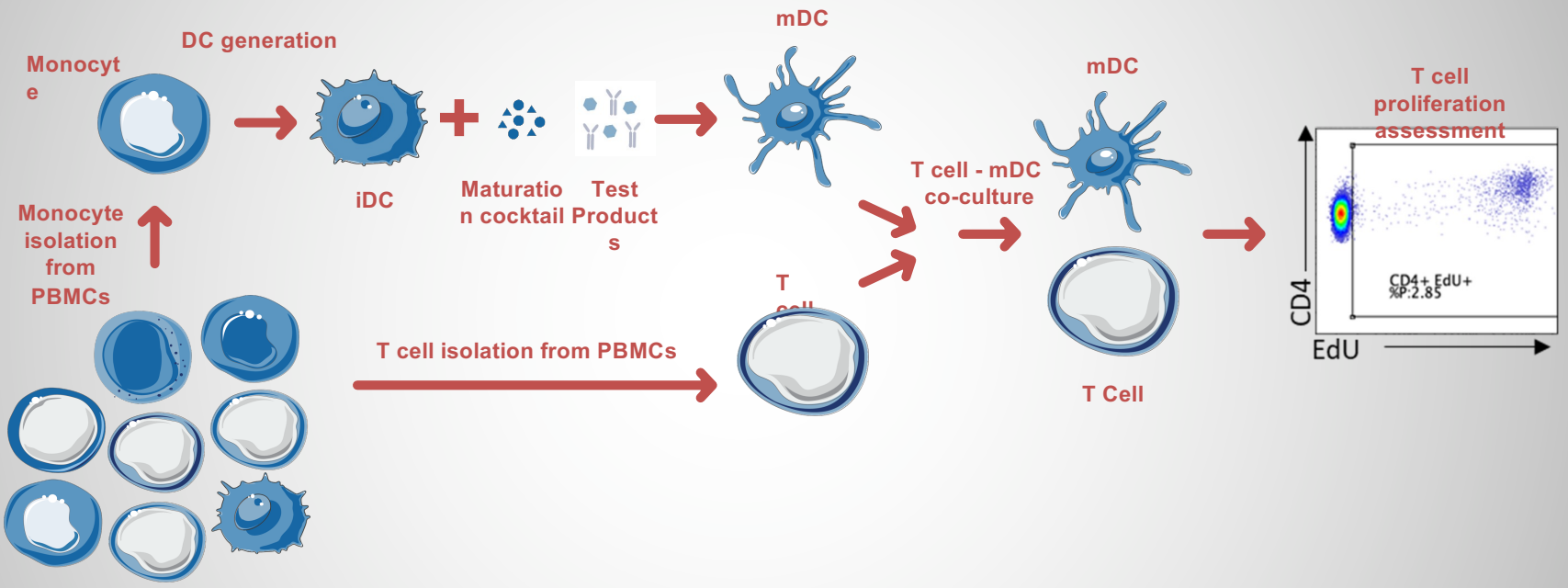
Adaptive response/late

DC:T cell assay



T cell activation and proliferation assays to assess and compare the immunogenicity potential of test molecules

Format depends on the nature and function of the test products:

DC-T cell format is used for test products with **immuno-modulatory functions**

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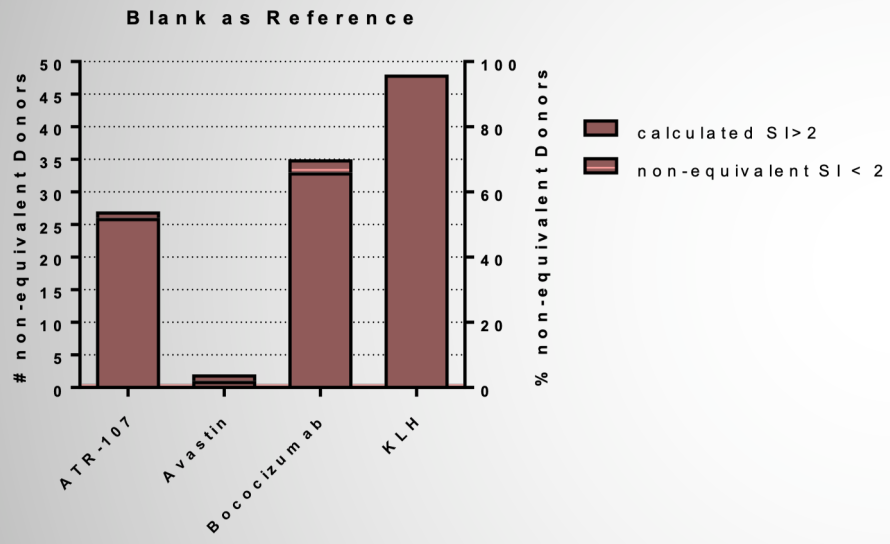
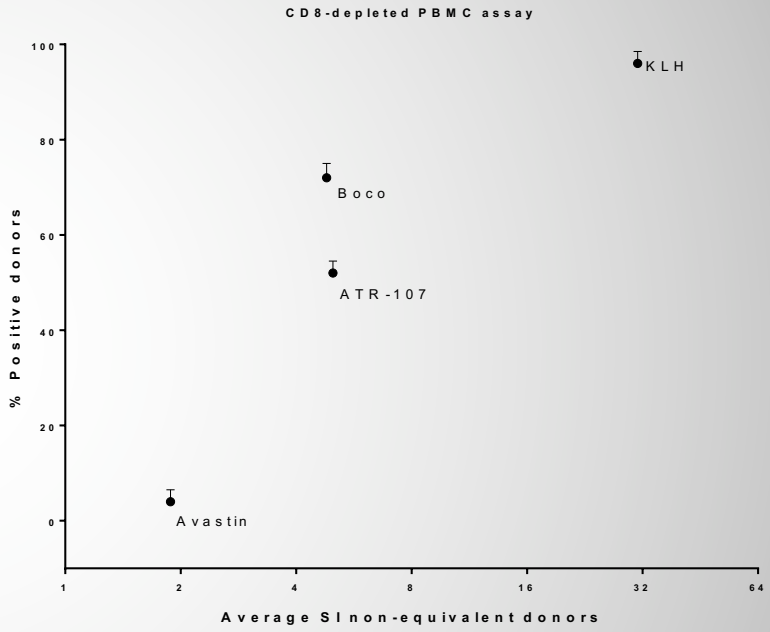
Adaptive response/late

T cell assays outcome





Per donor, a stimulation index (mean response in test condition/mean response in blank condition) is calculated

All reactions with a calculated SI > 2 are considered positive

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Correlations *in silico*, *in vitro* and *in vivo*

In silico: peptide binding

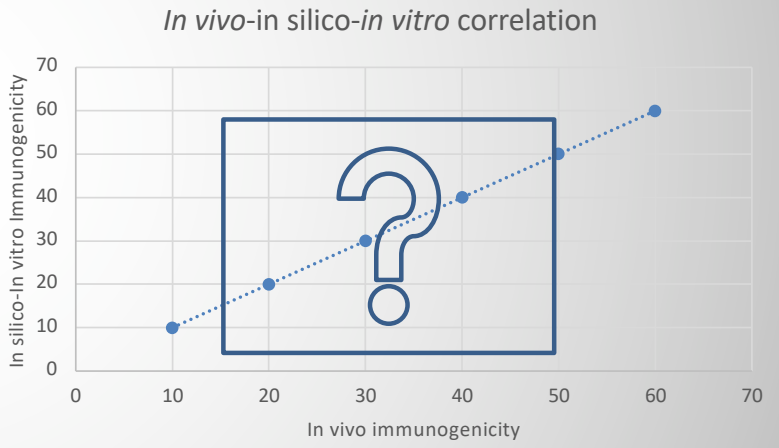
In vitro: T cell proliferation/ activation as a surrogate marker for antibody responses
50 donors

In vivo: anti-drug antibody responses
1000's of patients

Complexity of the human immune system

Unknown factors

Combination of drug-, patient and treatment-specific factors



EIP European Immunogenicity Platform

Tools to assess unwanted immunogenicity

EIP
European Immunogenicity Platform