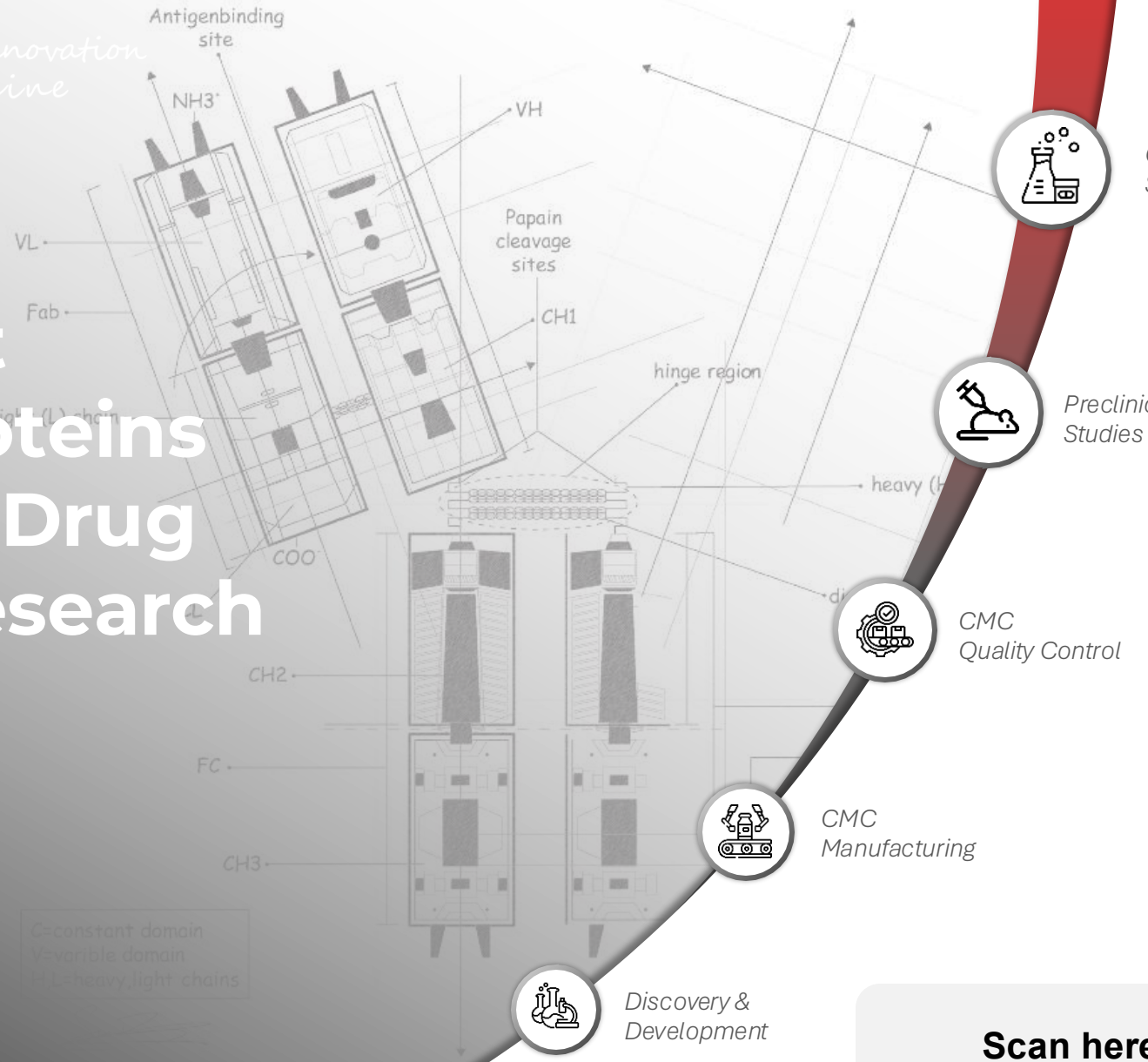
BIOSYSTEMS Acro | Where Proteins and Innovation Advance Biomedicine



Overcoming Challenges with Innovation
From Discovery to the Clinic

Clinical Studies

Preclinical Studies

CMC Quality Control

CMC Manufacturing

Discovery & Development

Designing Target Recombinant Proteins for Autoimmune Drug Discovery and Research

4th June, 2025

Ruiyuan Zheng

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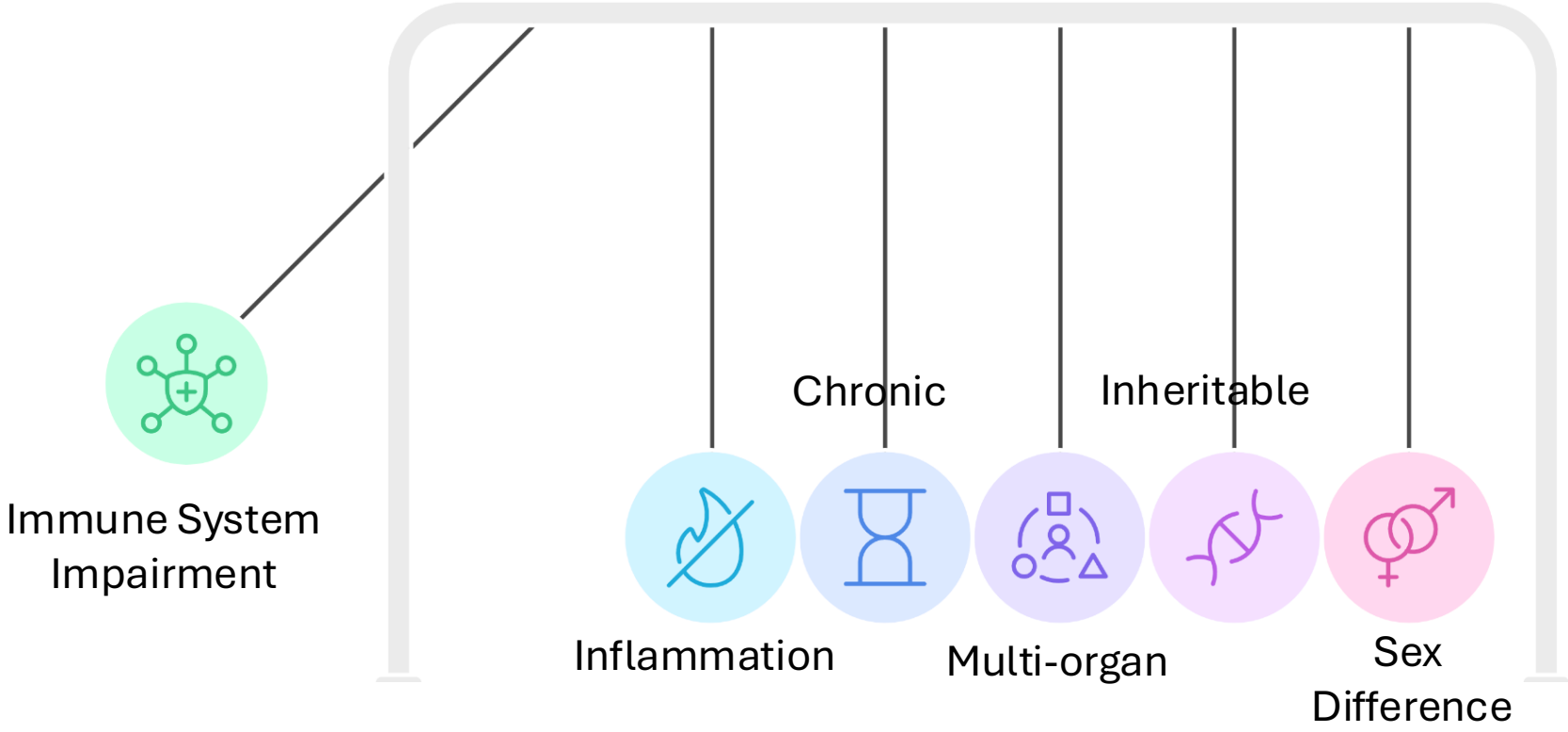
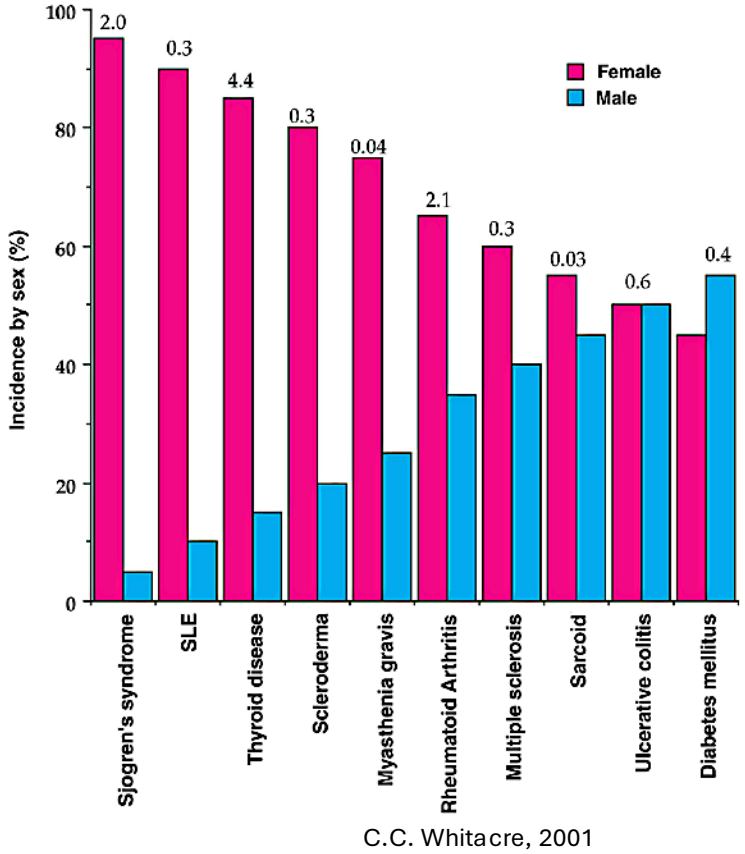
BIOSYSTEMS Acro | Where Proteins and Innovation Advance Biomedicine

CONTENT

Autoimmune Disease: A Brief Introduction

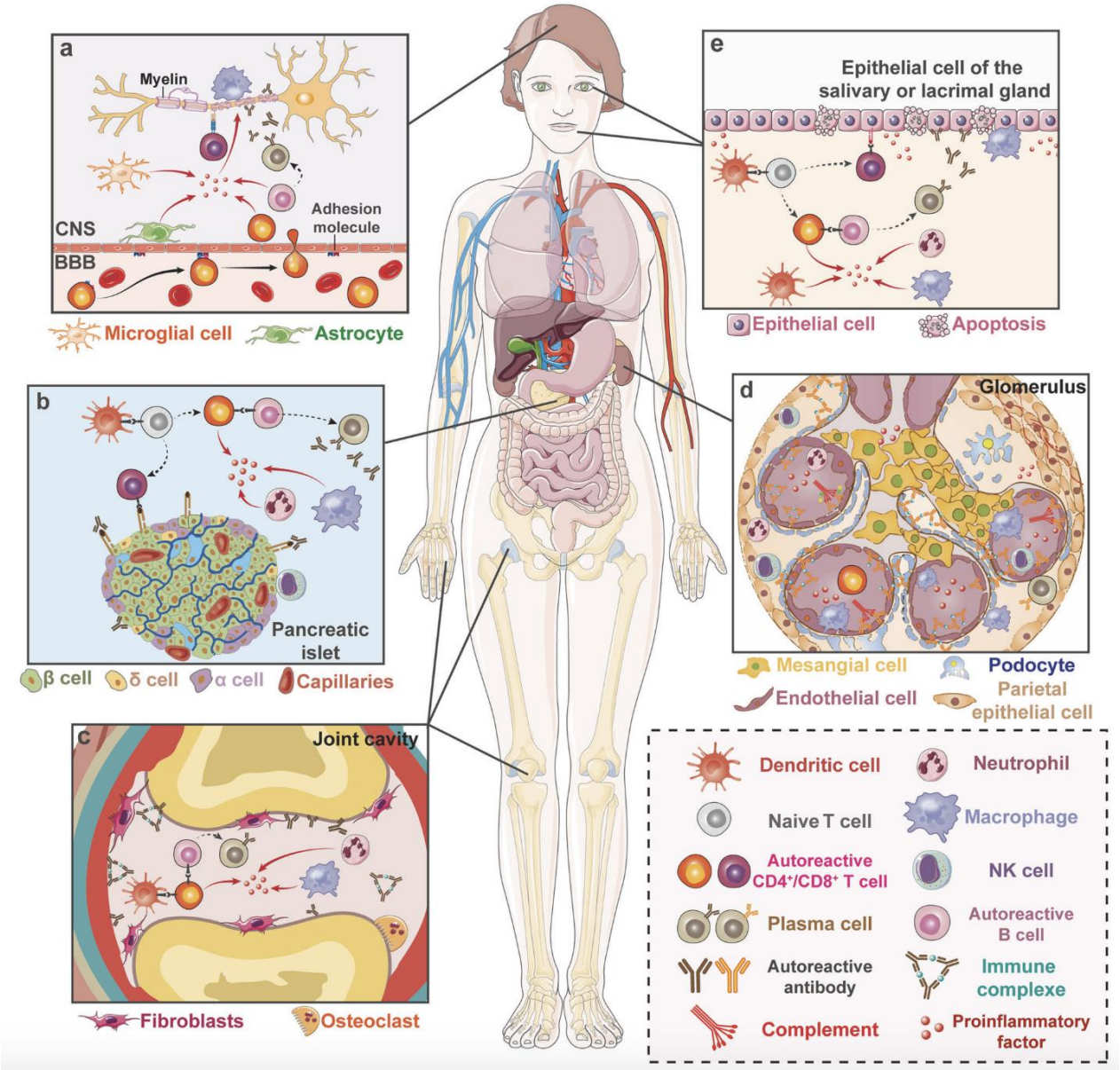
AUTOIMMUNE DISEASE: THE IMPAIRMENT AND REPAIR OF THE IMMUNE SYSTEM





C.C. Whitacre, 2001

- Autoimmune diseases have been shown to affect 3–5% of the population and become one of the most important public health problems.
- They are characterised by immune disturbances that cause the aberrant activation of autoreactive immune cells, resulting in tissue damage.



MULTIPLE ORGANS CAN BE AFFECTED AT A TIME.

A Multiple sclerosis: the brain, spinal cord, and optic nerves.

B Type I Diabetes: pancreas.

C RA: joints, lungs, heart, eyes, and skin.

D Systemic lupus erythematosus: skin, joints, kidneys, brain, and heart.

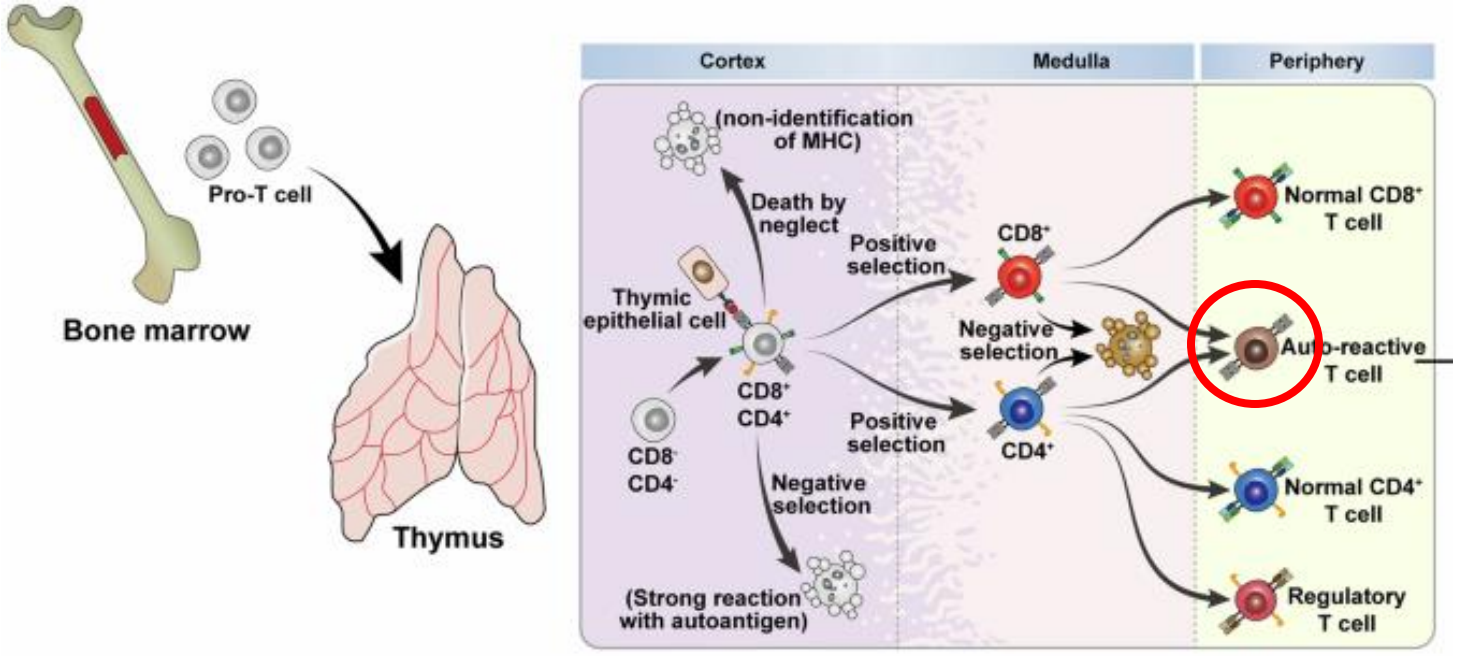
E Sjögren's syndrome: salivary and lacrimal glands, lungs, kidneys, nervous system, and skin.

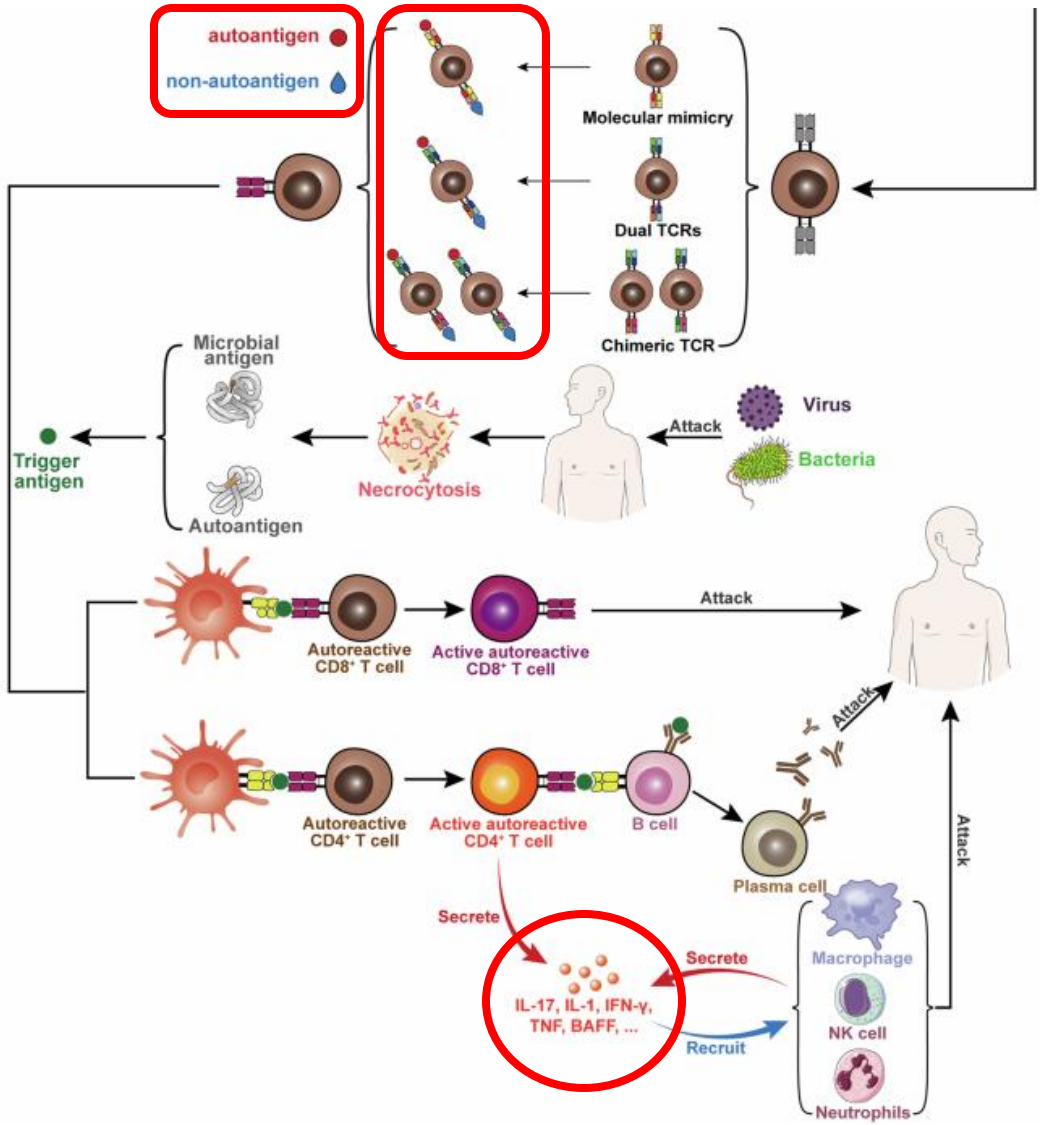
↓

How is that possible?

Y Song et al., 2024

THE MECHANISMS OF AUTOIMMUNE DISEASES

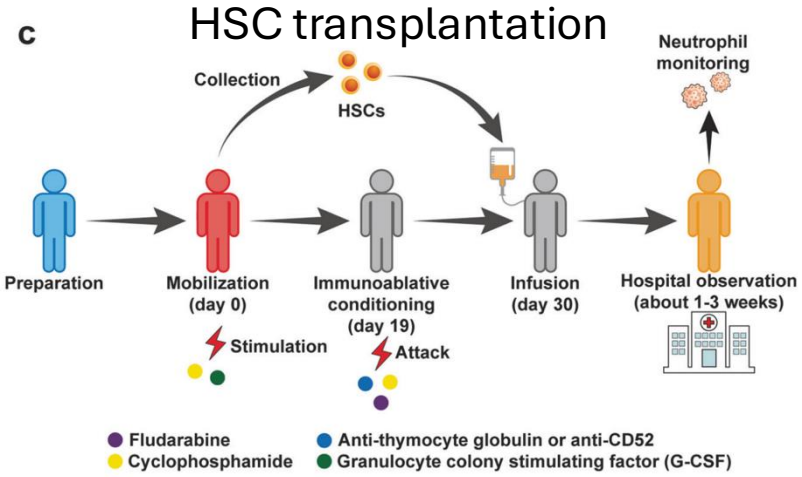


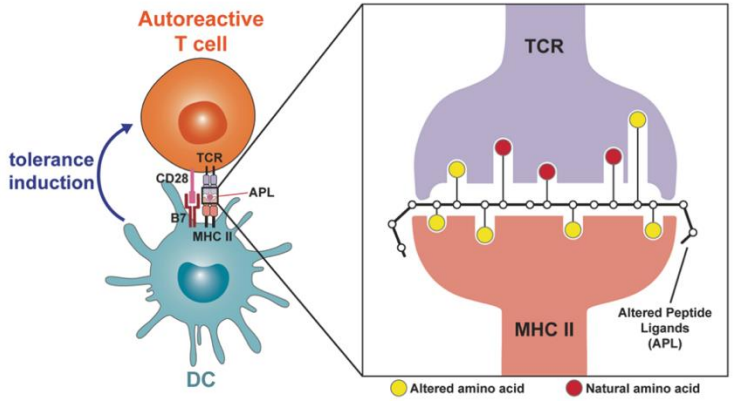


- Auto-reactive immune cells that escape the negative immune selection against autoantigens get activated by trigger antigens, and start to hunt 'self'.
- The immune reaction against 'self' gets amplified when activated auto-reactive CD4+ T cells secrete inflammatory cytokines.

Y Song et al., 2024

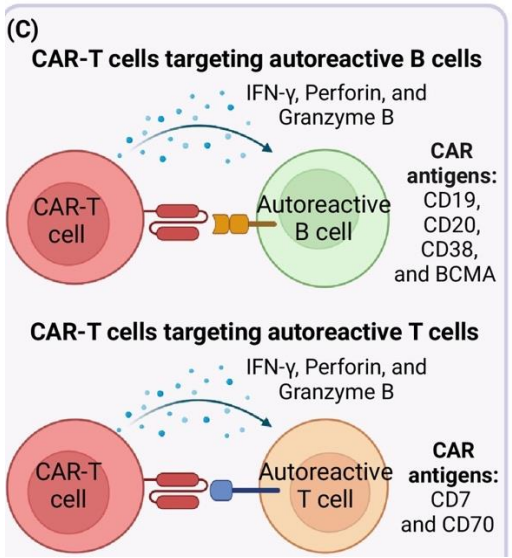
NEW THERAPEUTIC STRATEGIES FOR AUTOIMMUNE DISORDERS



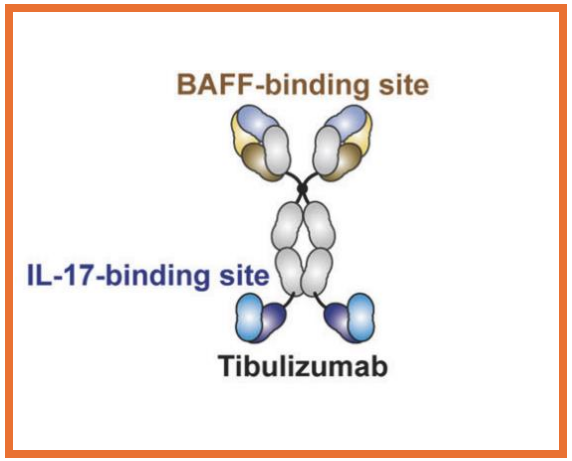


Altered Peptide Ligands

Several aa substitutions in key TCR identification positions cause obstacles in the signal transmission, affecting immune activation and inducing immune tolerance.



Y Song et al., 2024



Y r li et al., 2024

high specificity

precision

effectiveness

Most Frequently Used Solutions

From Adalimumab to FcRn Drug: Where Are We Now

FROM ADALIMUMAB TO ROSLINIMAB: THE WAY TO TARGETING OUR OWN IMMUNE SYSTEM.

1950s: Early use of immunosuppressants (e.g., corticosteroids)

1998: Approval of Etanercept and Infliximab for Crohn's disease and RA

2002: Approval of Adalimumab: first fully human anti-TNF mAb

2008: Approval of Tocilizumab targeting IL-6 receptor

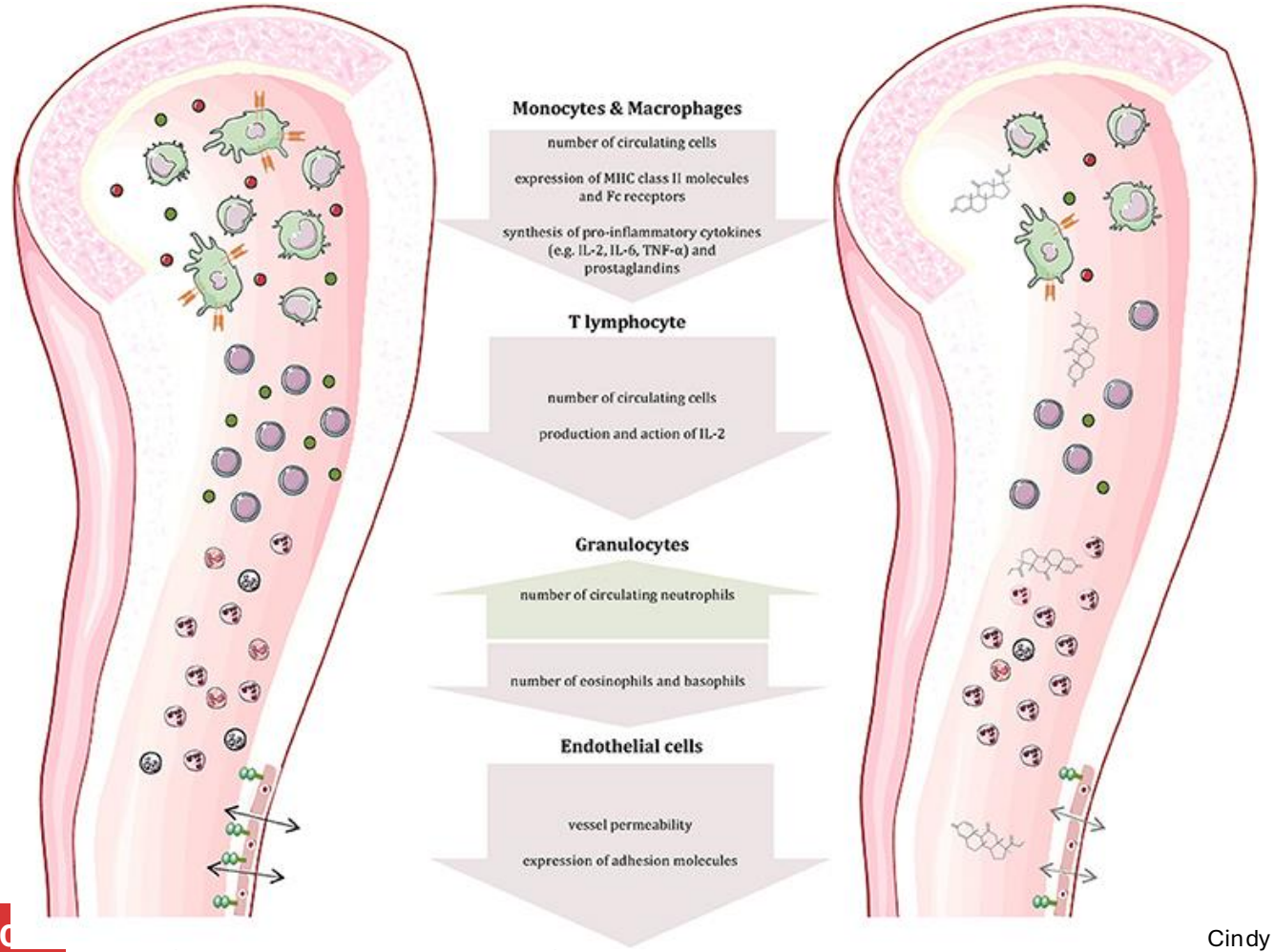
2015: Approval of Secukinumab targeting IL-17

2024: CAR-T therapies explored for autoimmune disorders

2025: Novel FcRn targeting therapies (e.g., Efgartigimod) show promise

THE STRONG IMMUNOSUPPRESSIVE EFFECT OF GLUCOCORTICOIDS AND THEIR SIDE-EFFECTS

Glucocorticoids affect the number and function of immune cells.



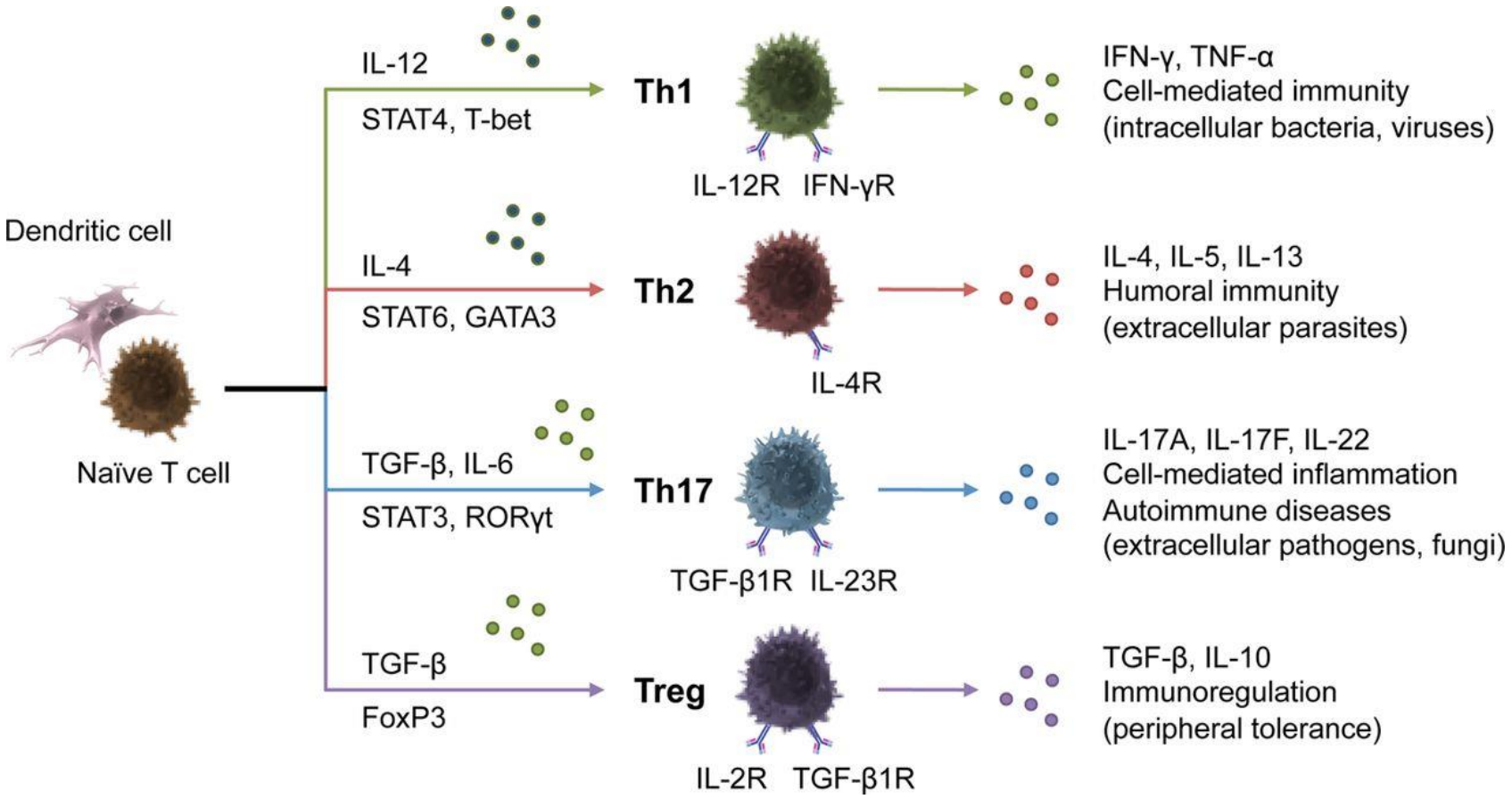
However...

- Casing swelling
- High blood pressure
- psychological effects
- Upset stomach
- Weight gain

Safer & More Effective Solutions Required

Cindy S et al., 2019

TARGETED DRUGS: ADALIMUMAB'S ADVANTAGES AND DISADVANTAGES

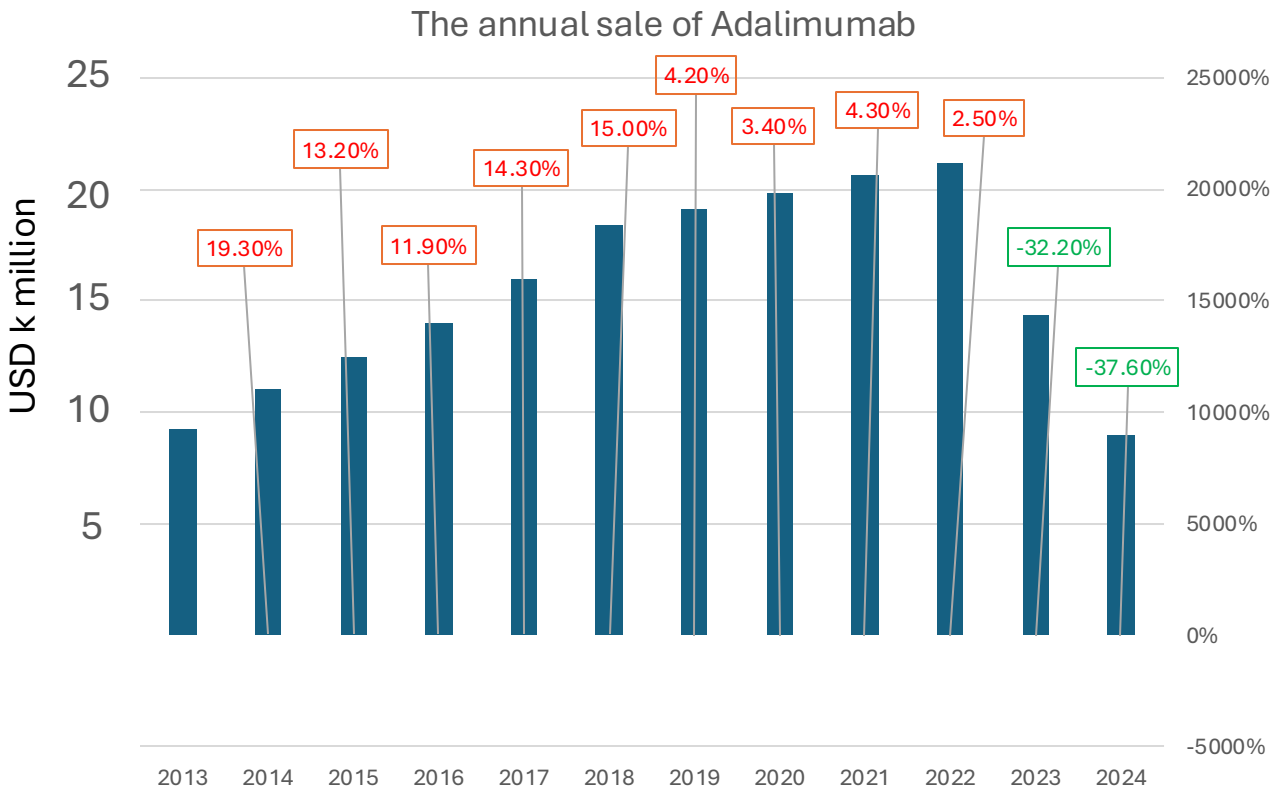


Dhavalkumar D P et al., 2012

- Distinct T-cell subsets drive the pathogenesis of autoimmune diseases.
- IL-12 promotes Th1 cells, which secrete IFN-γ and TNF-α.
- IL-6 promotes Th17 cells, which secrete IL-17A, IL-17F, and IL-22.
- Because each subset controls specific immunological pathways and is linked to particular autoimmune disorders, selectively targeting their signature cytokines offers a more precise therapeutic approach.

BIOLOGICS AGE: ADALIMUMAB'S ADVANTAGES AND DISADVANTAGES

Why do we need more targets beyond TNF-α?



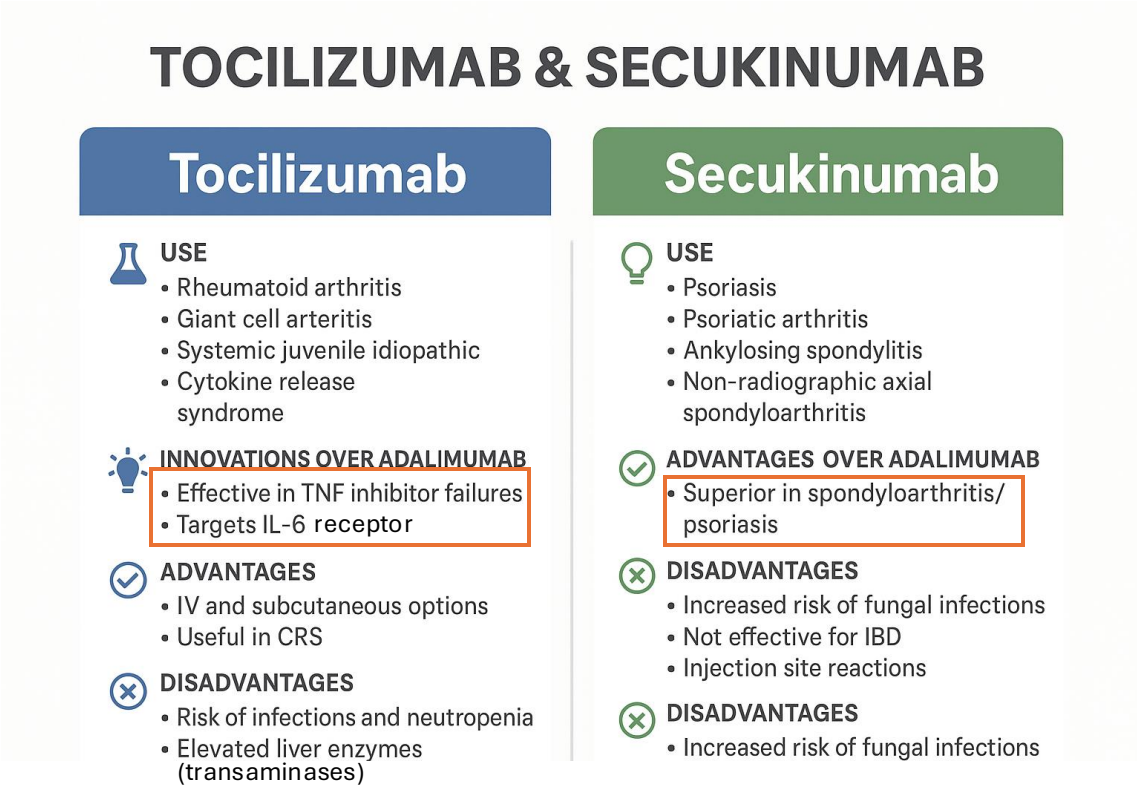
Advantages

- Effective across multiple autoimmune diseases
- Reduces inflammation quickly
- Long-term clinical experience

Disadvantages

- Immunogeneicity
- Not effective in all patients
- Autoantibody development
- Risk of infections

THE RISE OF INTERLEUKIN TARGETS: TOCILIZUMAB & SECUKINUMAB



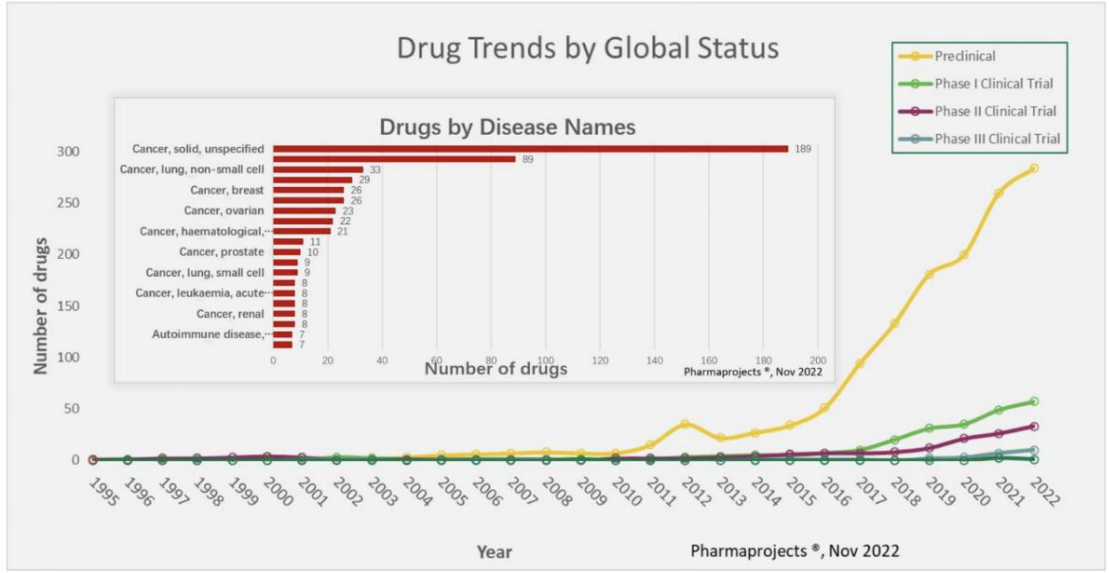
Drug	Target	Target Disease
Adalimumab	TNF-α	Broad inflammatory cytokine – central in many autoimmune diseases
Tocilizumab	IL-6 receptor	Key cytokine in RA, CRS, and systemic inflammation
Secukinumab	IL-17A	Central in psoriasis and spondyloarthropathies

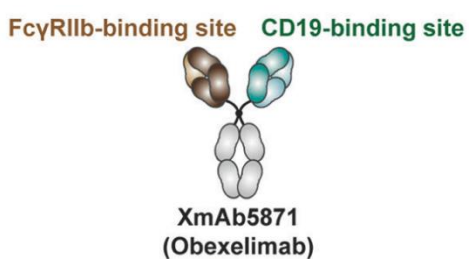
Yuji Y et al., 2014; Fcharztmagazine 2020; Dennis G M et al., 2019

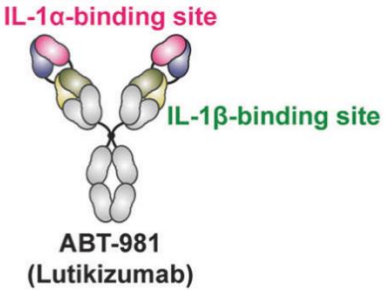
- Tocilizumab and Secukinumab offer greater specificity, alternative mechanisms of action, and effectiveness in TNF-refractory cases.

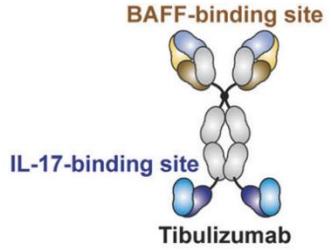
THE DEVELOPMENT OF BISPECIFIC mAb DRUGS

Rising trend of bispecific mAb drug development









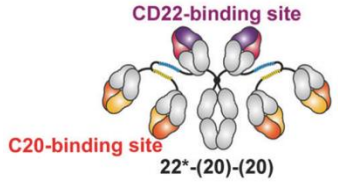
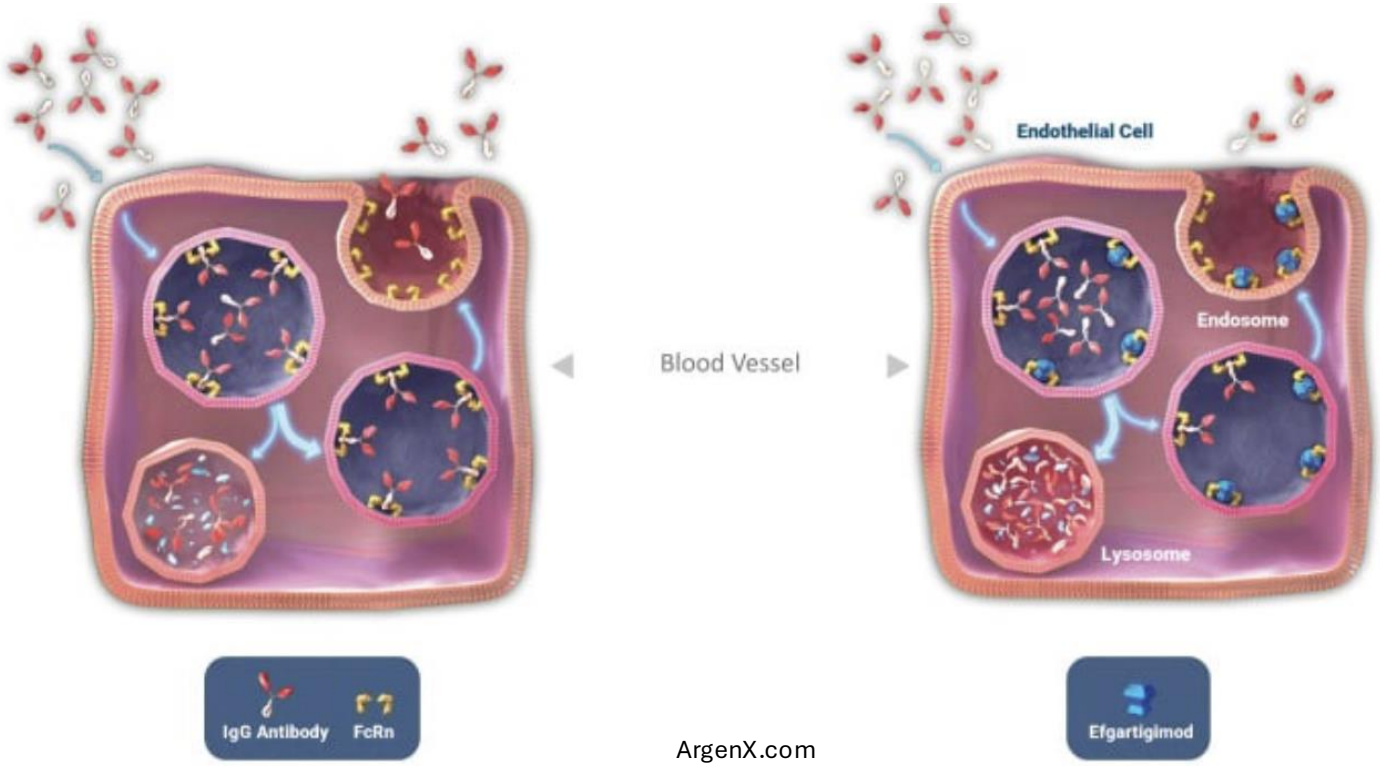


Figure 1. Trends from 1995 to 2022 for the development of bsAb drugs across the globe. (insert) bsAb target disease categories.

- **Dual targeting improves specificity and efficacy**, allowing simultaneous modulation of two disease-relevant pathways.
- **Potential to reduce immune overactivation more precisely** than single-target agents.
- **Lower doses may be effective**, reducing toxicity and improving safety.

FcRn DRUGS TO REDUCE AUTOANTIBODIES

Taking Efgartigimod as an example



ArgenX.com

Efgartigimod is a neonatal Fc receptor (FcRn) antagonist. It reduces the recycling of IgG and thereby lowers. pathogenic autoantibodies

The administration of Efgartigimod leads to a rapid and selective reduction in IgG levels without affecting other immunoglobulins or immune cells.

The mAB Drug Discovery & Manufacturing Pipeline

THERAPEUTIC mAb DISCOVERY WORKFLOW

1. Target Identification & Validation

•Select a disease-relevant antigen (e.g., receptor, cytokine).

•Validate its role using genomics, proteomics, or functional assays.

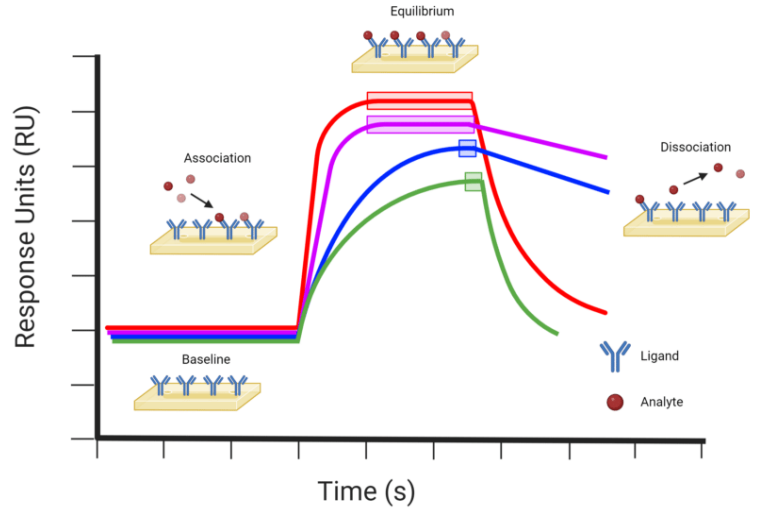
2. Immunisation / Antibody Generation

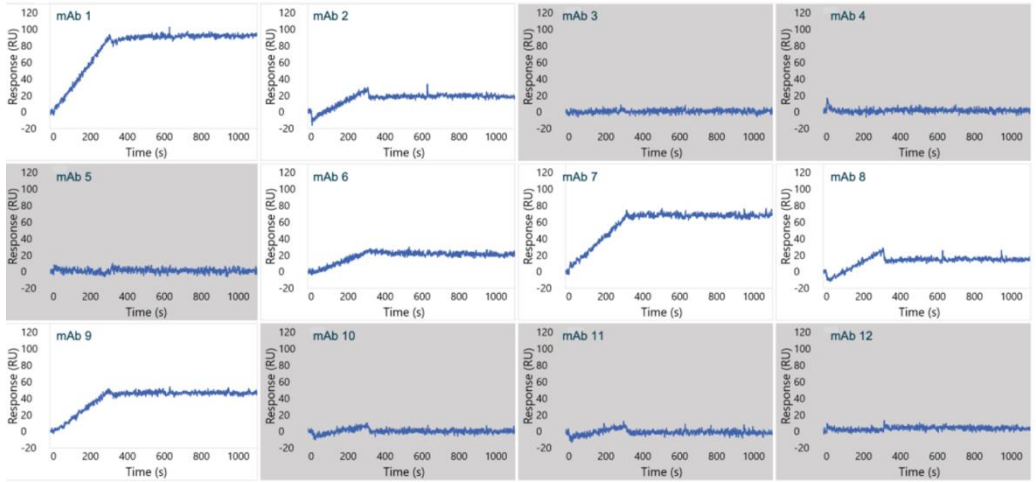
•You can immunise animals (typically mice) with the target antigen or use phage display libraries to generate a wide variety of antibody candidates.

3. Screening & Selection

•Use ELISA, flow cytometry, or high-throughput platforms to identify high-affinity antibodies specific to the target.

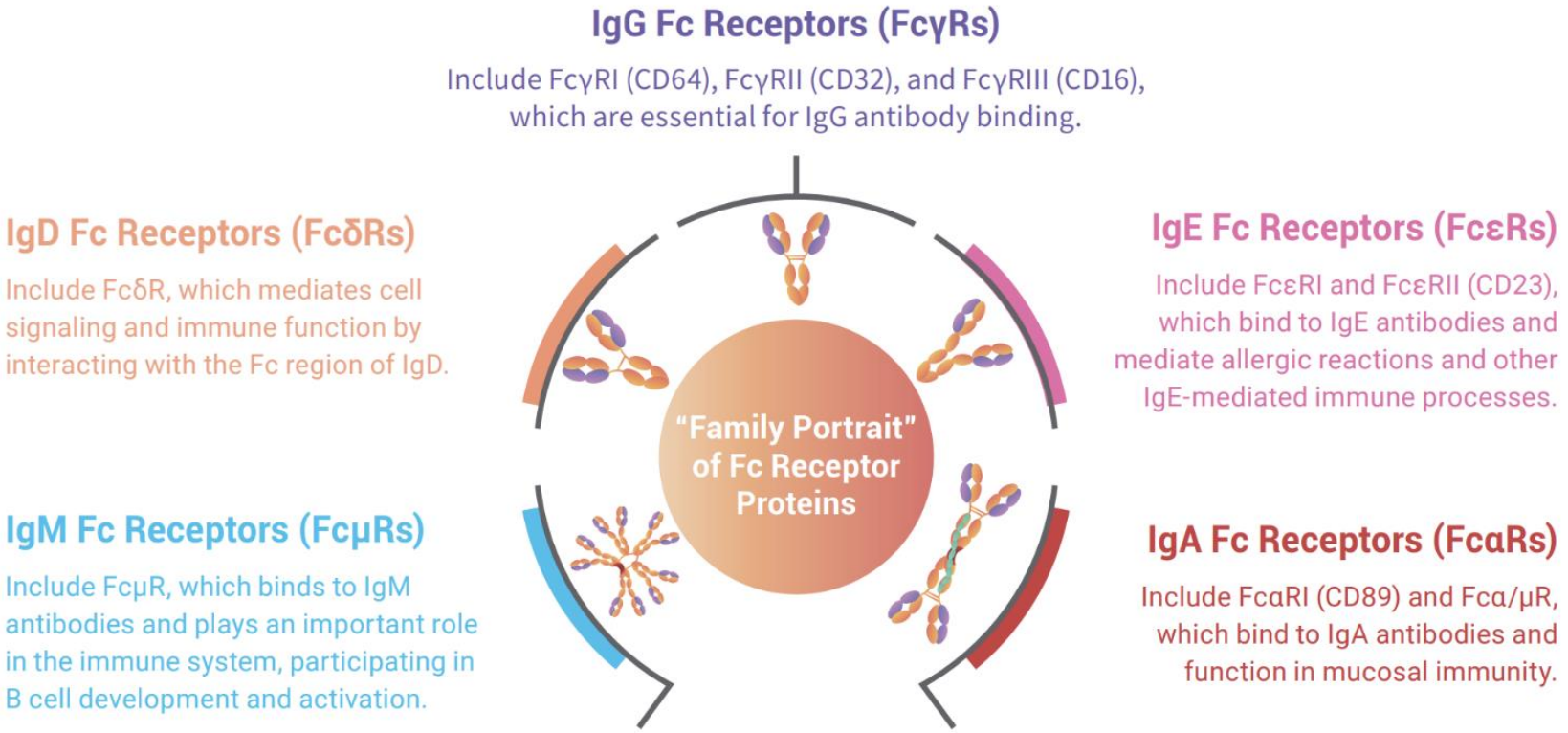
Real-life example 1: Screening of Therapeutic Candidates against CCR5 using HT-SPR





The figure shows representative sensorgram profiles of mAbs binding to CCR5. Note the avidity-driven kinetics exhibited by the very slow dissociation rate, and the sensitivity presented by the lack of binding in certain clones shown in the grey panels.

CHECKING THE EFFECT OF THE ANTIBODY DRUGS WITH SPECIFIC BINDING TESTS



The effects of antibody drugs often depend on the interaction of their Fc segment with Fc receptor proteins on target cells. This interaction can trigger a series of signal transduction processes such as antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC), thus achieving the biological functions of antibody drugs such as immunomodulation and cell killing.

REAL-LIFE EXAMPLE 3: HT-SPR EVALUATION OF FC-GAMMA RECEPTORS (FcγRs) BINDING

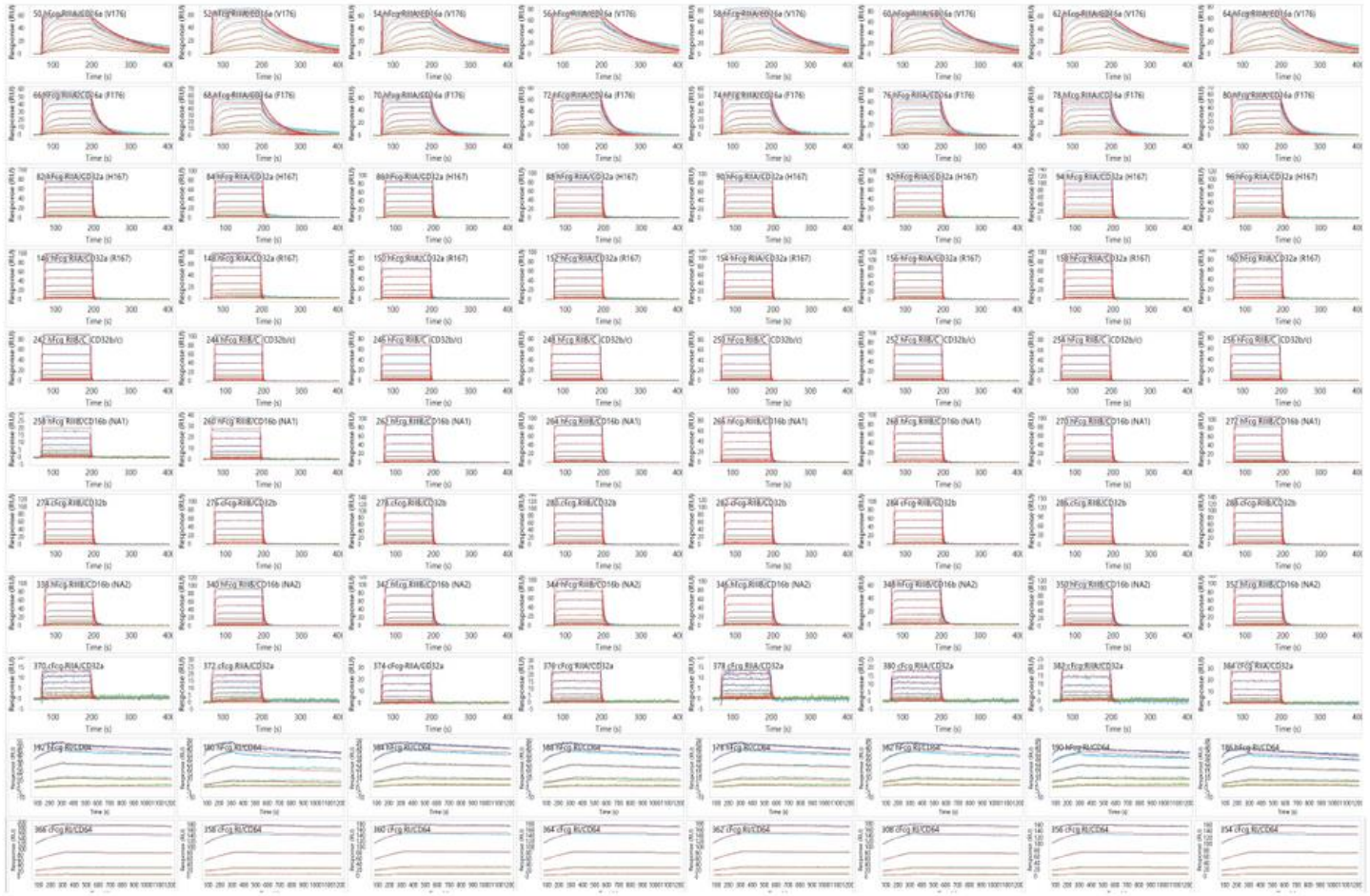


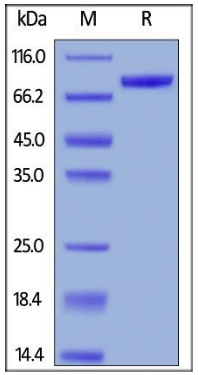
Figure 3. Binding profiles of trastuzumab against a panel of 11 FcγRs with 8 replicates of each.

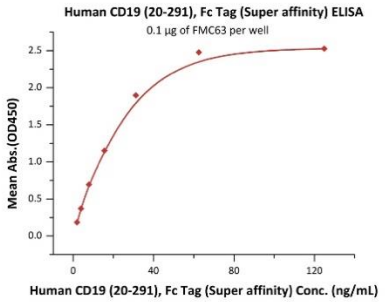
- FcγRs act as the primary method of cell signalling between IgG antibodies and our immune systems.
- The figure illustrates HT-SPR data uniformity across replicate FcγRs for both slow and rapid interactions, demonstrating how high-quality reagents and robust assay design can result in highly confident measurements.

RECOMBINANT PROTEIN ANALYTICAL METHODOLOGY

High Purity

>90% as determined by SDS-PAGE.



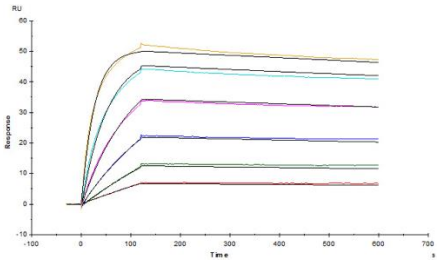


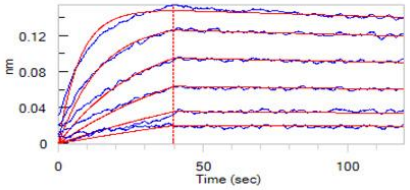
Verification by ELISA

Immobilized FMC63 at 1 μg/mL (100 μL/well) can bind Human CD19 (20-291), Fc Tag with a linear range of 1-31 ng/mL

Affinity by SPR

Human CD19 (20-291), Fc Tag can bind FMC63 MAb (mouse IgG2a) with an affinity constant of 0.17 nM as determined in a SPR assay (Biacore T200).



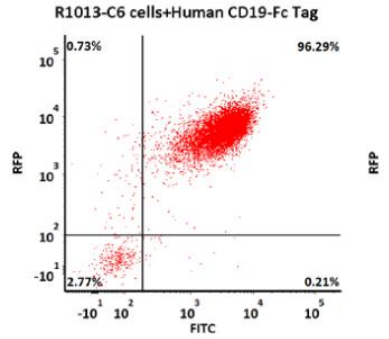


Affinity by BLI

Human CD19 (20-291), Fc Tag can bind MC63 MAb (mouse IgG2a) with an affinity constant of 0.483 nM as determined in BLI assay (ForteBio Octet Red96e).

Binding Activity

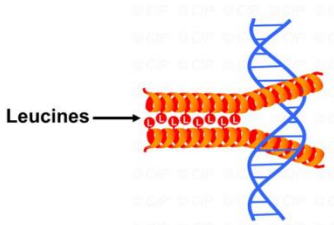
The data showed that the expression level of anti-CD19 scFv on the surface of anti-CD19-CAR 293 cells was 96.29%.



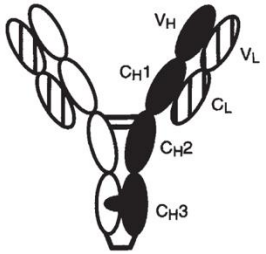
- **Verified by ELISA, SPR, BLI, etc.**
- **High Sensitivity & Specificity**
- **Stable with lot-to-lot consistency**
- **Near-native or native Protein Conformations**

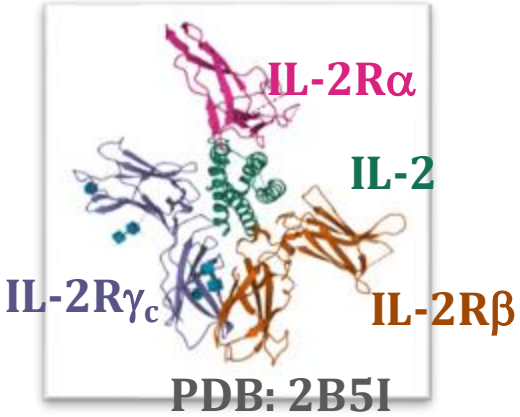
REAL-LIFE EXAMPLE 2: IL-2 RECEPTOR COMPLEX

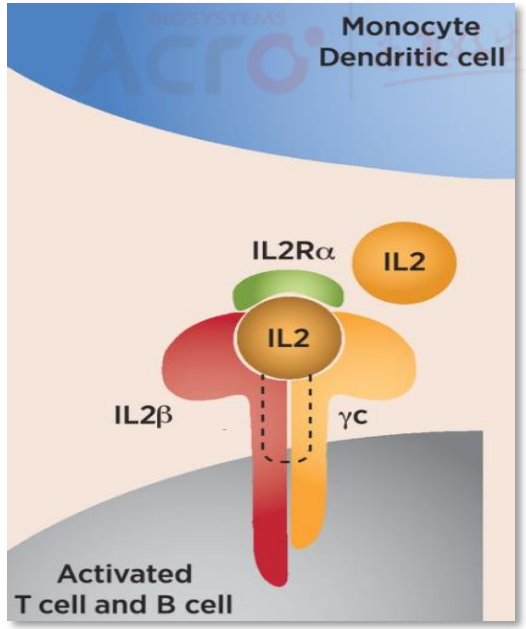
Leucine zipper



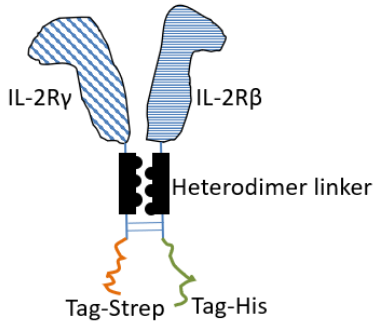
Fc-Knob into hole



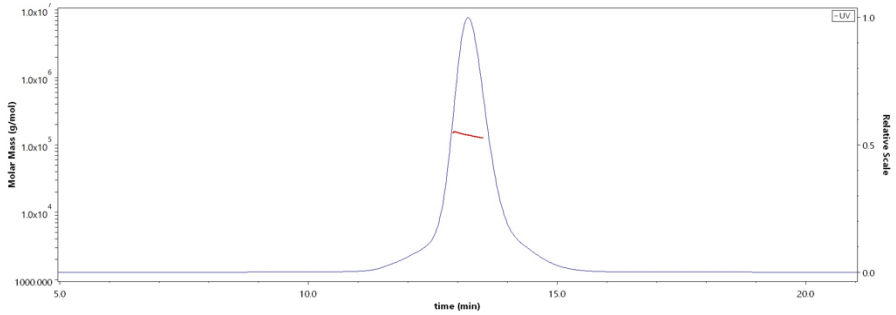




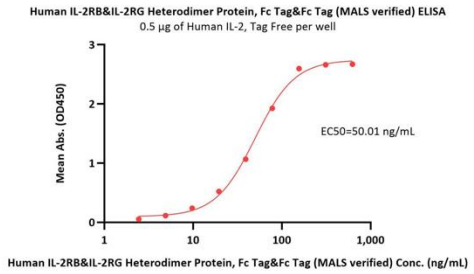
IL-2 R beta & IL-2 R gamma Heterodimer



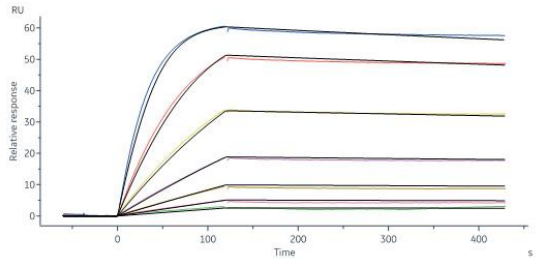
High-Performance Liquid Chromatography



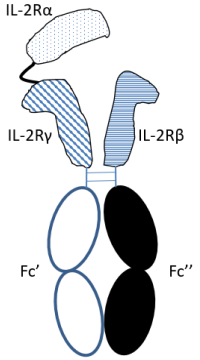
ELISA

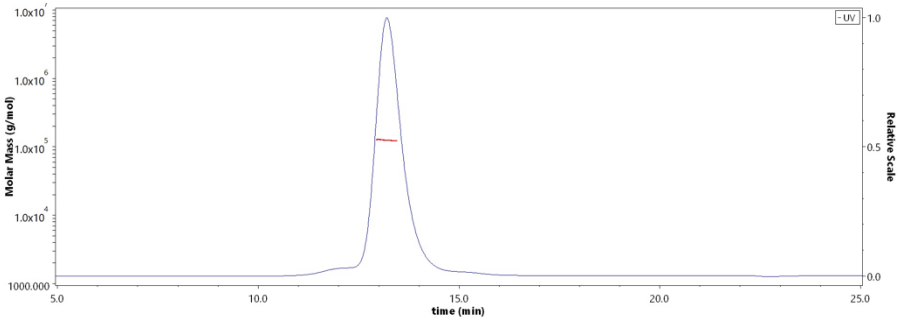


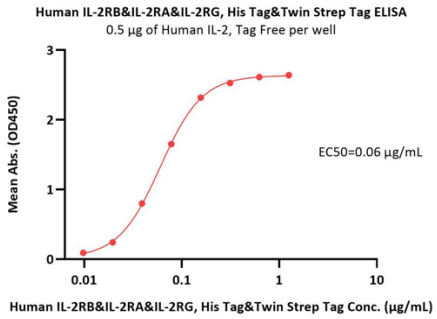
SPR

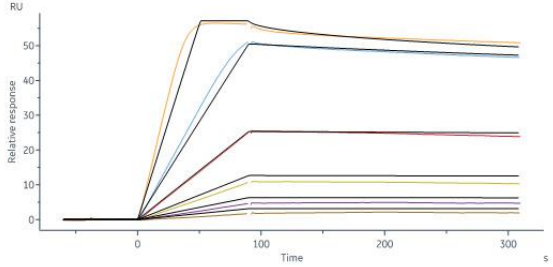


IL-2 R beta & IL-2 R alpha & IL-2 R gamma



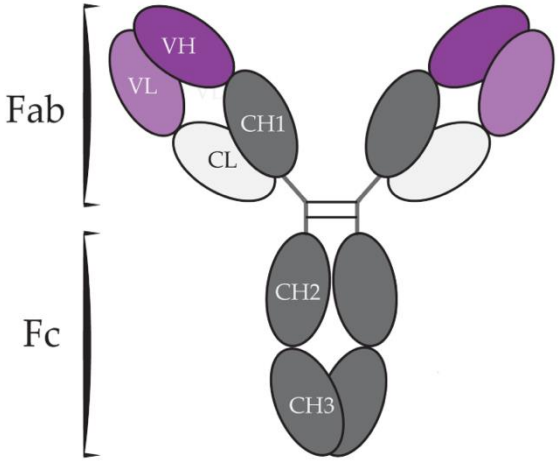






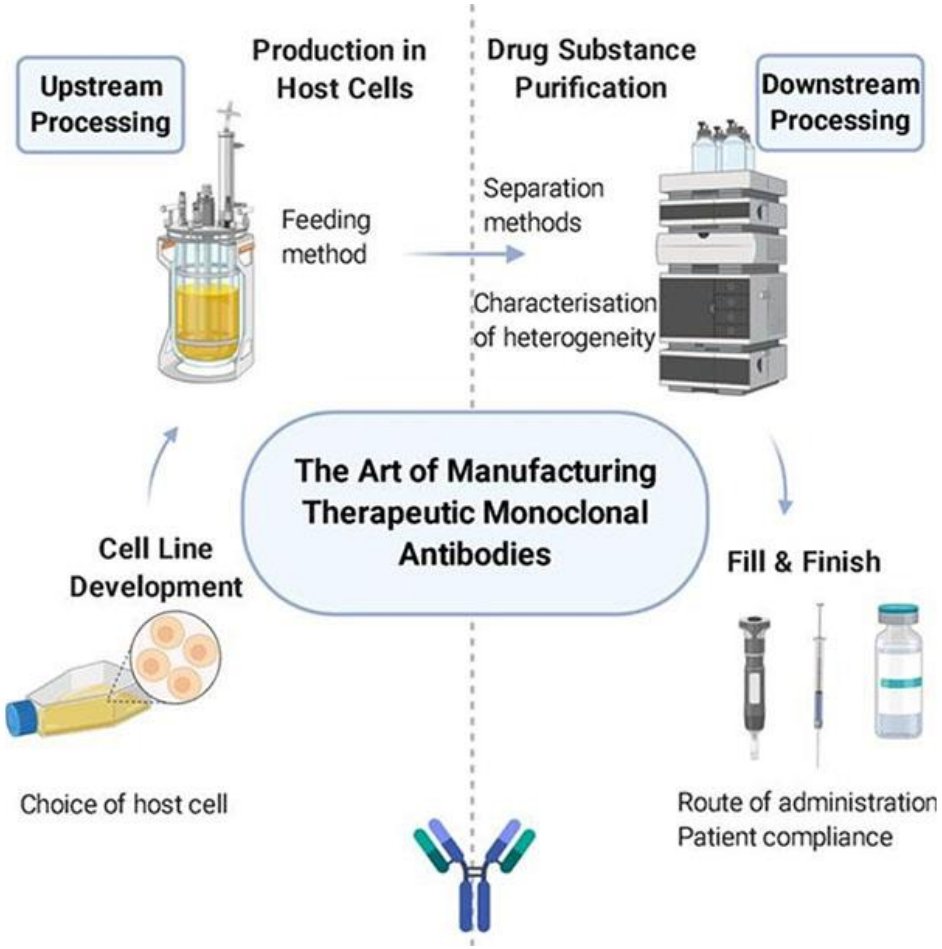
IL-2受体复合物及其制备方法与应用, CN112553256B

THE INTACT PROCESS OF THERAPEUTIC ANTIBODY PRODUCTION STARTS WITH CELL LINE OPTIMISATION



naked whole mAb

A whole mAb consists of an antigen-binding fragment and a crystallisable fragment.



Stefania C. Carrara et al., 2020

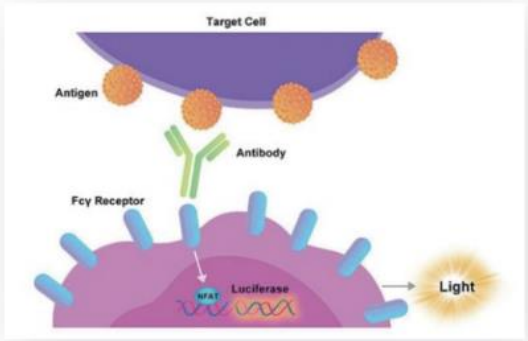
USE FUNCTIONAL CELL LINES FOR QUALITY CONTROL

Generate plasmid carrying the designed antibody → Introduce plasmid into host cells → Identify high-expression clonal cell lines → Select cell lines maintaining high levels of expression through scale-up and bioreactor processes

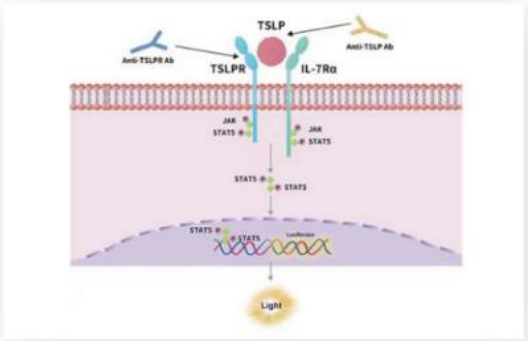
M M Zhu et al., 2017

The use of reporter cell lines for production evaluation

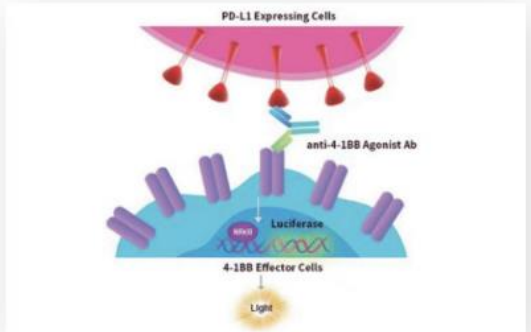
ADCC/ADCP Functional Valification



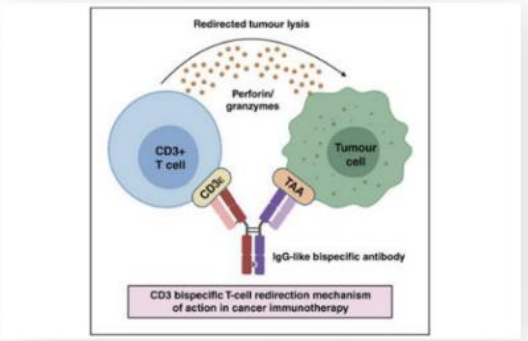
Target & Transduction Research



Cross-linking Drug Evaluation

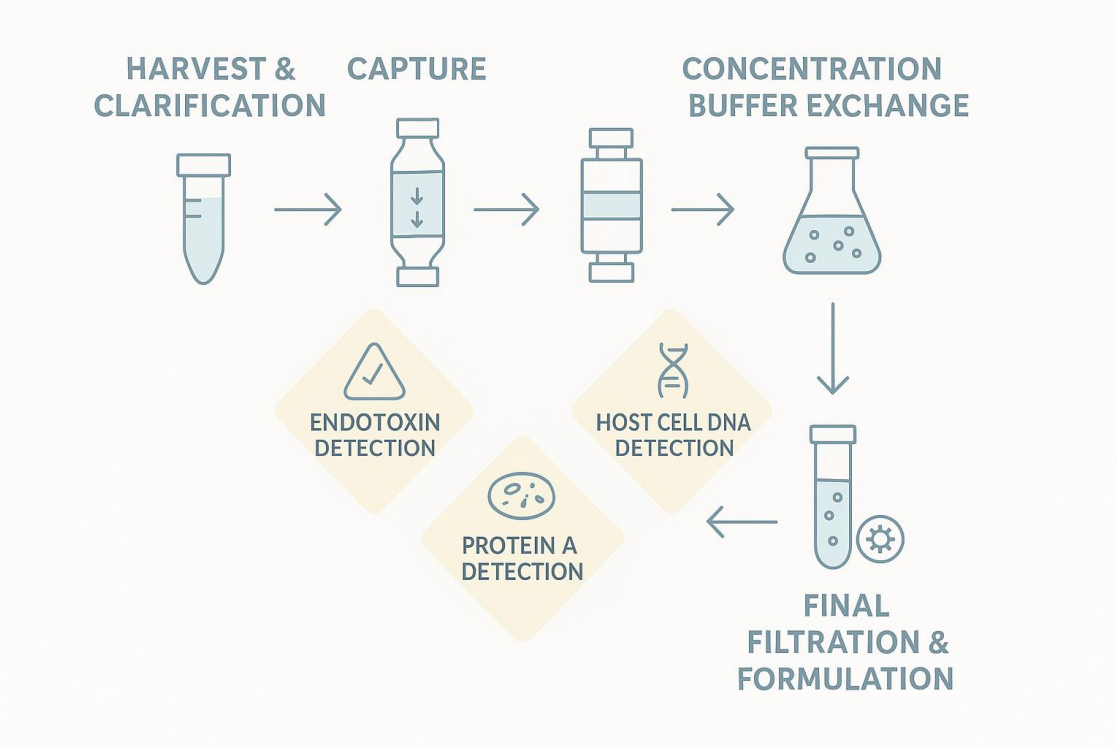


CD3/TAA BsAb Evaluation



DOWNSTREAM PURIFICATION & QUALITY CONTROL

Purification & saftey tests



- The downstream process of therapeutic antibody production includes harvest, protein A affinity capture, polishing chromatography, ultrafiltration/diafiltration, and sterile filtration before final formulation and fill-finish.
- Core purification steps (e.g., Harvest, Capture, Polishing, UF/DF, Filtration)
- Critical analytical/detection checkpoints:
  - ✅ Endotoxin Detection
  - ✅ Host Cell DNA Detection
  - ✅ Protein A Detection
  - ✅ HCP Detection
  - ✅ Viral Clearance

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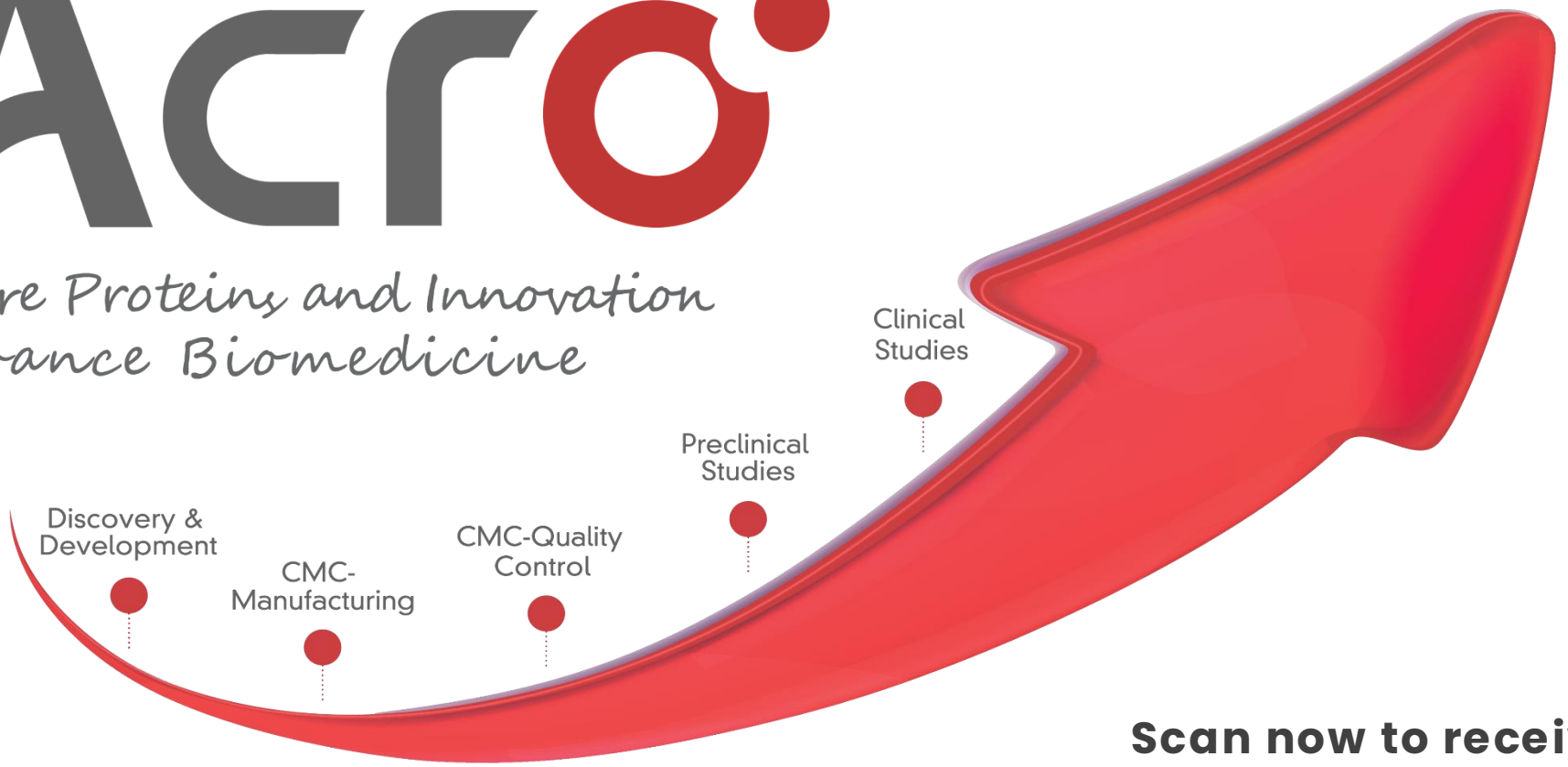

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