Trends in chemical biology and drug discovery: Biologics

Markus Enzelberger

My protein journey

1990-1997

Universität Stuttgart

Study:
Chemistry
Technical
Biochemistry

1997-1999

BASF

PhD:
Directed evolution of enzymes for chiral synthesis of epoxides

1999-2001

California Institute of Technology 1891

Postdoc:
(Francis Arnold/Steve Quake)
uHTS microfluididc screening of large protein libraries

2001-2002

Fluidigm

Senior Scientist:
Microfluidic HT crystallization of protein variants

2002-2020

morphosys

CSO:

- Development of the HuCAL library a fully synthetic human combinatorial antibody library
- >100 therapeutic antibody projects
- Number of clinical and marketed antibodies

2020-2025

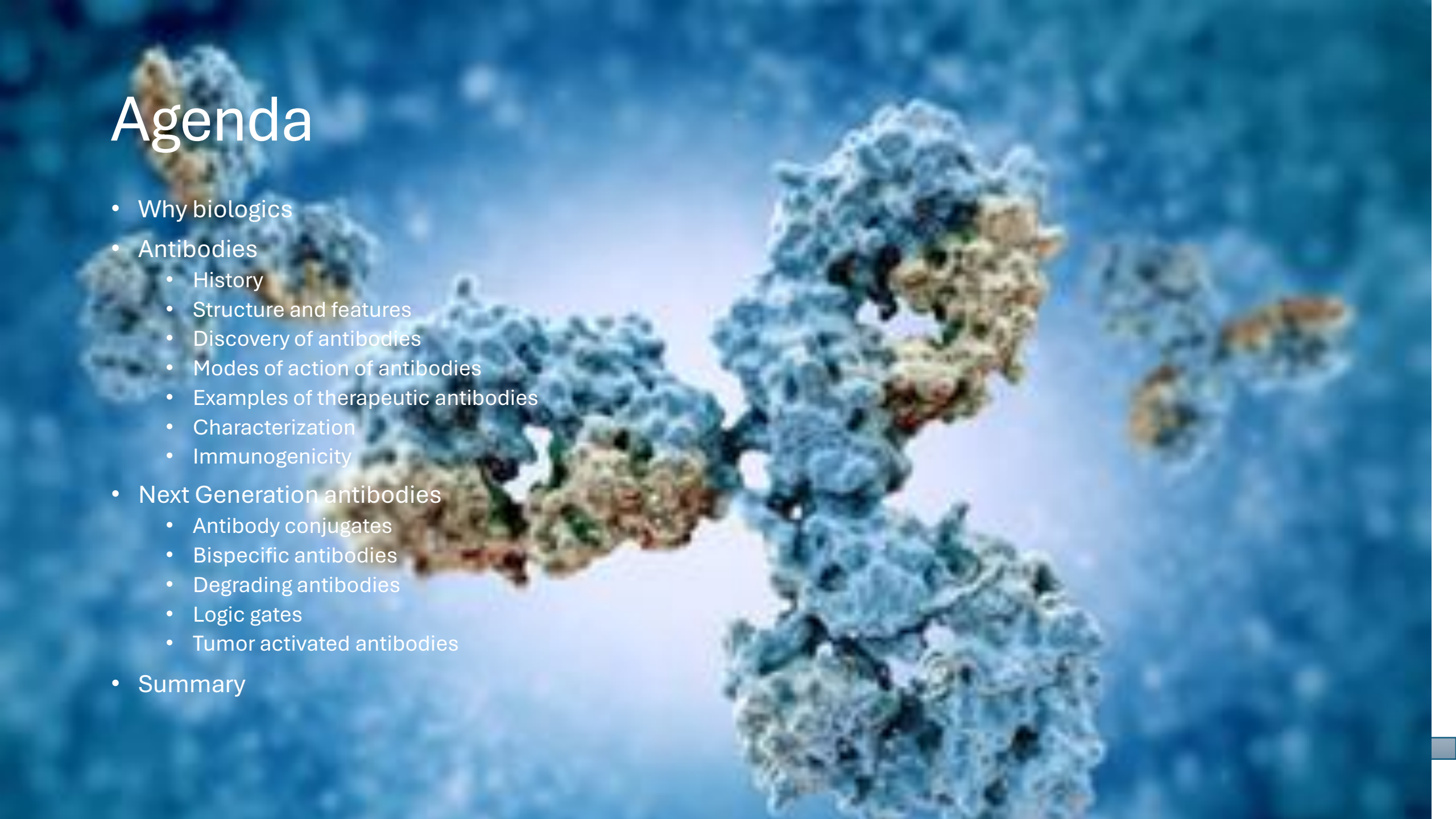
VERSANT ventures

Investor

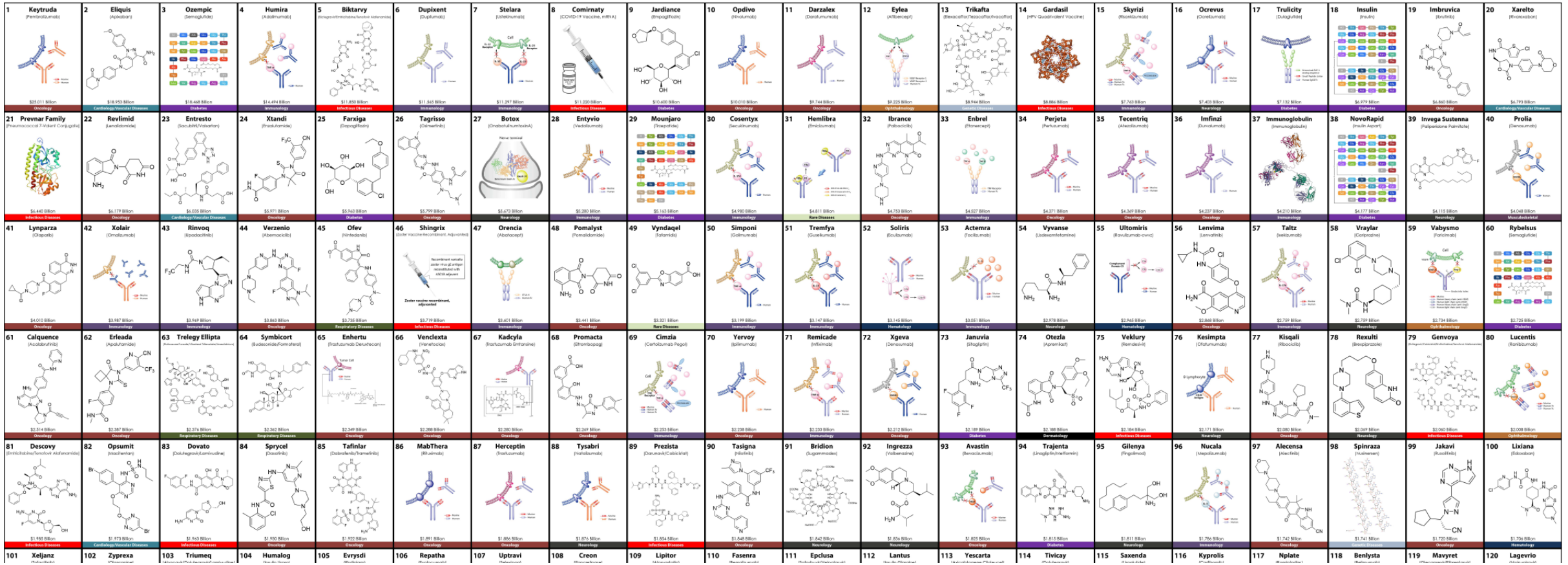
- Companies with new (protein) technologies and drug candidates
- Running antibody group in Versant's discovery engine Ridgeline

Agenda

- Why biologics
- Antibodies
  - History
  - Structure and features
  - Discovery of antibodies
  - Modes of action of antibodies
  - Examples of therapeutic antibodies
  - Characterization
  - Immunogenicity
- Next Generation antibodies
  - Antibody conjugates
  - Bispecific antibodies
  - Degrading antibodies
  - Logic gates
  - Tumor activated antibodies
- Summary



The best selling drugs….



Compiled and Produced by Ryan E. Williams and Hayden M. Leatherwood from the Njarðarson Group (The University of Arizona)

are dominated by Biologics

Compiled and Produced by Ryan E. Williams and Hayden M. Leatherwood from the Njarðarson Group (The University of Arizona)

Biologics

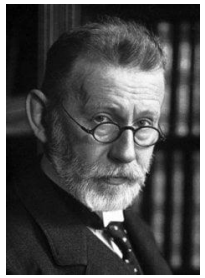
- **Center for Biologics Evaluation and Research (CBER) (FDA)** definition: Biologics can be composed of sugars, proteins, or nucleic acids or complex combinations of these substances, or may be living entities such as cells and tissues.
- Examples:
  - Vaccines
  - (long) peptides (insulin)
  - Blood and blood components
  - Gene therapy
  - Cell therapy
  - Protein therapeutics:
    - Monoclonal antibodies
    - Nanobodies
    - Antibody drug conjugates
    - Bi-specific antibodies

Why Biologics

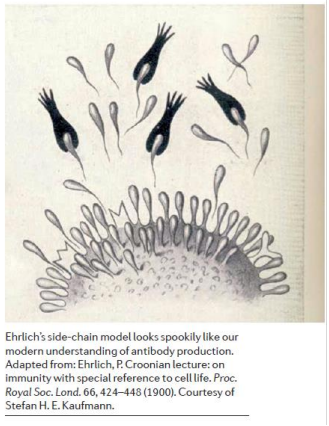
	Biologic (Antibody)	Small molecule
Inhibition of Protein / Protein interaction		
Inhibition of active site (enzyme, kinase)		
Intracellular targeting		
Oral availability		
Target selectivity		
Rational discovery		
Toxcicity		
Effector function		
Cost of goods		
Half life		
Immunogenicity		

Antibodies: The dream of the magic bullet

Magic Bullet / Key lock hypothesis



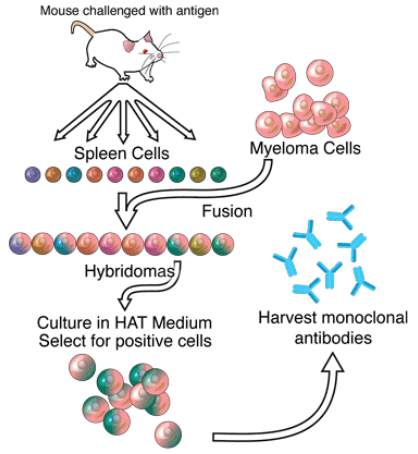
- Paul Ehrlich (1854-1915)
- Hypothesis: Body produces selective molecules to defend against pathogens
- These antibodies are highly selective to one target like a



Ehrlich's side-chain model looks spookily like our modern understanding of antibody production. Adapted from: Ehrlich, P. Croonian lecture: on immunity with special reference to cell life. *Proc. Royal Soc. Lond.* 66, 424–448 (1900). Courtesy of Stefan H. E. Kaufmann.

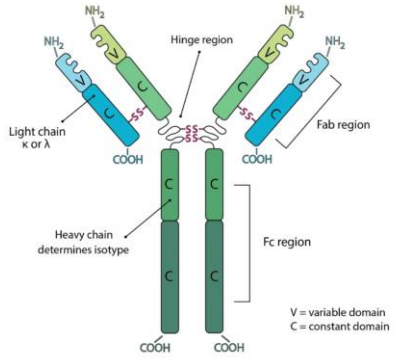
Monoclonal antibodies

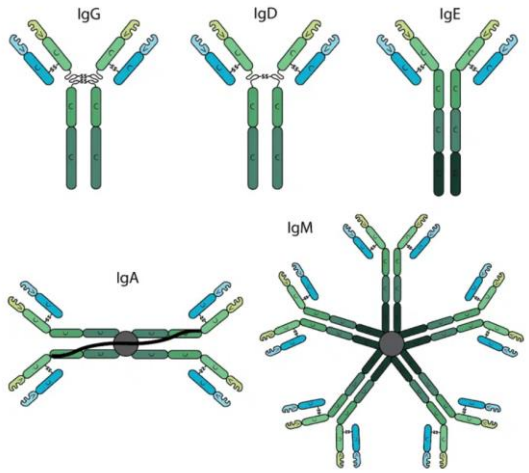
- Cesar Milstein and Georg Koehler (Noble price 1984)
- Hybridoma method for isolation of monoclonal antibodies from mice
- Key step is to immortalize the single antibody producing B-cells so they can be proliferated and analyzed
- The never filed IP however Wistar institute US did, what lead to a big scandal in the early 80s.



https://www.biomol.com/resources/biomol-blog/invention-of-the-hybridoma-technologyey to a lock

Structure and Feature of Antibodies





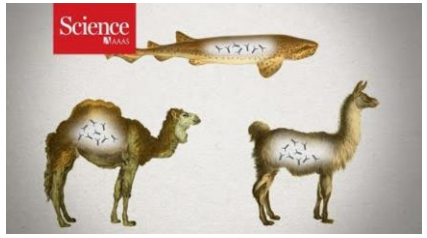
Structure:

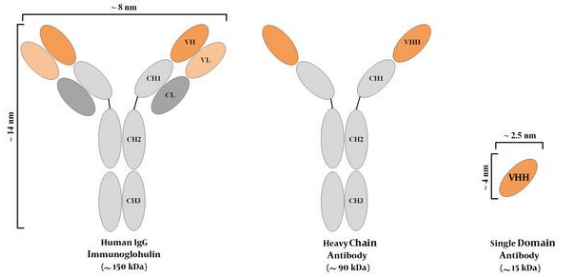
- 4 polypeptide chains
- Variable regions responsible for antigen recognition (human repertoire (single individual) 10^7, but fully accessible are 10^15(?) via somatic hypermutation)
- Antigen selectivity determined by 6 CDRs (Complementarity determining regions) which are highly variable and only partially germline encoded (VDJ recombination)
- Constant (Fc) part responsible for long half life and effector function
- 2 light chains (kappa or lambda) 2 heavy chains (different isotypes IgG (1,2,3,4), IgA, IgD, IgE, IgM) for drug development only IgG important
- MW: 150 kDa (Fab 50 kDa)
- 4 inter-chain disulfide bridges (used for disulfide coupling of toxins)
- Glycosylated (important for some functions)
- The binding side on the antigen is called **epitope**, the structure binding on the antibody side is called **paratope**

https://bioxcell.com/educational-articles/antibody-structure/

Alternative formats

VHH, single domain antibodies



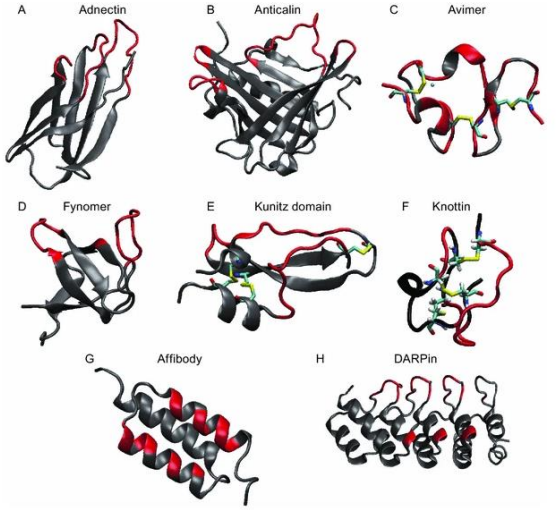


Key Features:

- Only one chain, easier to express, very easy for multi-specifics (no heavy/light chain pairing issue)
- Different epitopes due to long HCDR3s and evolutionary distance (shark)
- Worse affinity (3 instead of 6CDRs)
- Sometimes production issues due to long HCDR3

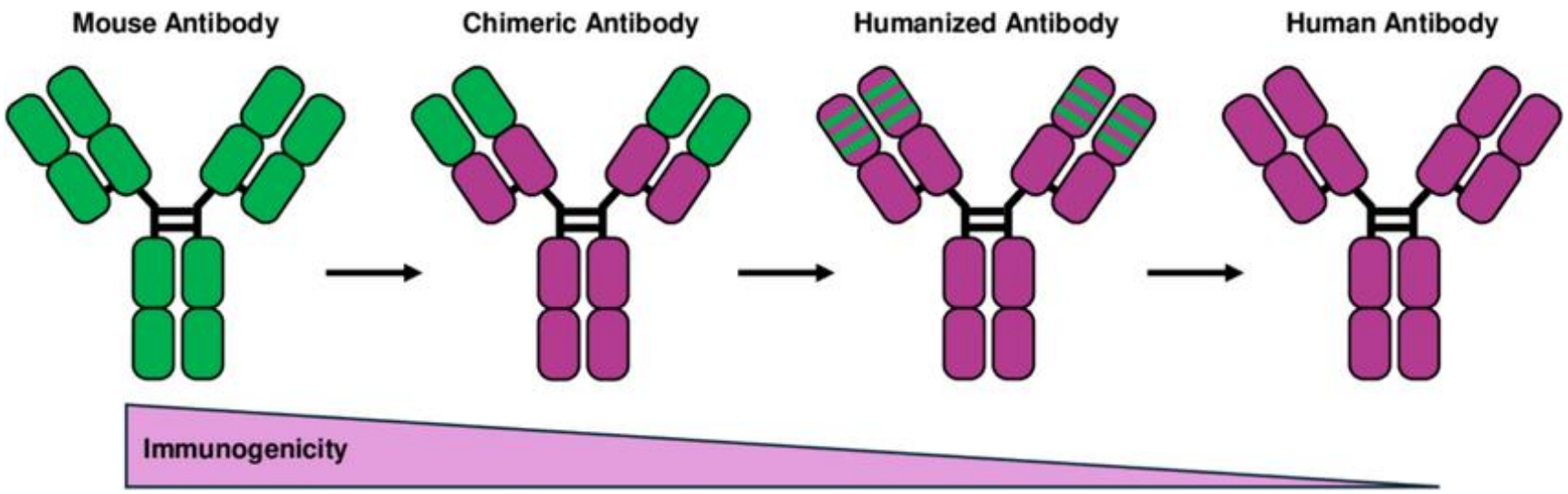
Alternative synthetic binding scaffolds

(only limited importance in drug development, immunogenicity issues)

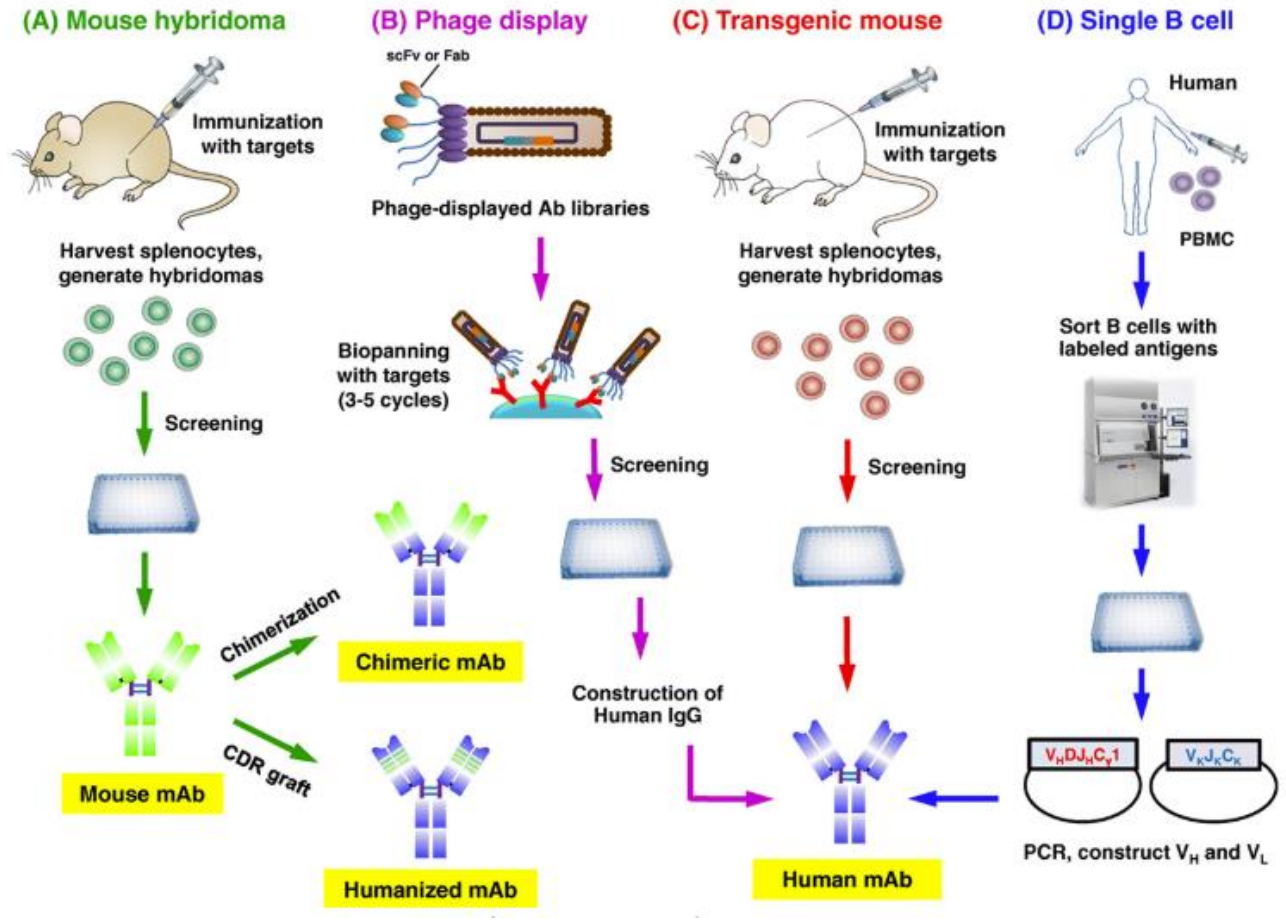


Simeon, R., Chen, Z. In vitro-engineered non-antibody protein therapeutics. Protein Cell 9, 3–14 (2018). https://doi.org/10.1007/s13238-017-0386-

Humanization

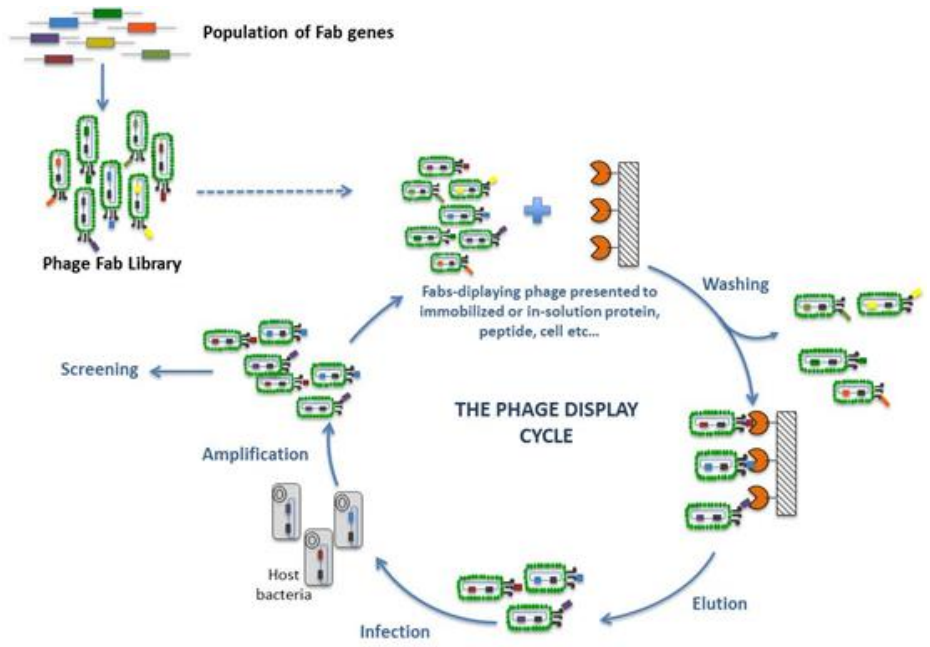


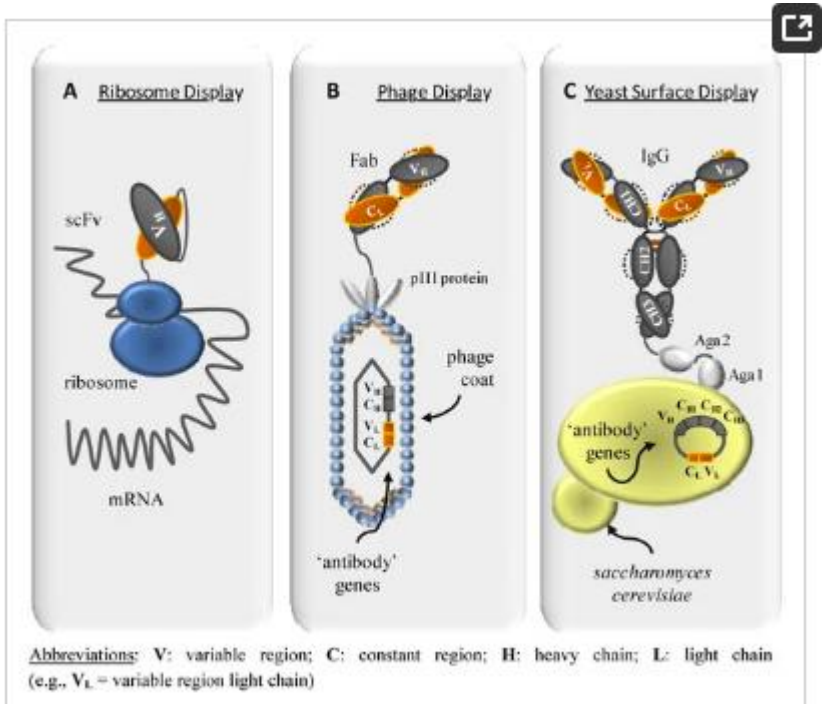
Discovery of antibodies



Lu, RM., Hwang, YC., Liu, IJ. *et al.* Development of therapeutic antibodies for the treatment of diseases. *J Biomed Sci* **27**, 1 (2020). https://doi.org/10.1186/s12929-019-0592-z

Display and Libraries (in vitro selection methods)

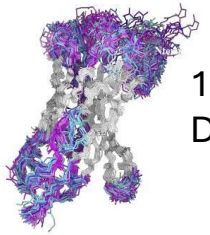




https://www.creative-biolabs.com/blog/wp-content/uploads/2016/12/phage-display.png

Example for a synthetc library:
Human Combinatory Antibody Library (HuCAL) Concept

Structures / Folds



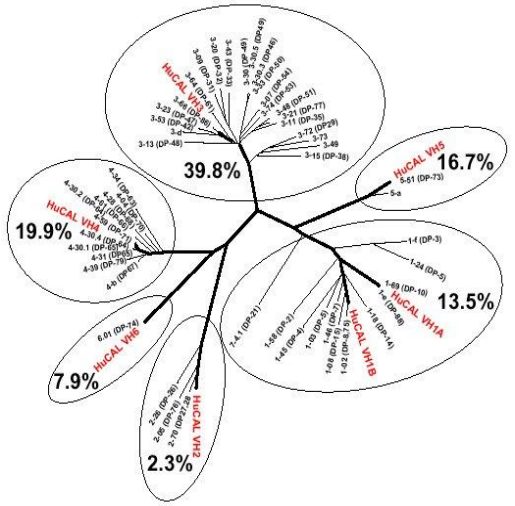
132 Ig Variable Domain Structures

Germline Genes

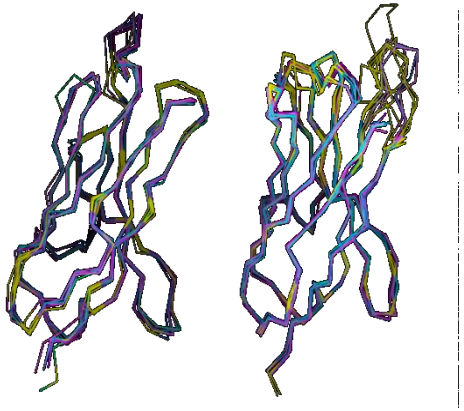
~ 51 VH Genes

~ 40 Vk Genes

~ 30 Vl Genes



HuCAL Master Frameworks

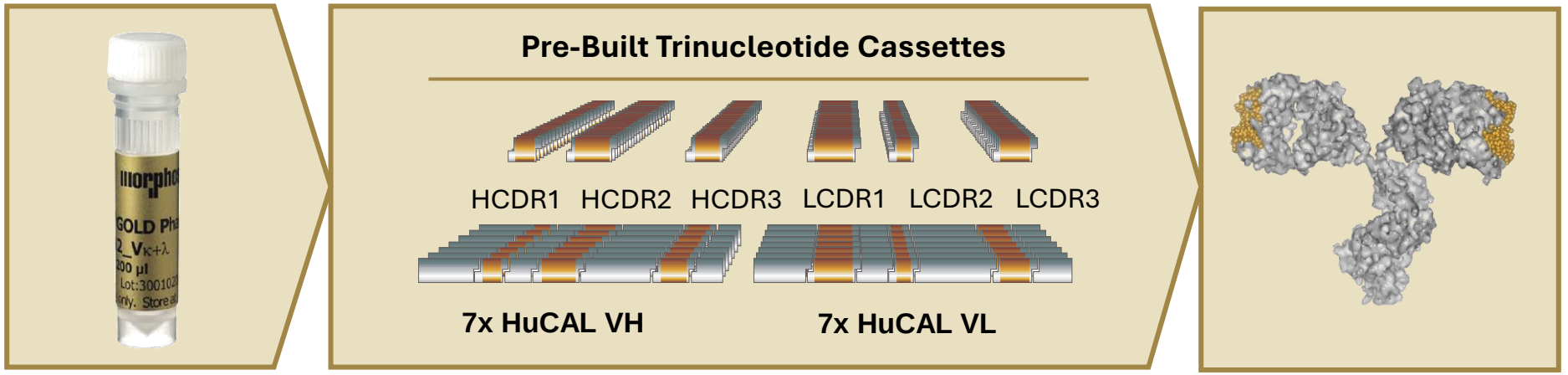


Bioinformatics	Analysis	Gene Synthesis
Analysis of human antibody germline and rearranged genes	Grouping into VH/VL sub-families according to sequence homology	Combination of VH and VL HuCAL Master Genes covers structural diversity

morphosys

Tiller T, Schuster I, Deppe D, Siegers K, Strohner R, Herrmann T, Berenguer M, Poujol D, Stehle J, Stark Y, Heßling M, Daubert D, Felderer K, Kaden S, Kölln J, Enzelberger M, Urlinger S. A fully synthetic human Fab antibody library based on fixed VH/VL framework pairings with favorable biophysical properties. MAbs. 2013 May-Jun;5(3):445-70. doi: 10.4161/mabs.24218. Epub 2013 Apr 9. PMID: 23571156; PMCID: PMC4169037.

Human Combinatory Antibody Library (HuCAL)



A 1.6x 10^{10} fully human Fab library

Master genes are close to human germline sequences

- Cover structural diversity in man using consensus 7x VH and 7x VH frameworks (49 framework combinations)

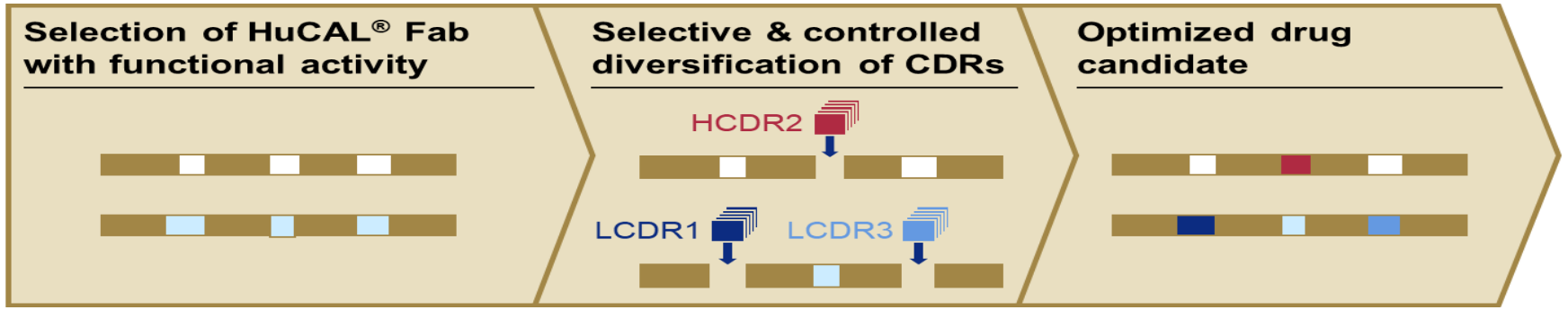
Diverse repertoires in all 6 CDRs

- Diversity designed according to natural distribution in man
- Tailor-made using TRIM technology

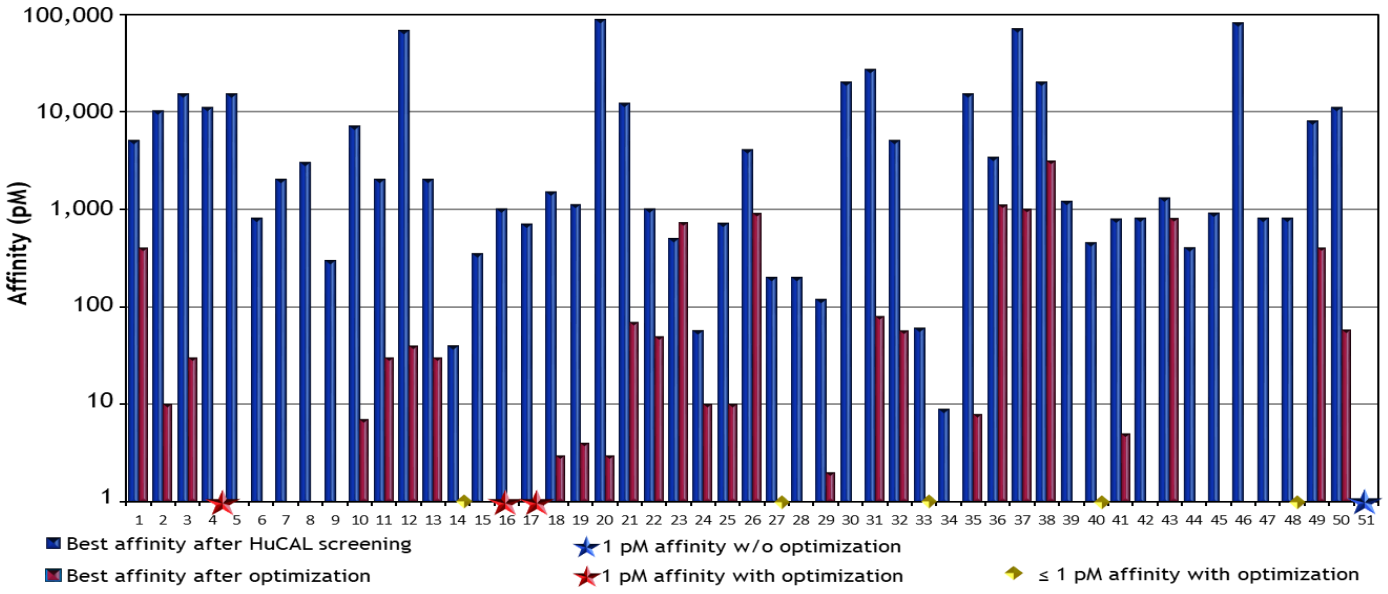
Fully modular

- Engineering, easy optimization and affinity maturation

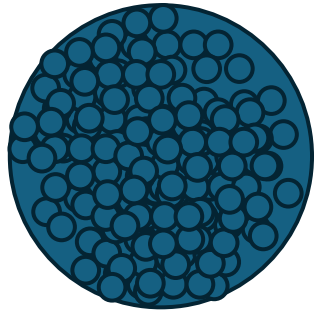
Affinity Maturation



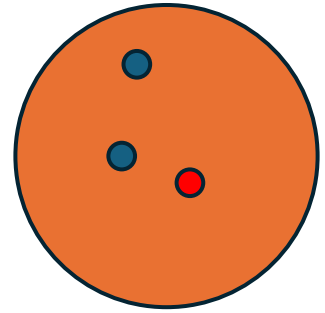
Best monovalent affinities of therapeutic candidates with & w/o optimization from HuCAL



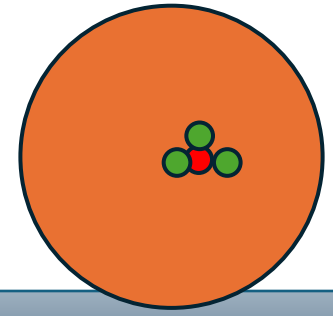
The sequence space concept:



Theoretical CDR diversity: 10^30 (not screenable)

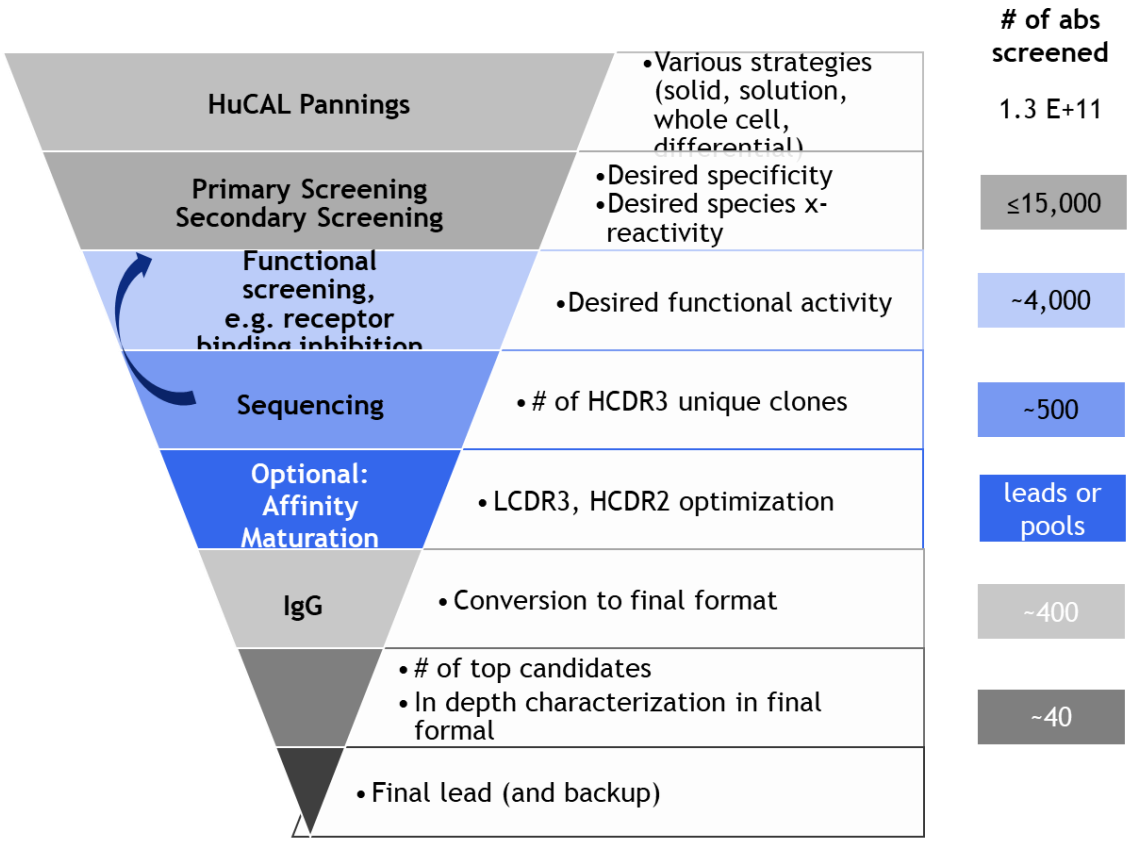


Library “shotgun”: 10^10
Binder
Non-binder



Affinity library 10^10
Binder
Improved binder

Antibody screening funnel

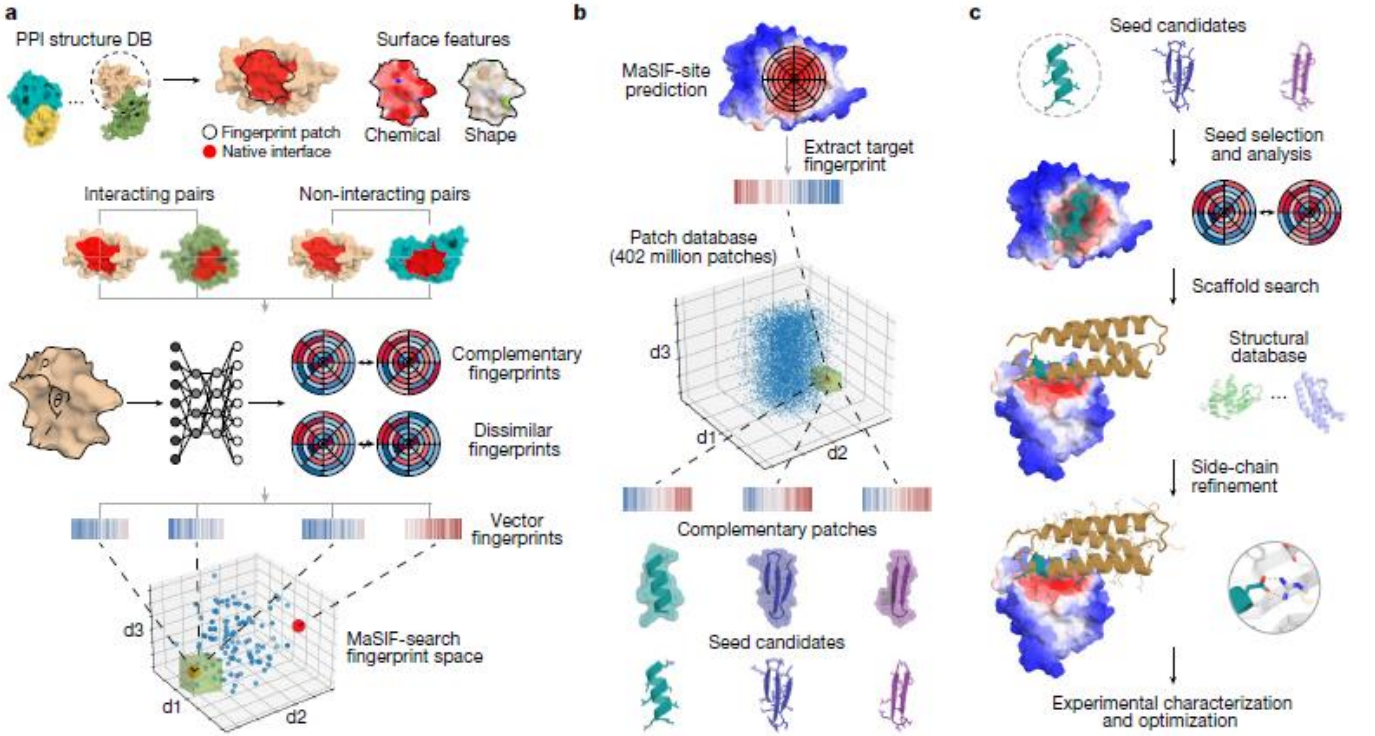


Alternative antibody discovery methods

Method	Features
Yeast Display	Full IgG display/selection Limited library size (10^9)
Ribosomal Display	Very large libraries (10^13) Only scFV Difficult IgG conversion
Alternative animals (Chicken, rabbit)	Higher epitope diversity (evolutionary distance) Humanization difficult
B-cells from deceased	E.g. Spanish flu survivors
Full de novo / in silico	Still lacking final prove

Full de novo generation of binders

- The concept of interactable surface fingerprints, the “druggable” epitope



Article

De novo design of protein interactions with learned surface fingerprints

https://doi.org/10.1038/s41586-023-05993-x

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Accepted: 21 March 2023

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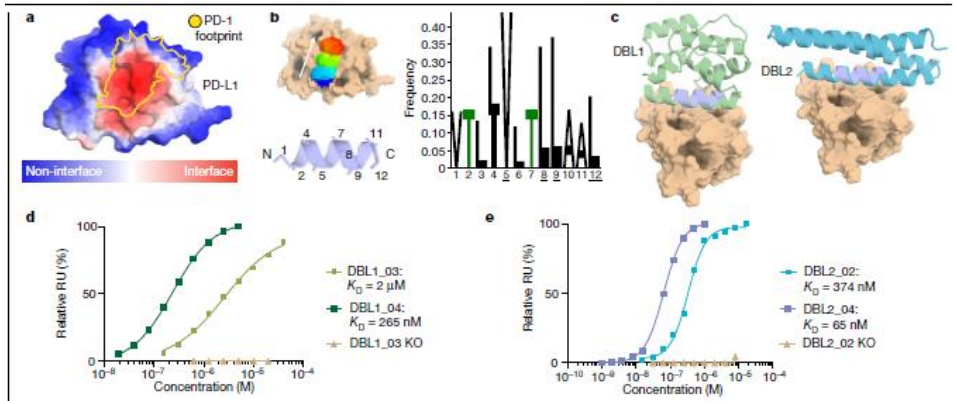
Open access

Check for updates

Pablo Gainza[1,2,10,11], Sarah Wehrle[1,2,11], Alexandra Van Hall-Beauvais[1,2,11], Anthony Marchand[1,2,11], Andreas Scheck[1,2,11], Zander Harteveld[1,2], Stephen Buckley[1,2], Dongchun Ni[3,4], Shuguang Tan[5], Freyr Sverrisson[1,2], Casper Goverde[1,2], Priscilla Turelli[6], Charlène Raclot[6], Alexandra Teslenko[7], Martin Pacesa[1,2], Stéphane Rosset[1,2], Sandrine Georgeon[1,2], Jane Marsden[1,2], Aaron Petruzzella[8], Kefang Liu[5], Zepeng Xu[5], Yan Chai[5], Pu Han[5], George F. Gao[5], Elisa Oricchio[6], Beat Fierz[7], Didier Trono[6], Henning Stahlberg[3,4], Michael Bronstein[9] ✉ & Bruno E. Correia[1,2] ✉



Gainza, P., Wehrle, S., Van Hall-Beauvais, A. et al. De novo design of protein interactions with learned surface fingerprints. Nature 617, 176–184 (2023). https://doi.org/10.1038/s41586-023-05993-x

Outlook, the in silico challenge

Where are we with full in silico generation of antibodies?

Correspondence

https://doi.org/10.1038/s41587-024-02469-9

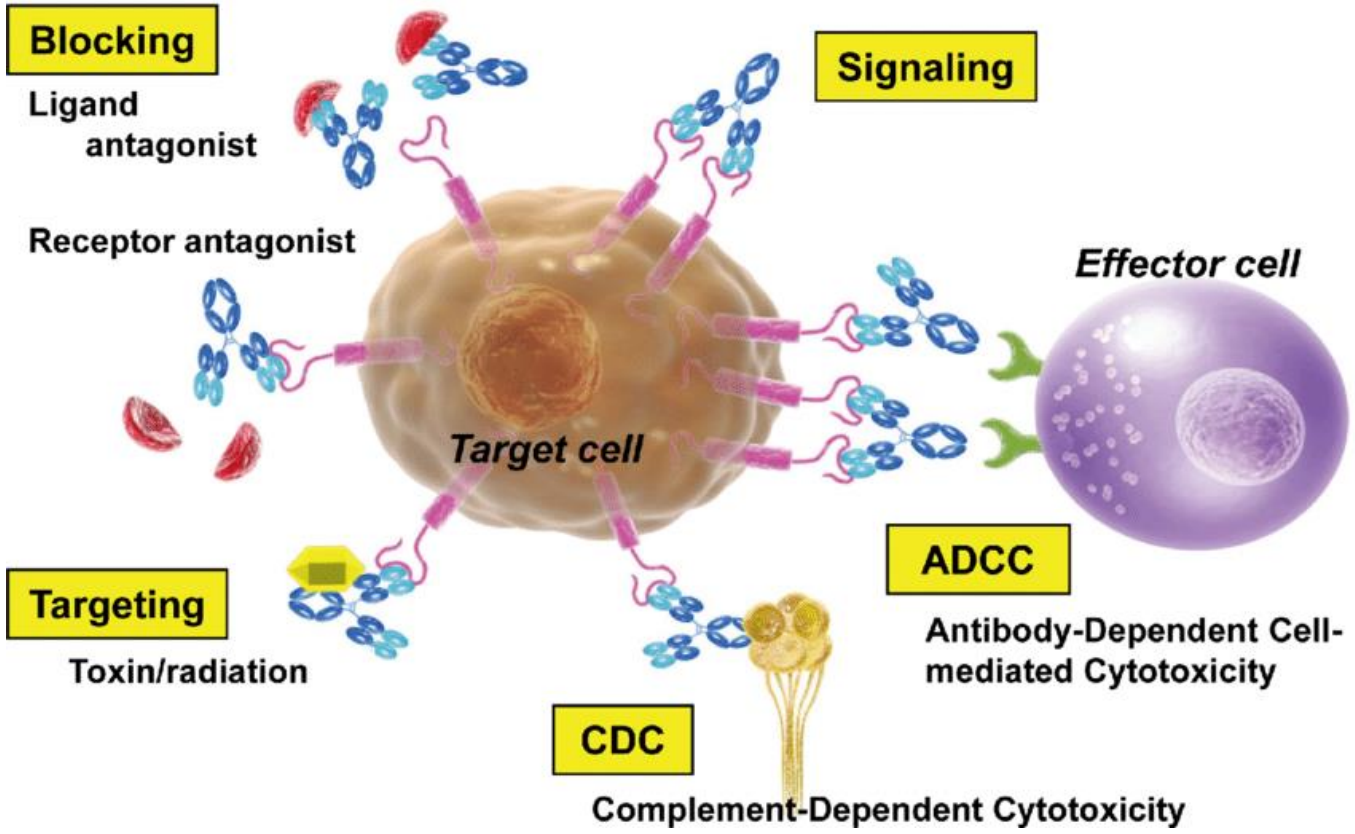
AIntibody: an experimentally validated in silico antibody discovery design challenge

Check for updates

Science is frequently subject to the Gartner hype cycle[1]: emergent technologies spark intense initial dramatically in 2018[7] and 2020[8]. Although AlphaFold has not competed since, many methods today are inspired by the AlphaFold via the eponymous AIntibody.org website) that will present a series of escalating AI/ML challenges in antibody discovery, calibrated

1. Given an antibody sequence with known affinity to a target, generate candidates with improved affinities.
2. Given an antibody sequence with known affinity to a target, with poor developability properties (for example, thermal stability, polyreactivity), generate candidates with equal or better affinities that lack the issues of poor developability.
3. Given the sequence of a target with known structure, and structurally similar targets in the PDB, generate specific antibodies binding to the target.
4. Given the sequence of a target with unknown structure, but similar targets in the PDB, generate specific antibodies binding to that target.
5. Apply Challenge 3 above to a specific predefined epitope.
6. Given a set of antibody sequences known to bind distinct epitopes on a given target, predict which antibodies bind to different epitopes.
7. Given a set of antibody sequences known to bind distinct epitopes on a given target, predict which antibodies will allow sandwich binding.
8. Given the sequence of a target, and a specific epitope within that target, generate de novo antibodies binding to that specific epitope.
9. Given a set of diverse antibodies, predict their epitopes.
10. Given the sequence of a target with unknown structure, generate antibodies binding to that target.
11. Given an antibody sequence to a known human target epitope, create antibody designs that are cross-reactive to cynomolgus monkey and mouse with epitopes identical to or spatially close to the parental human epitope in their 3D structure.
12. Create de novo or rationally guided antibody designs which do not bind any known targets (isotype controls).

Antibodies Modes of Action

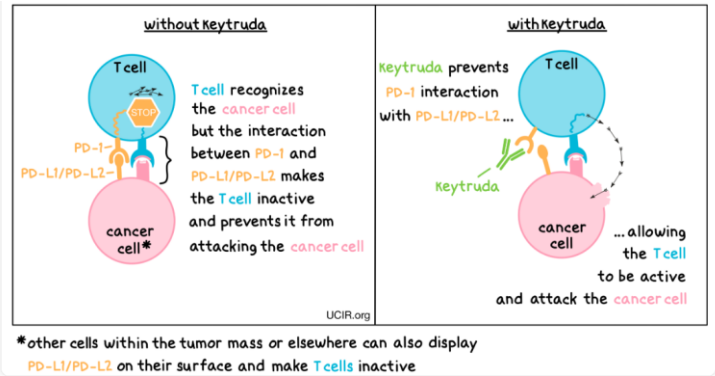


Suzuki, Masami & Kato, Chie & KATO, Atsuhiko. (2015).

Examples of antibody blockbusters

Keytruda (Pembrolizumab)

- Humanized mouse antibody against PD-1 silent IgG4 (not depleting)
- Mode of action

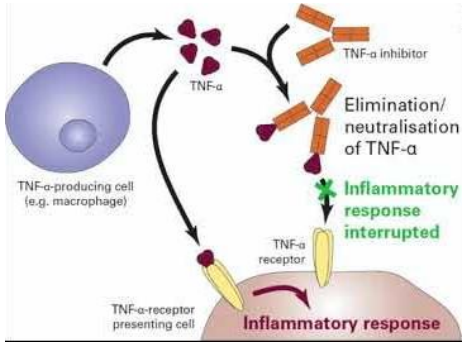


https://www.ucir.org/immunotherapy-drugs/pembrolizumab

- Indications: TNBC, NSCLC, Melanoma, Kidney, Colorectal
- USD 20.5b sales in 2023

Humira (Adalimumab)

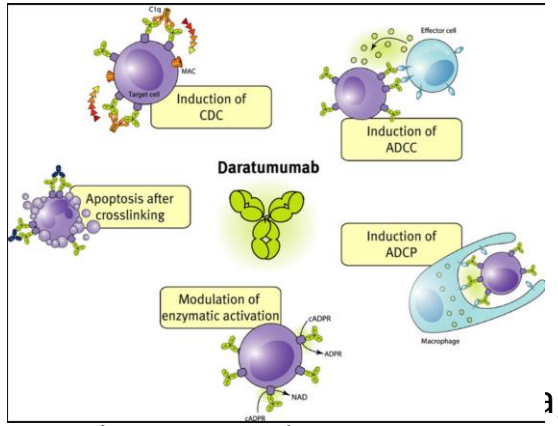
- Human antibody (1st phage display mAb) against TNFalpha IgG1
- Mode of action:



- Indications: Crohn's, Rheumatoid arthritis, Plaque Psoriasis
- Sales: USD 14b sales in 2023

Darzalex (Daratumomab):

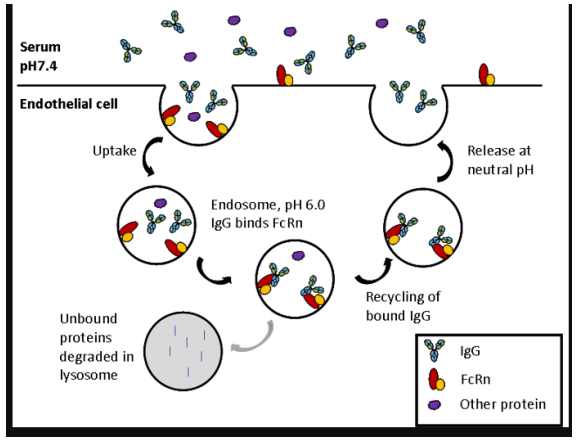
- Human antibody (from humanized mouse) against CD38 IgG1 (full ADCP, ADCC and CDC active)
- Mode of action:



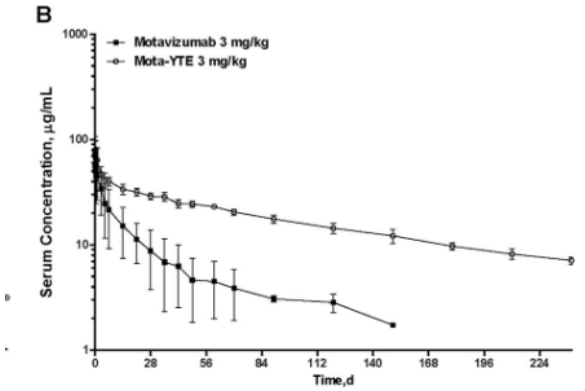
- autoimmune diseases
- Sales: USD 10b in 2023

Antibody characterization Half Life

- Like albumin antibodies (IgG) bind to the neonatal Fc receptor (FcRn) and are recycled
- This leads to serum half life of around 14d enabling 2-4 weekly dosing of antibodies
- Antibody half life can be tuned by mutations in the Fc region, mostly used is the so called YTE mutation increasing half life to approx. 4 weeks.
- Certain application (e.g. radio pharmaceuticals) prefer very short half life, mutations for this also exist.



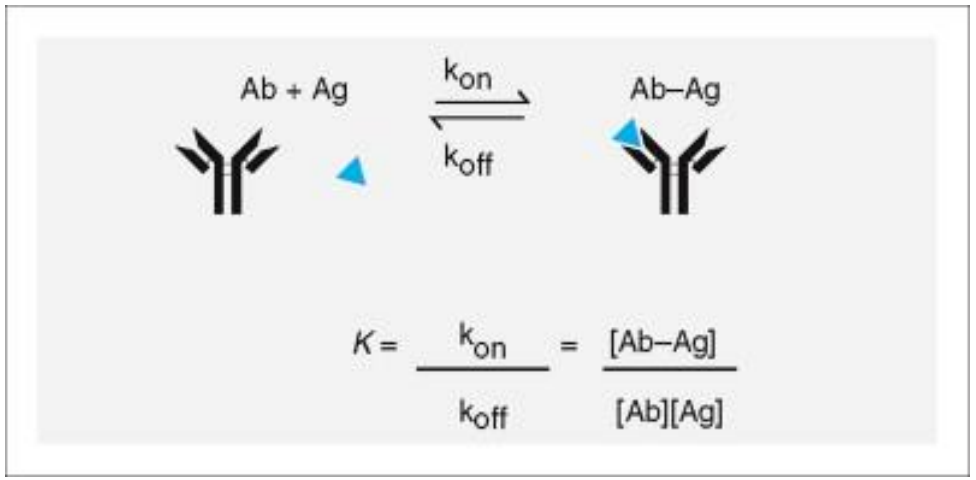
https://absoluteantibody.com/wp-content/uploads/2014/03/Recycling.png



Robbie GJ, Criste R, Dall'Acqua WF, Jensen K, Patel NK, Losonsky GA, Griffin MP. 2013. A Novel Investigational Fc-Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-Life in Healthy Adults. Antimicrob Agents Chemother 57:.

Antibody characterization (I)

Affinity



Typical (monovalent) affinities of antibodies:

- Soluble antigens: 0.1-300pM
- Surface antigens: 0.1-10nM
- ADCs: 1-50nM

Avidity

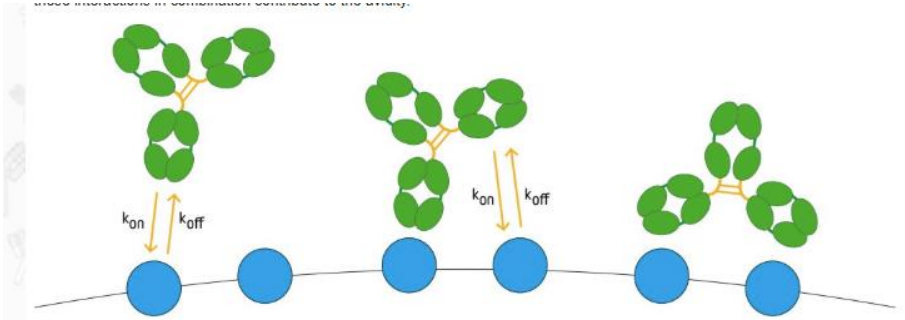


Figure 1: Valency refers not only to the number of antigen binding sites on the antibody, but also to the number of residues contributing to the paratope. (Image adapted from JBC 291 (21) 2016 p11337).

Factors influencing avidity:

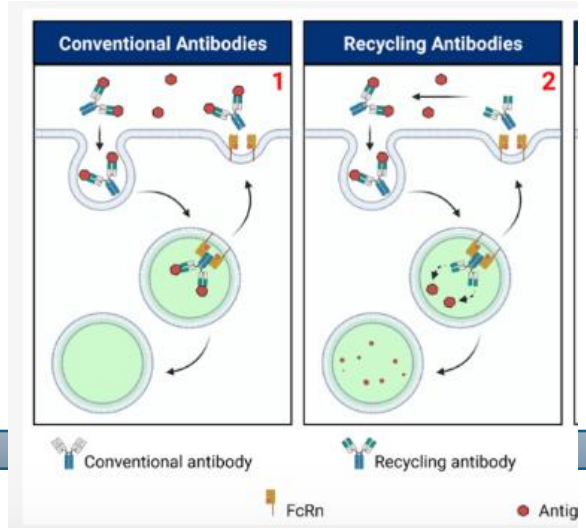
- Kon and koff
- Antigen density
- Epitopes

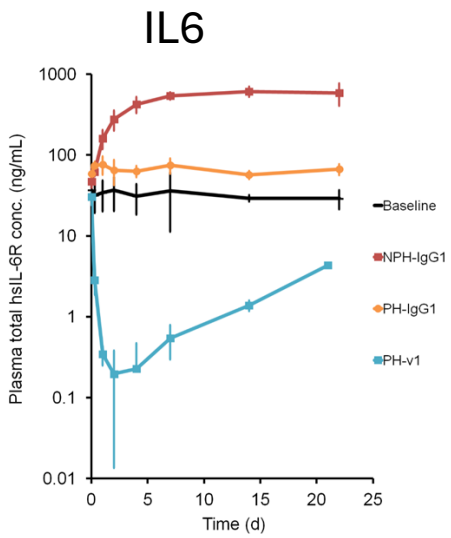
Affinity: points to consider for soluble antigens

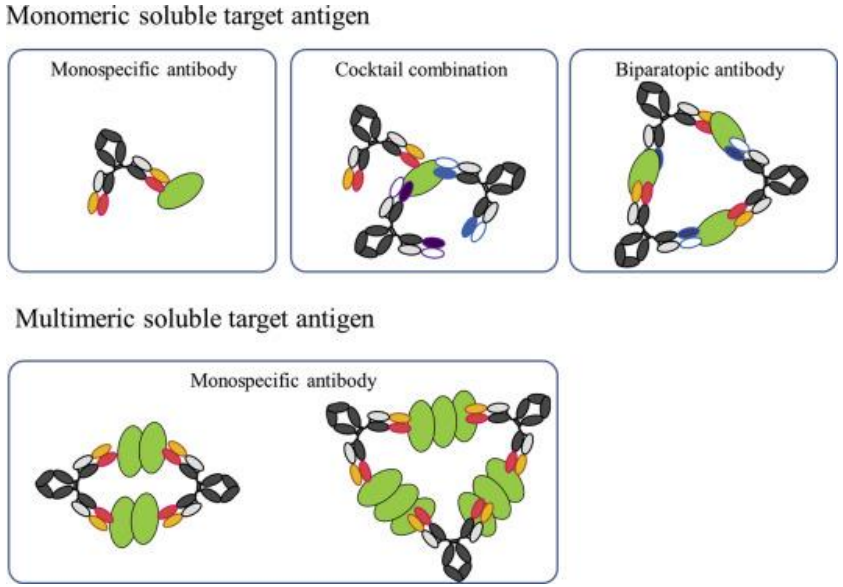
- Homo-multimeric soluble antigens (e.g. TNFalpha, Il23..) form immune-complexes and are easily cleared via the liver

However….

- Monomeric soluble targets (CCL2, IL6, GCSF) cannot be eliminate *in vivo* … instead target plasma concentration increases in serum
  - Key reason for many antibodies failing: e.g. MCP-1 antibodies worsen because plasma levels rise over time (depot effect)
- Solutions:
  - Extreme slow off-rates
  - Bi-paratopic antibodies
  - pH sensitive binding (recycling antibody)



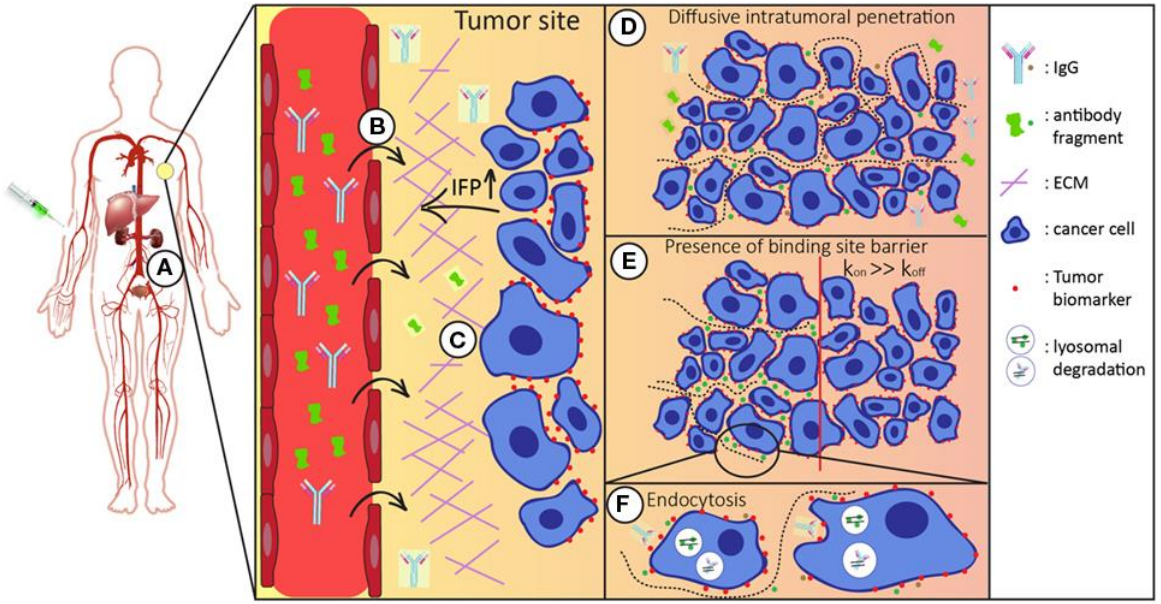




Kenta Haraya, Tatsuhiko Tachibana, Tomoyuki Igawa, Improvement of pharmacokinetic properties of therapeutic antibodies by antibody engineering, Drug Metabolism and Pharmacokinetics, https://doi.org/10.1016/j.dmpk.2018.10.003.

Affinity: points to consider in oncology

- Barrier effect (under debate):
  - Very high affinity might prevent deep tumor penetration
- Triangle to be optimized:
  - Affinity sweet spot between tumor penetration and barrier
  - Size: as smaller as better (tumor penetration) however as smaller as shorter half life
  - Half life: as longer as better (more passes through tumor) however as larger as less penetration



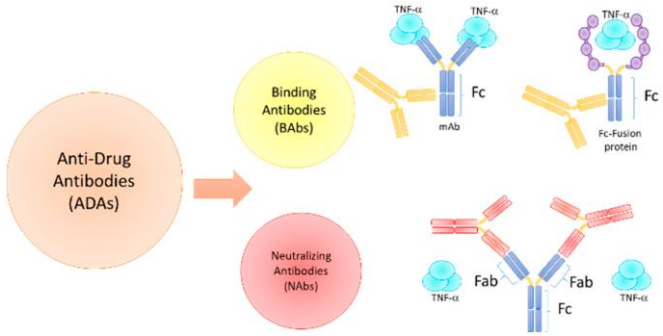
Front. Immunol. , 12 October 2017
Sec. Vaccines and Molecular Therapeutics
Volume 8 - 2017 | https://doi.org/10.3389/fimmu.2017.01287

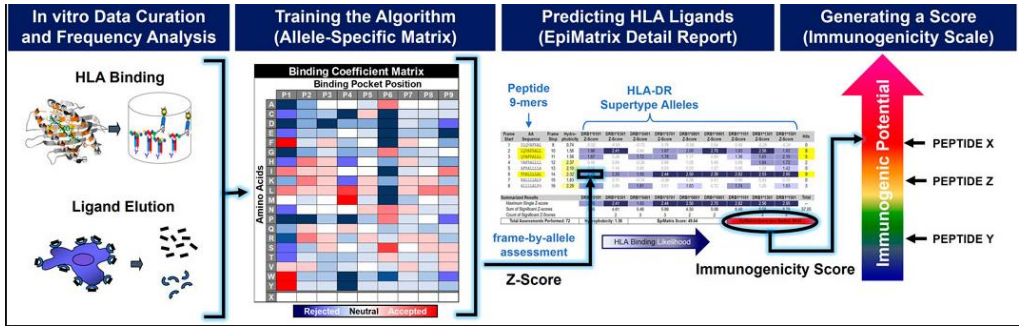
Antibody characterization Biophysics(II)

Feature	Issues	Measurements
Monomeric content	Aggregation (immunogenicity)	Size exclusion chromatography (SEC); Light scattering (MALS)
Hydrophobicity	Aggregation, high viscosity (for s.c), unspecific binding	Hydrophobic interaction chromatography (HIC)
Melting temperature	Stability, storage	CD, Thermoflour
Posttranslational modifications (glycosylation, isomerisaton, etc)	Heterogeneity (dose finding)	Mass spectrometry (MS)
ADCC/ADCP	Functionality	Reporter cells
Yield	Cost of goods	

Immunogenicity

- The formation of (neutralizing) anti-drug-antibodies (ADAs) is one of the key issues in the development of biologics.
- Key drivers for immunogenicity:
  - Primary protein sequence (foreign) , T-cell epitope
    - In silico tools to predict potentially immunogenic epitopes
    - Ex vivo testing in human antigen presenting cells
  - Biophysical properties (B-cell activation):
    - Aggregation, stickiness
  - Target selection:
    - Targets on immune cells (e.g. cytokine receptors) tends to have greater potential for immunogenicity
  - Pre existing antibodies:
    - Certain e.g. glycan structures, homology to vaccines etc. might have triggered





Front. Drug Discov. , 10 October 2022 | https://doi.org/10.3389/fddsv.2022.952326

Immunogenicity against Cetuximab a scientific mystery story

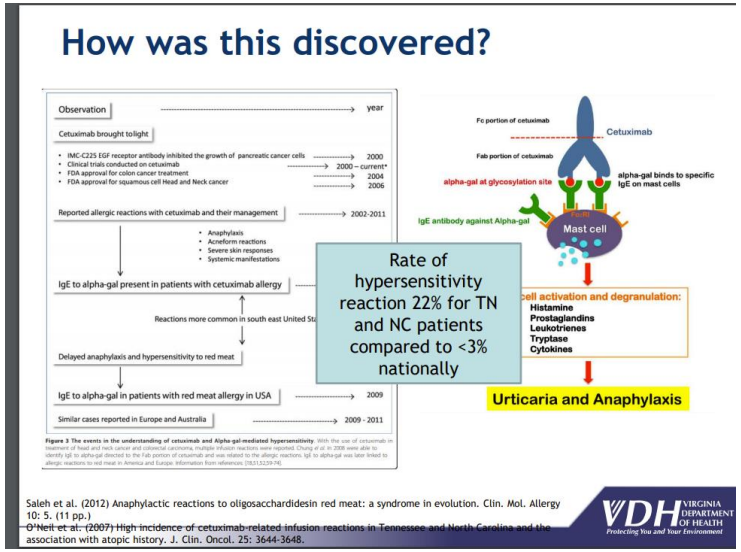
Distribution of Cetuximab hypersensitivity

https://img1.wsimg.com/blobby/go/da7047b1-e51a-4d03-8cbd-ead35925ac6e/downloads/Alpha-gal%20Syndrome.pdf?ver=1604077519690

Immunogenicity against Cetuximab a scientific mystery story

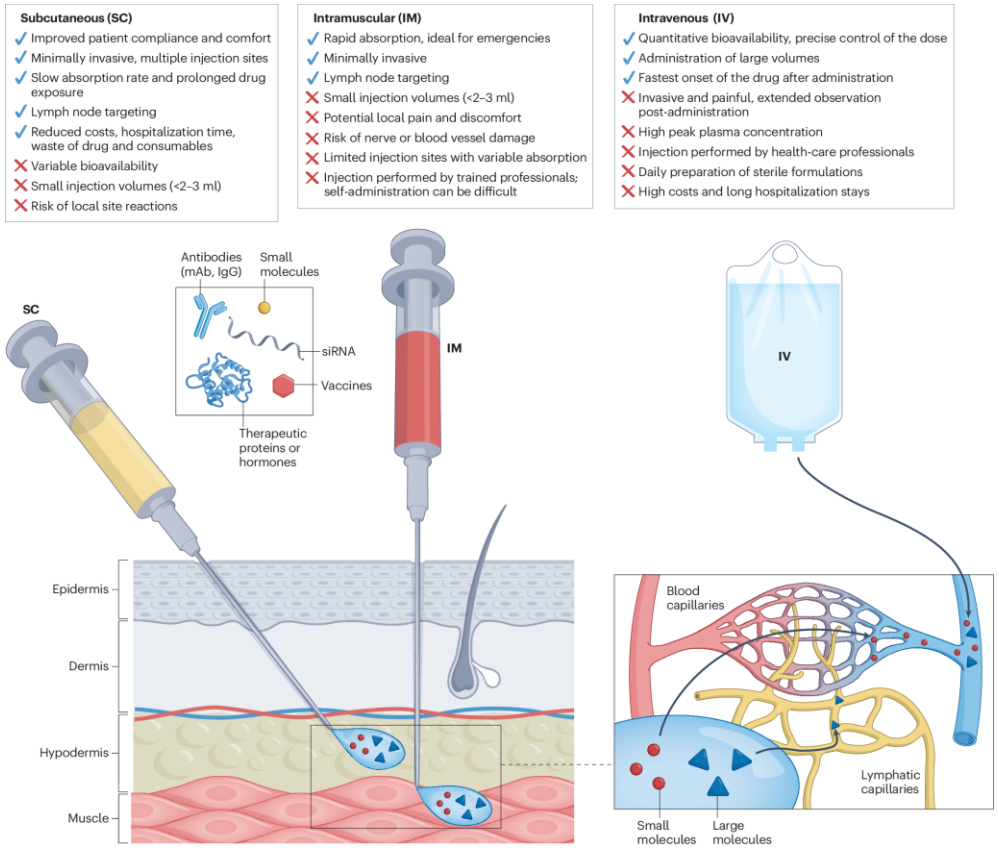
Distribution of Cetuximab hypersensitivity

- In contrast to most other monoclonal antibodies (which are produced in CHO (Chinese hamster ovary) cells, Cetuximab is still produced in a mouse hybridoma , this cell line adds an unusual sugar (a-gal) to the Fc part.
- The same sugar structure is part of the toxin of a tick homed in this area



https://img1.wsimg.com/blobby/go/da7047b1-e51a-4d03-8cbd-ead35925ac6e/downloads/Alpha-gal%20Syndrome.pdf?ver=1604077519690

Antibody administration



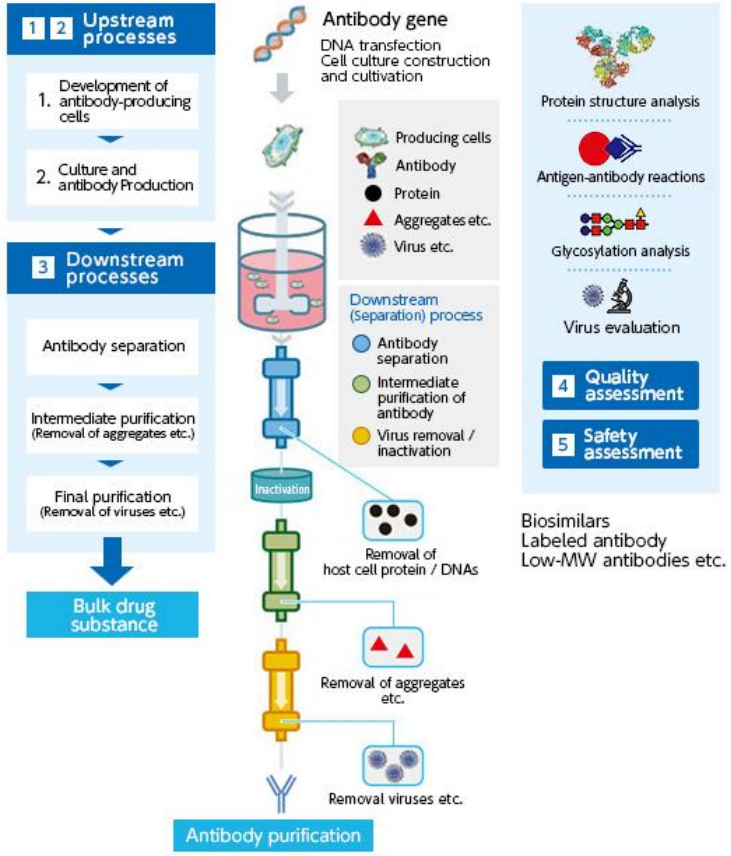
S.c. vs. i.v.

- S.c. application esp. in pens or pre-filled syringes becomes differentiation factor esp. in chronic disease (atopic dermatitis, RA, Crohn's, allergy, asthma…)
- However: antibodies have to be very well behaved (solubility, stability, aggregation) to be formulated to high concentrations which is needed for s.c.
- For cost reasons, often early clinical trials are done with i.v. and then s.c. is developed
- For Humira the device design is considered pivotal for commercial success because patients with rheumatoid arthritis can handle it.

Tomasini, L., Ferrere, M. & Nicolas, J. Subcutaneous drug delivery from nanoscale systems. Nat Rev Bioeng 2, 501–520 (2024). https://doi.org/10.1038/s44222-024-00161-w

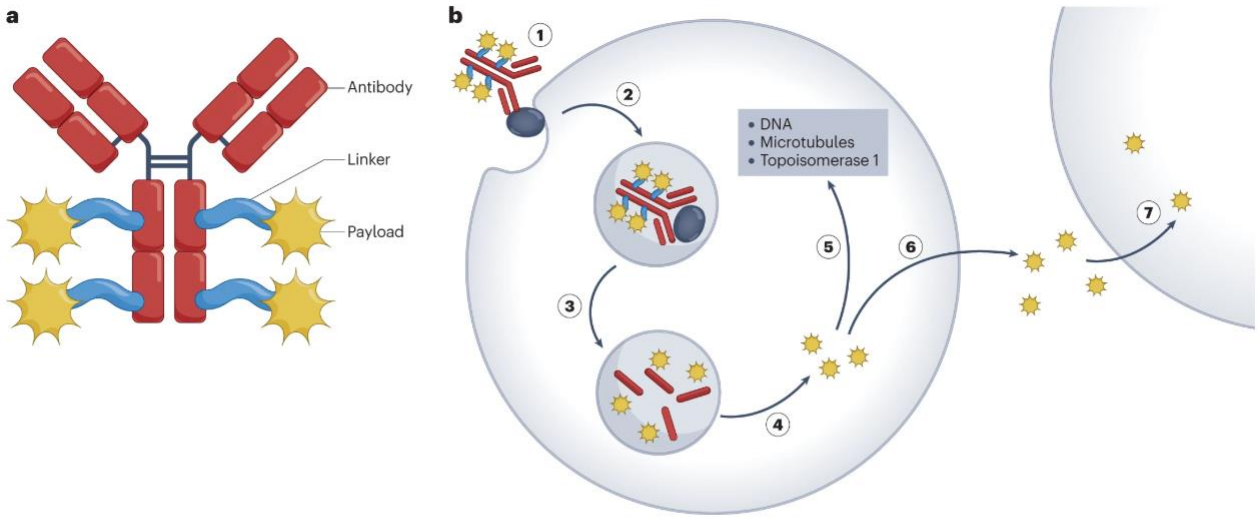
Antibody production

- Standard process:



- Production Systems:
  - CHO (standard)
    - Right glycosylation
  - Yeast
    - Cheap, but glycosylation difficult
  - E.coli
    - Very cheap, No glycosylation
  - Plants: Tabacco, potato, algae, moss
    - Not established but discussed for: very high-volume production, (oral) vaccination, third world countries, and highly toxic fusion proteins

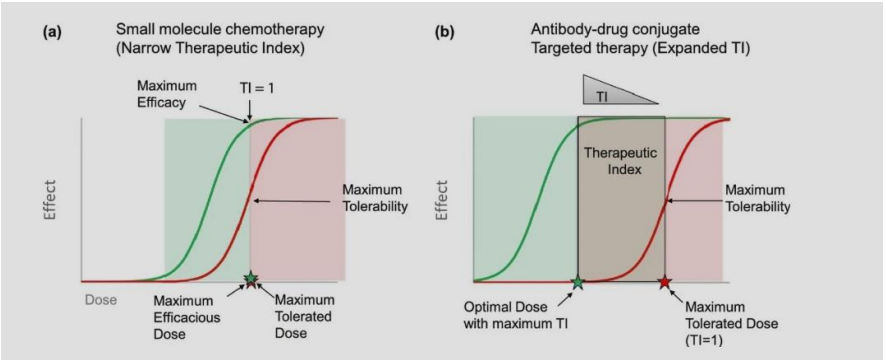
Antibody Drug Conjugates (ADCs)



a, Antitumour ADCs are composed of three key elements: a monoclonal antibody moiety that binds to an antigen preferentially expressed on the tumour cell surface, thereby ensuring specific binding to tumour cells; a covalent linker that ensures that the payload is not prematurely released in the blood but is released within the tumour cell; and a cytotoxic payload that will induce tumour cell apoptosis through the targeting of key components (DNA, microtubules, topoisomerase 1). **b**, ADC cytotoxicity requires key sequential steps: (1) binding to cognate antigen; (2) internalization of the ADC–antigen complex; (3) lysosomal degradation of the antibody portion; (4) release of payload within the cytoplasm; and (5) interaction with target. A fraction of the payload may be released in the extracellular environment (6) where it can be taken up by neighbouring cells (7), a process known as the bystander effect.

Dumontet, C., Reichert, J.M., Senter, P.D. et al. Antibody–drug conjugates come of age in oncology. Nat Rev Drug Discov 22, 641–661 (2023). https://doi.org/10.1038/s41573-023-00709-2

- “Selectively” deliver toxic payloads to cancer cells with a specific target sparing healthy cells
- Goal: Increase therapeutic index (typical TI are around 3 for ADCs)



Antibody Drug Conjugates (ADCs) II

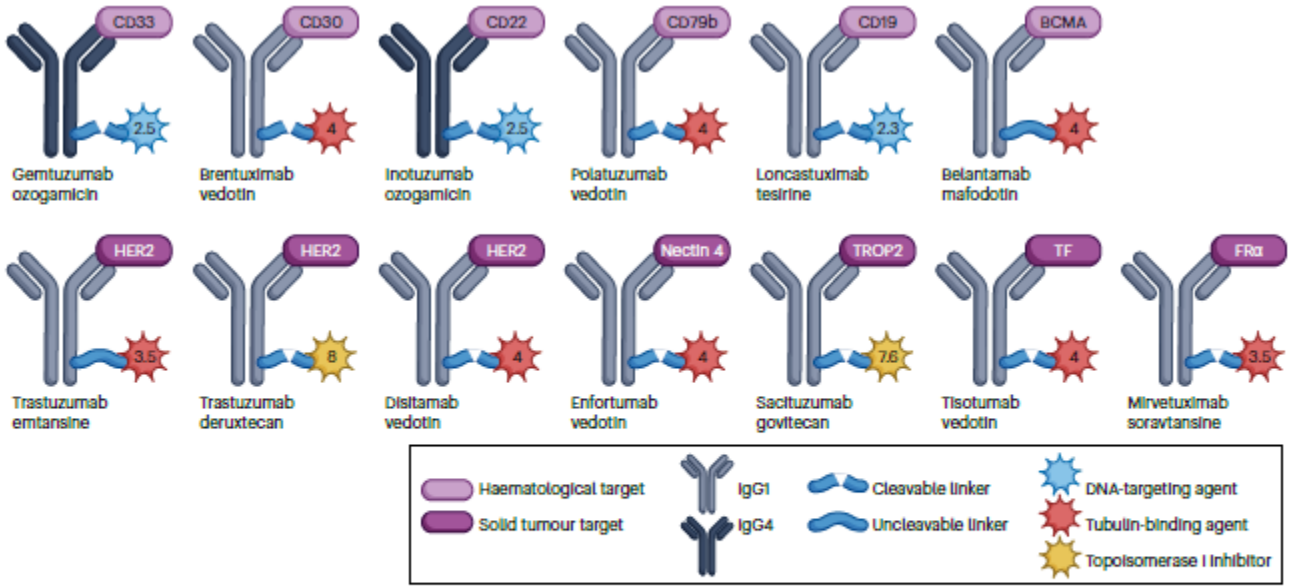
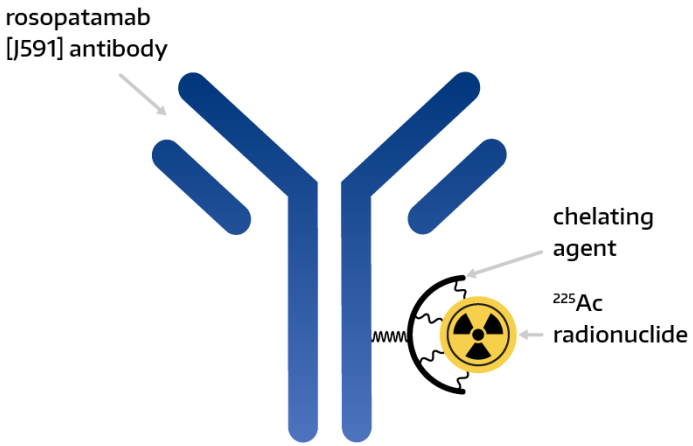


Fig. 2 | Main characteristics of approved ADCs. The similarities and differences among approved ADCs. Of the 13 approved ADCs, 6 target haematological indications (top row) and 7 target solid-tumour indications (bottom row), including 3 that target HER2 antigen. Eleven of these ADCs belong to the IgG1 subclass, which has a crystallizable fragment (Fc) portion that can effectively bind to and activate Fcγ-receptor-expressing cells, whereas others belong to the IgG4 subclass, which naturally has a lower affinity for Fcγ receptors. Various linker technologies have been used. These linkers are categorized as being cleavable (broken chain) or uncleavable (continuous chain). Payload colour indicates DNA-targeting agents in blue, tubulin binders in red and topoisomerase I inhibitors in yellow. The values given for the payloads indicate the DARs. BCMA, B cell maturation antigen; TF, tissue factor; TROP2, tumour-associated calcium signal transducer 2.

- Important parameters:
  - Target: typical examples: HER2, cMet, Trop2, CD79b (liquid tumor) (as “cleaner” and “homogenous”) as better
  - Target: internalization important
  - Toxin (Tubulin inhibitors, topoisomerase inhibitors, PDBs…)
  - Linker, cleavable vs. non cleavable
  - Drug antibody ratio (DAR): typically, 2-8
  - Site directed vs. random conjugation

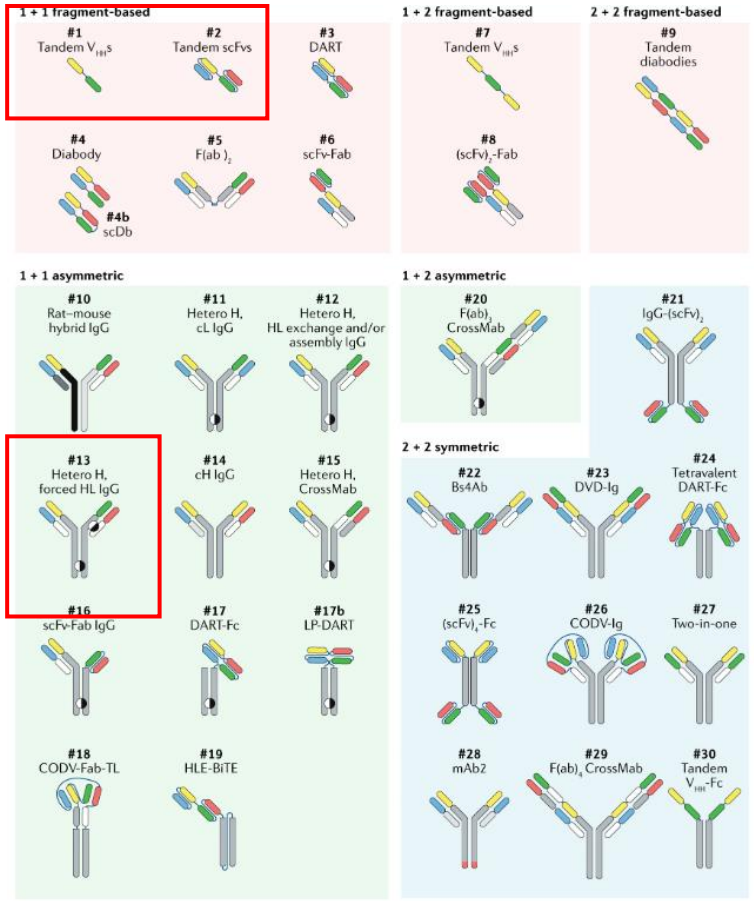
Other conjugates

- Antibody radio conjugates
  - Deliver alpha, beta or gamma emitter to tumor side (most used isotopes 225 Actinium,
  - Very efficient
  - Low resistance
  - Short half life needed
  - Secondary tumors
  - Difficult supply and waste chain
  - Difficult production (heating for chelator load)
- Antibody cytokine conjugates
  - Deliver “toxic” immunomodulatory cytokines in inflammatory or cancer lesion
- Antibody ASO (antisense oligo), siRNA etc.. conjugate
  - Silence genes in target cells

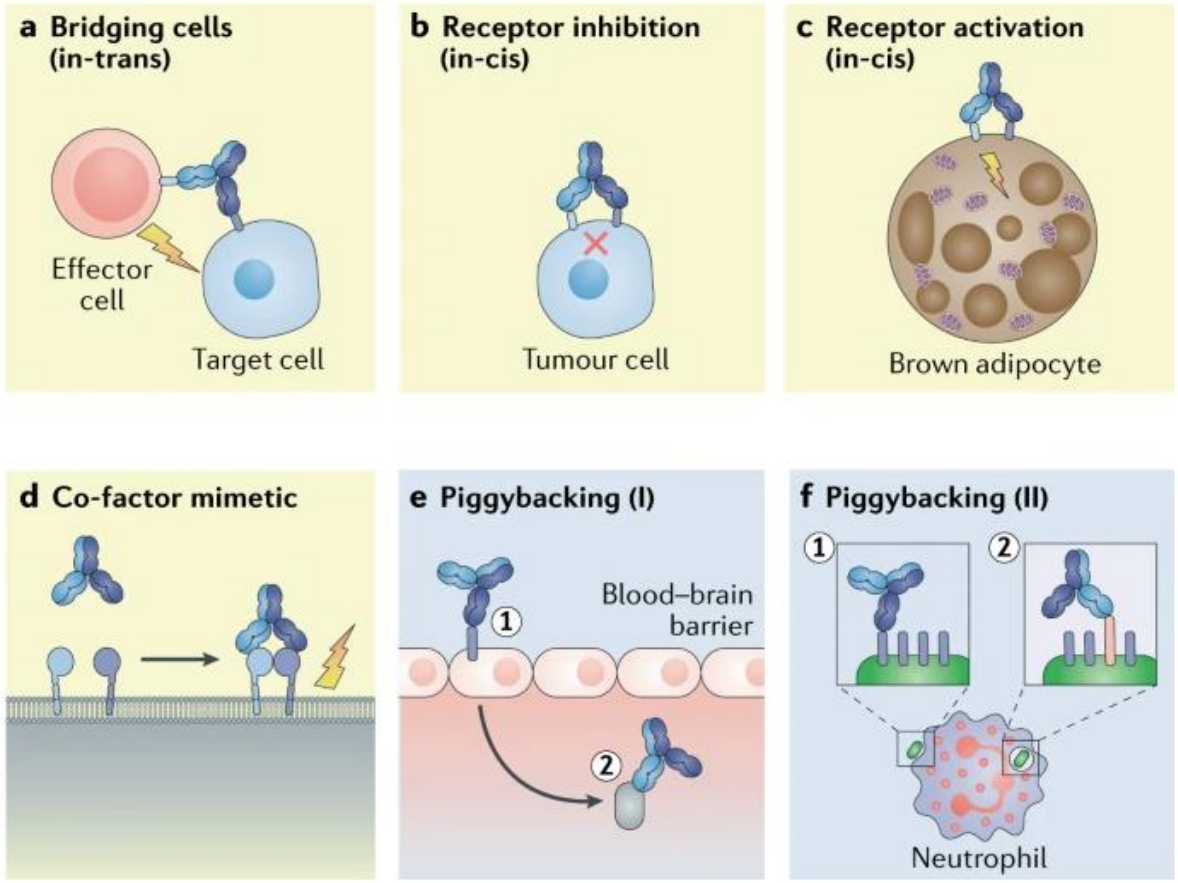


Bi-specific Antibodies

A zoo of formats:

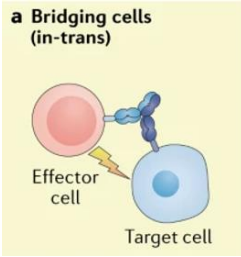


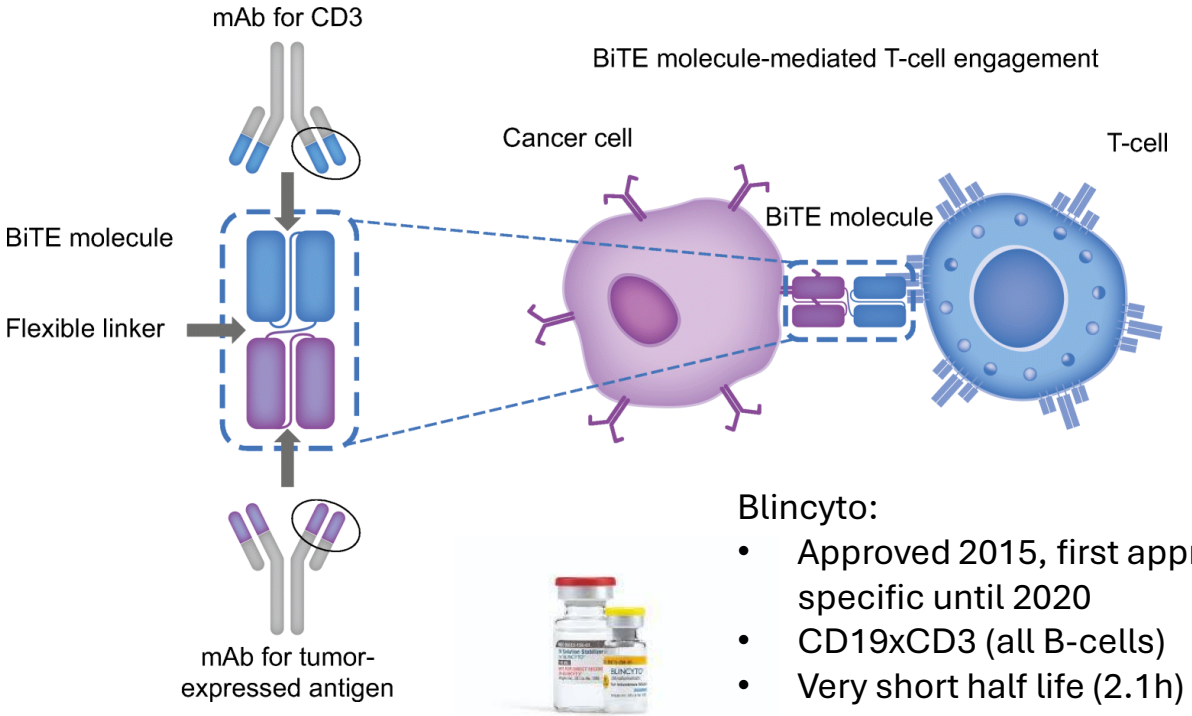
Modes of action:



Labrijn, A.F., Janmaat, M.L., Reichert, J.M. *et al.* Bispecific antibodies: a mechanistic review of the pipeline. *Nat Rev Drug Discov* **18**, 585–608 (2019). https://doi.org/10.1038/s41573-019-0028-1

T-cell engager





Blincyto:

- Approved 2015, first approved bi-specific until 2020
- CD19xCD3 (all B-cells)
- Very short half life (2.1h) continuous infusion needed
- Strong cytokine release

T cells are very effective killer cells (more efficacious than NK cells), recruiting them to (tumor) cells, clustering the CD3 and trigger the killing.

CRS:

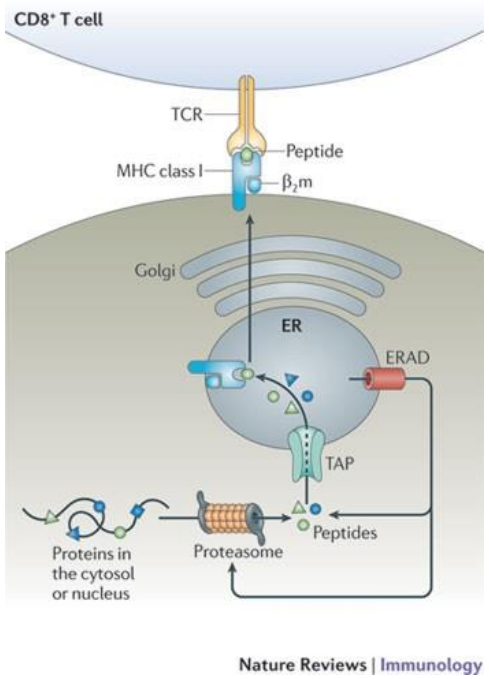
- Early generation lead to massive release of cytokines (CRS (Cytokine release syndrome))
- Solution: Decrease affinity forCD3

- Applications:
  - Early applications were liquid cancer only
  - Increasing use in autoimmune diseases (deplete all antibody producing cells)

Viardot, A., Locatelli, F., Stieglmaier, J. *et al.* Concepts in immuno-oncology: tackling B cell malignancies with CD19-directed bispecific T cell engager therapies. *Ann Hematol* **99**, 2215–2229 (2020). https://doi.org/10.1007/s00277-020-04221-0

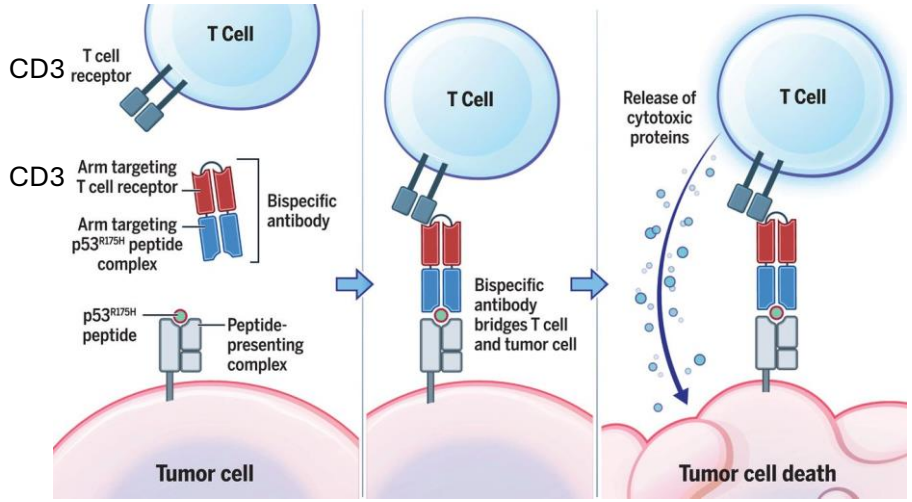
How to adress intracellular targets

TCR or TCR mimicking antibodies bispecifics



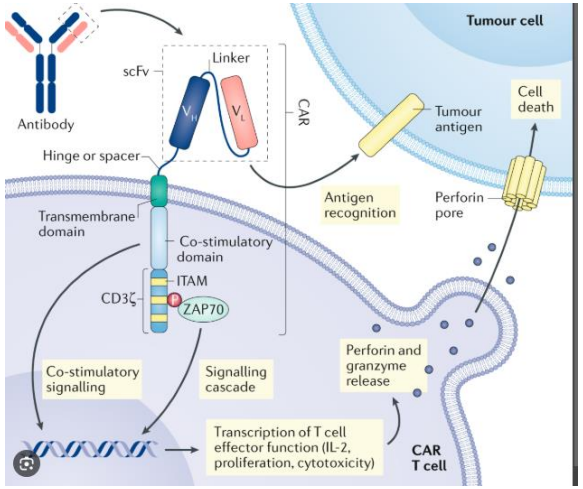
Neefjes, J., Jongsma, M., Paul, P. *et al.* Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol* **11**, 823–836 (2011). https://doi.org/10.1038/nri3084

- Fragments (9 aa) of (all) cellular proteins (also viral etc.) are presented on the surface of (most) cells on MHC molecules
- MHC/peptide complex can be selectively recognized by T cell receptors on T-cells
- T cell recognize foreign (or mutated) peptides and kill cell
- Recombinant TCRs or antibodies raised against MHC/peptide can be used with T-cell recruiters to target specific MHC/peptides and so make intracellular proteins accessible.
- Complexities: different MHC allotypes, low copy numbers, binders difficult to generate
- One product (Tebentafusp) on the market

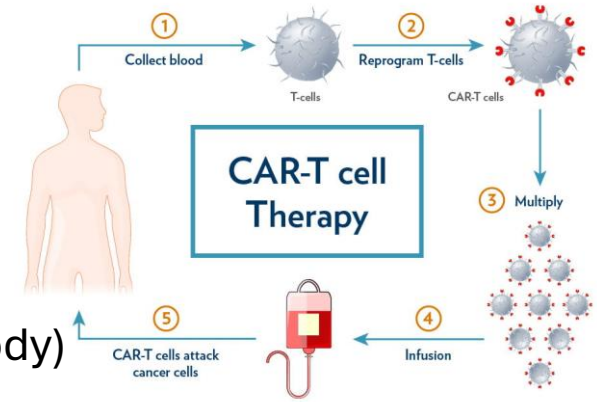


Emily Han-Chung Hsiue *et al.*
Targeting a neoantigen derived from a common *TP53* mutation.*Science***371**,eabc8697(2021).DOI:10.1126/science.abc8697

CAR-T cells



Emily Whitehead 10years after treatment of her pediatric AML with the firs CAR-T therapy (2012)



Features of CAR-T cells:

- Very effective (brings very efficient killer with antibody)
  - Fast onset of action
  - Very deep deletion
- Living drug:
  - As long antigen is present, cell proliferate
  - Long persistence
  - Long duration of response
- Effective fast killing might lead to cytokine release syndrome (CRS)
- Very complex process (patient specific)
- Very expensive (approx. USD 1m/treatment)
- Pretty much restricted to liquid tumors
- Main products: anti-CD19
- Recent use also in autoimmune disease

Larson, R.C., Maus, M.V. Recent advances and discoveries in the mechanisms and functions of CAR T cells. Nat Rev Cancer 21, 145–161 (2021). https://doi.org/10.1038/s41568-020-00323-z

Extracellular targeted protein degradation: an emerging modality for drug discovery

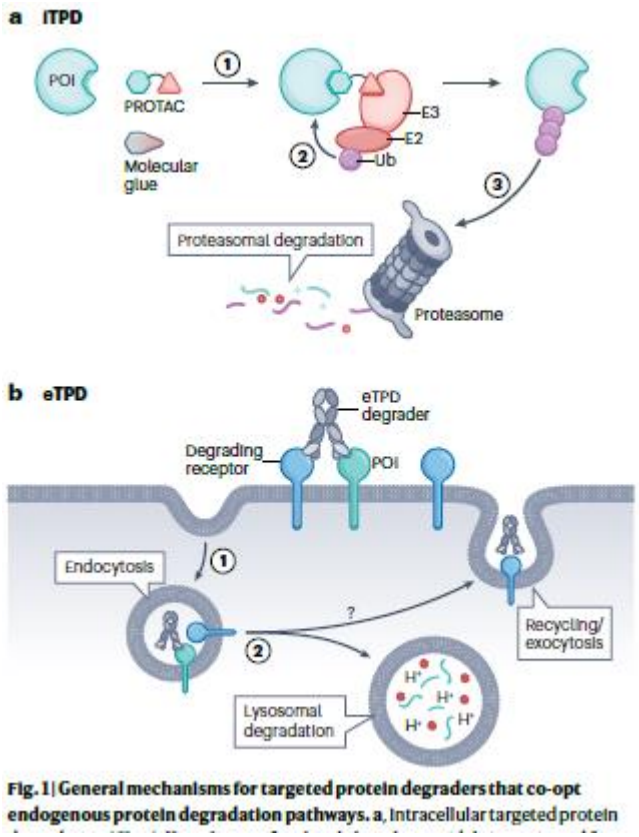
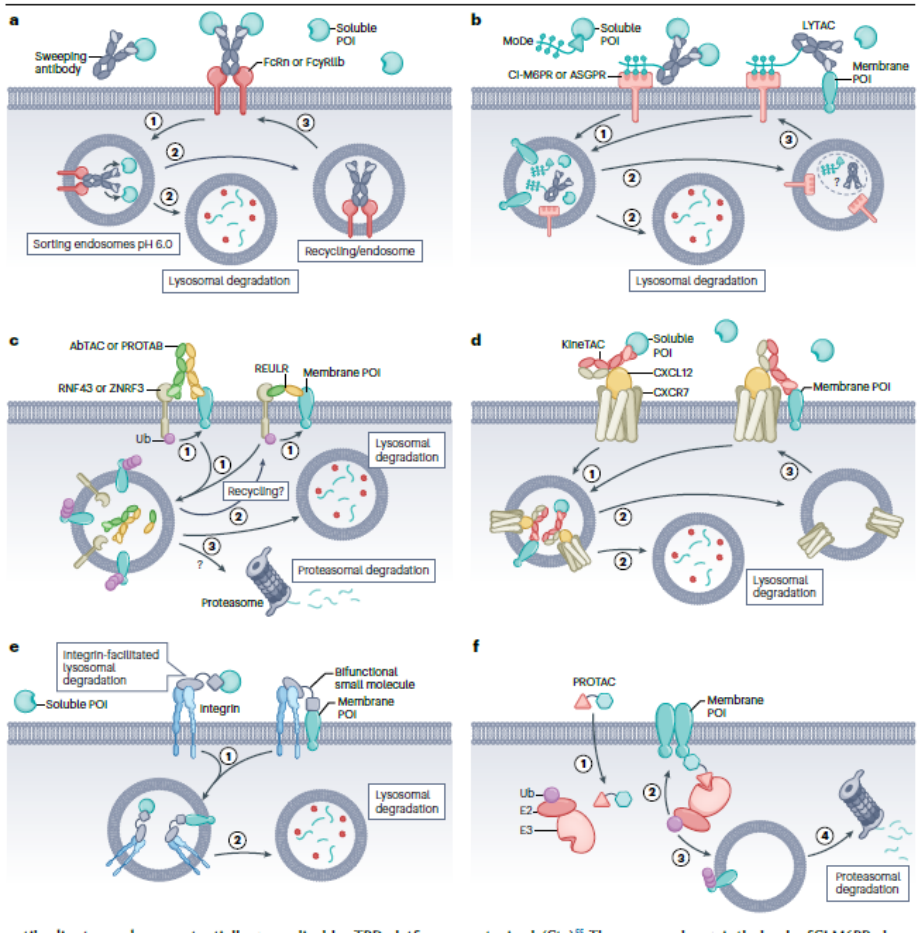


Fig. 1 | General mechanisms for targeted protein degraders that co-opt endogenous protein degradation pathways. a, Intracellular targeted protein

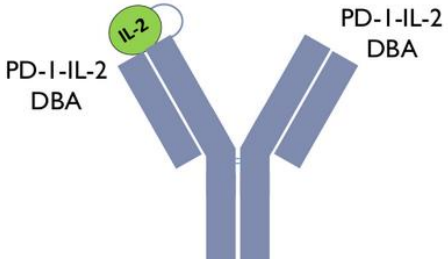


Wells, J.A., Kumru, K. Extracellular targeted protein degradation: an emerging modality for drug discovery. *Nat Rev Drug Discov* **23**, 126–140 (2024). https://doi.org/10.1038/s41573-023-00833-z

Smart antibodies:

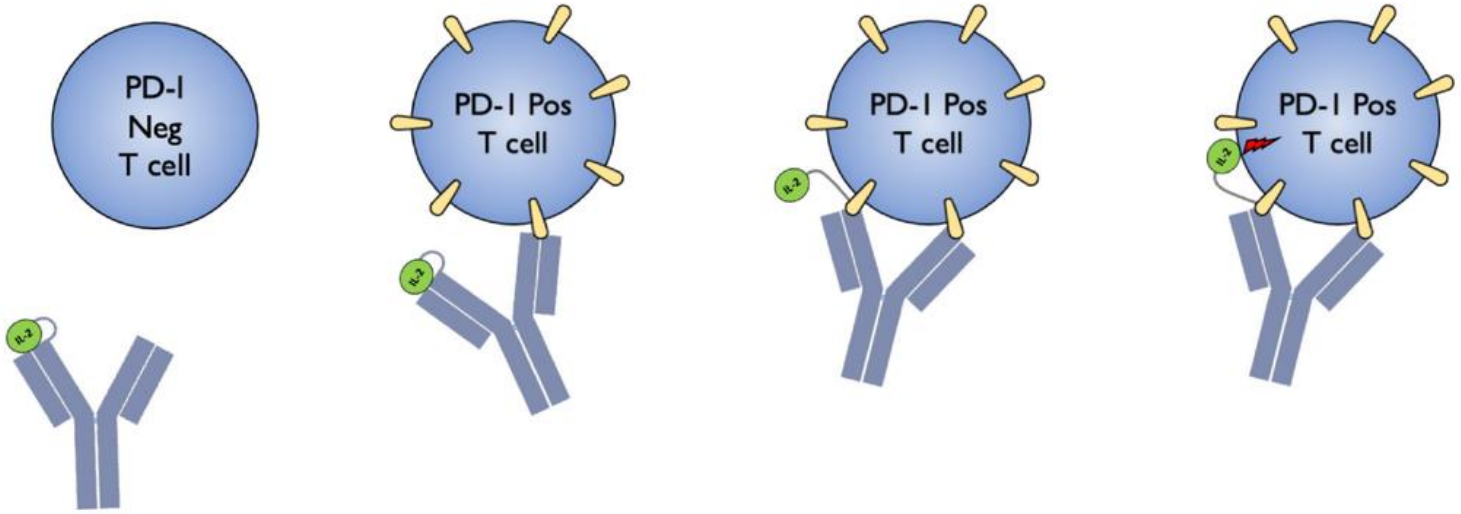
Good Therapeutics Concept: Regulated Immunocytokines (PD-1/IL2)

IL-2 only becomes active when antibody binds to PD-1 on the surface of T cells

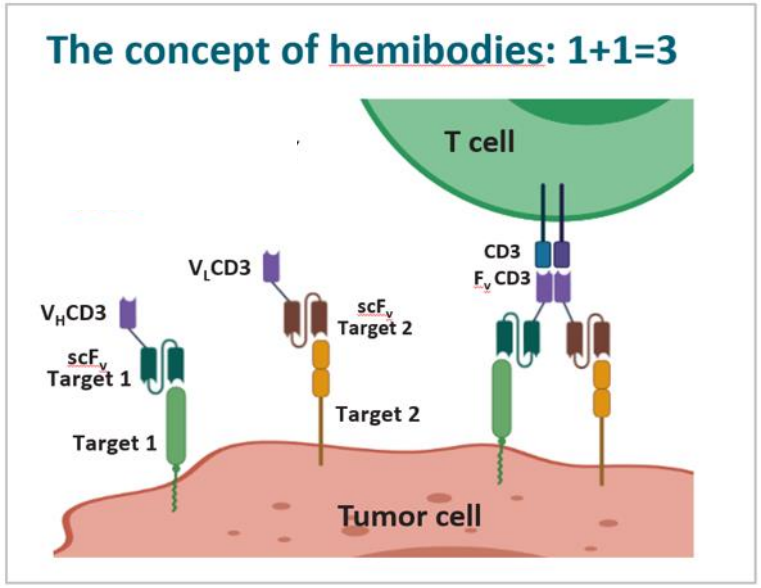


DBA: Dual-binding antibody for PD-1 and IL-2

- Binds PD-1 with high affinity
- Binds IL-2 with medium affinity
- Flexible linker connects IL-2 to Fab arm



Smart Antibodies: AND Gates

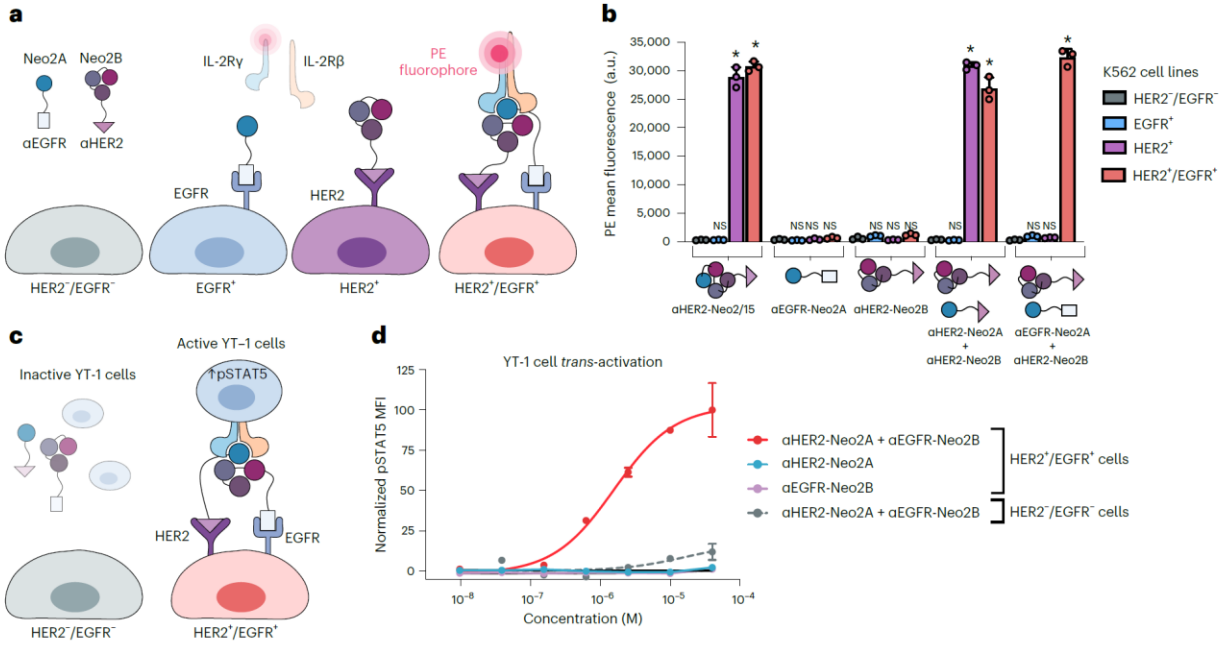


Key Features:

1. **Increased tumor selectivity by "AND Gate"**
2. **Decreased CRS and wider therapeutic window due to T-cell activation at tumor site only**
3. **Broad potential applications beyond T-cell engagers**

https://www.cherrybiolabs.com/technology/

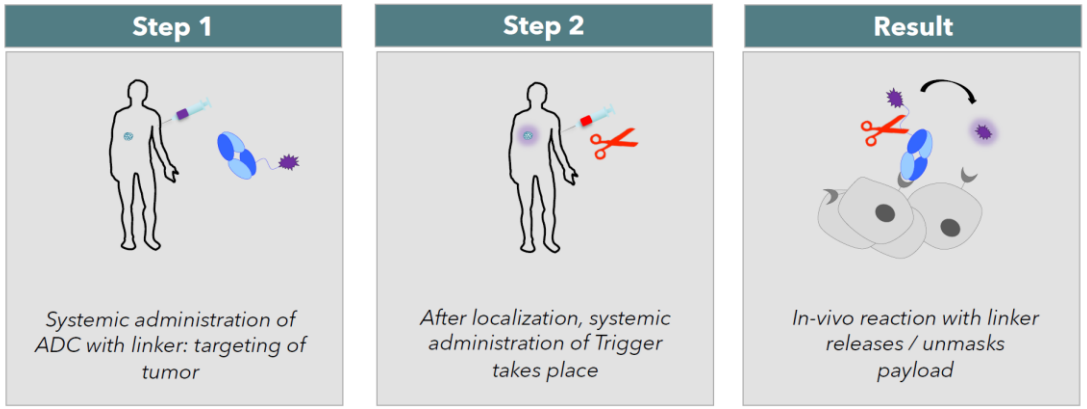
Baker's split cytokine approach

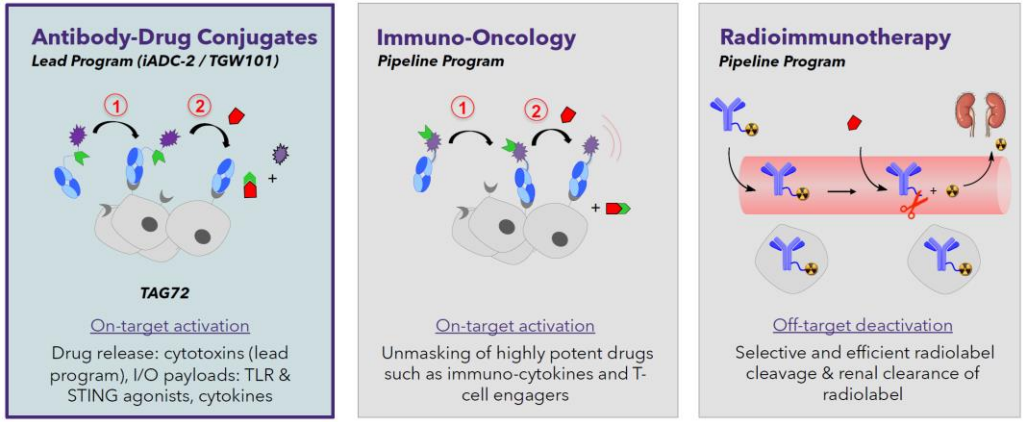


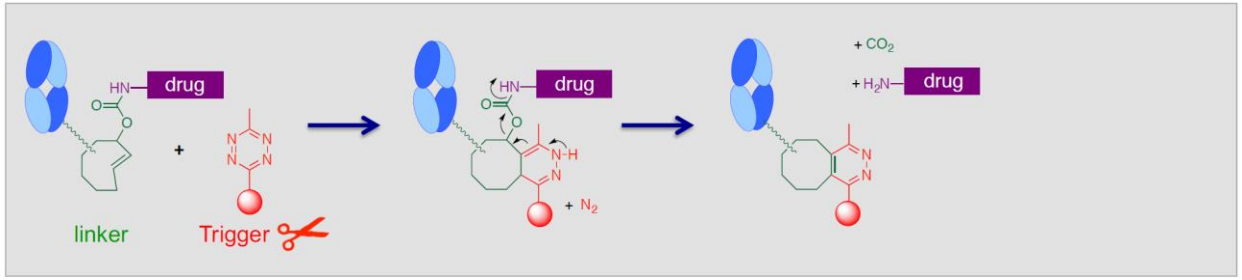
Quijano-Rubio, A., Bhuiyan, A.M., Yang, H. *et al.* A split, conditionally active mimetic of IL-2 reduces the toxicity of systemic cytokine therapy. *Nat Biotechnol* **41**, 532–540 (2023). https://doi.org/10.1038/s41587-022-01510-z

Switchable Biologics

Activation by click chemistry



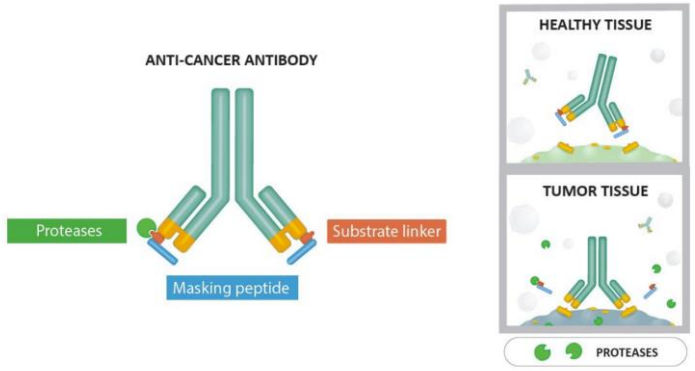




https://www.tagworkspharma.com/pipeline

Tumor activated antibodies

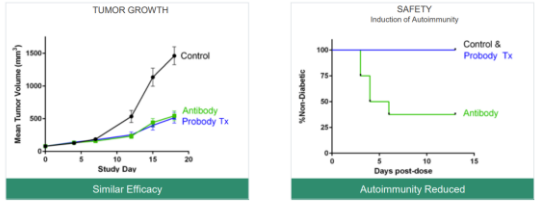
Tumor protease induced (irreversible)



CytomX Therapeutics

13

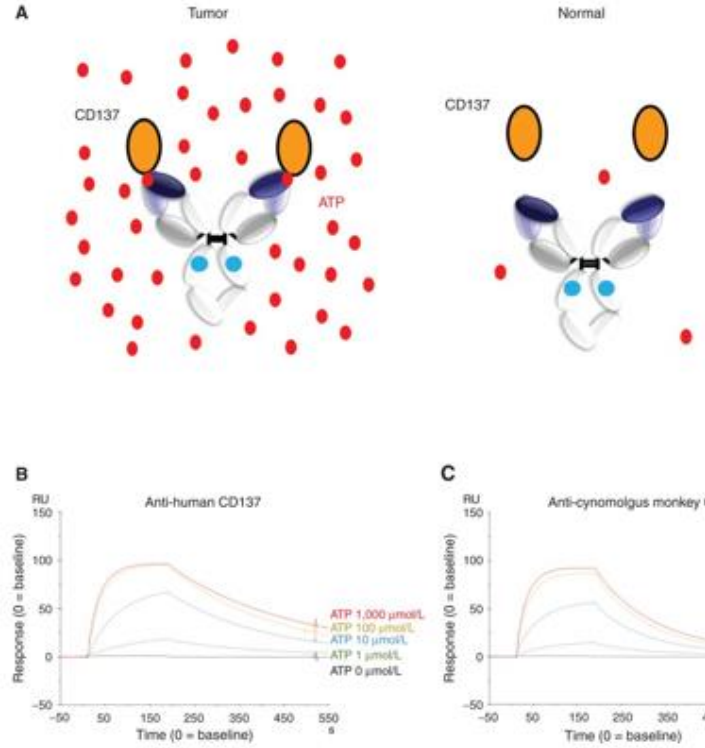
Preclinical Proof of Concept for CX-072:
A PD-L1 Probody Therapeutic with Antitumor Efficacy, Improved Safety



CytomX Therapeutics

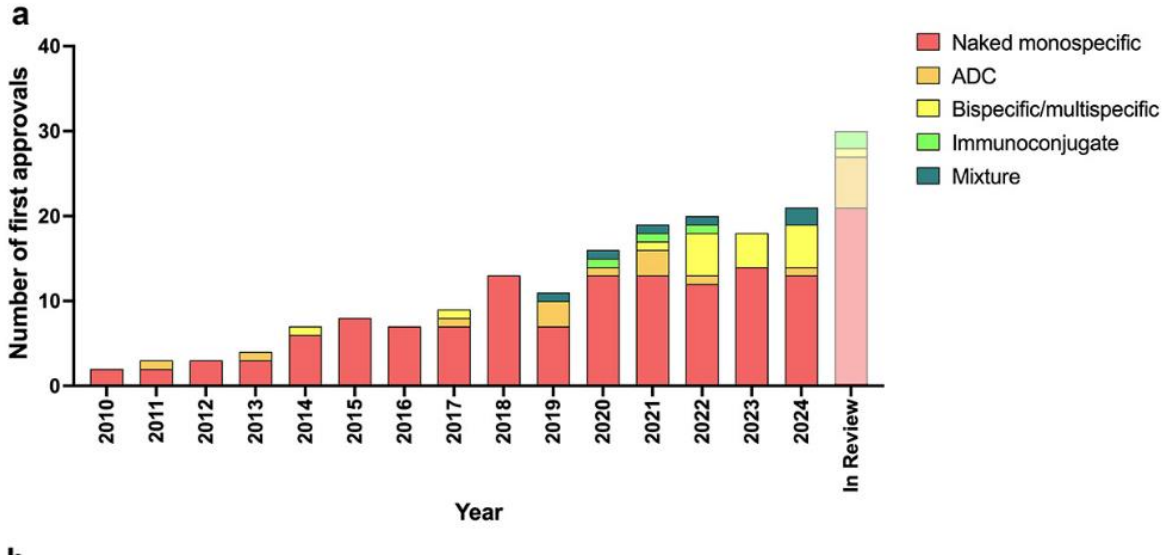
15

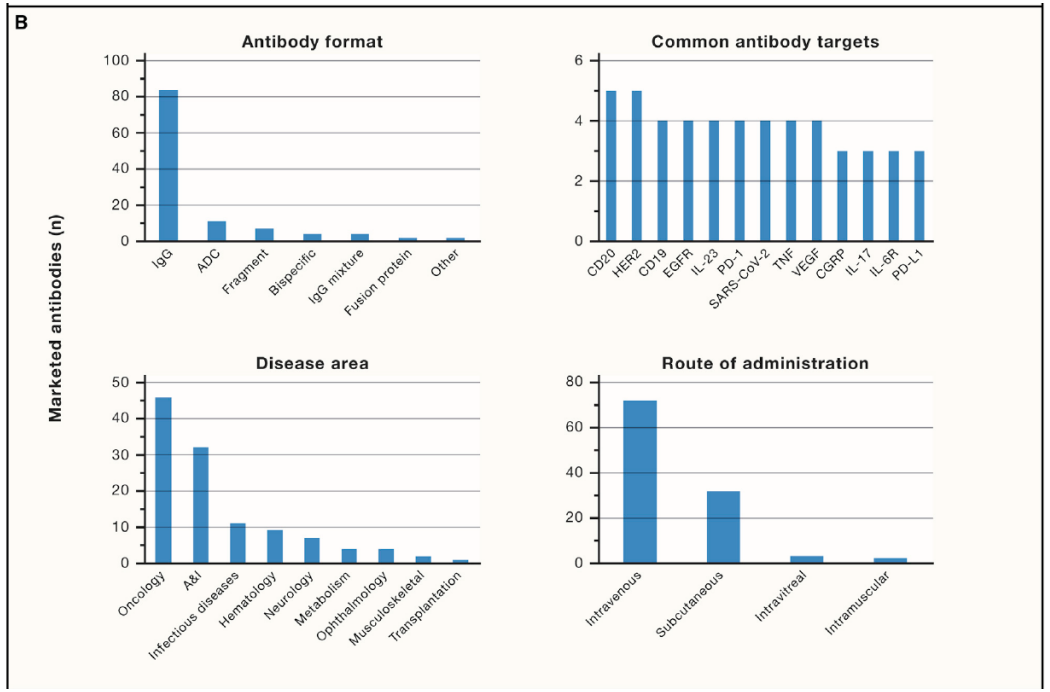
Tumor metabolite (ATP) triggered (reversible)



Cancer Discov. 2021;11(1):158-175. doi:10.1158/2159-8290.CD-20-0328

Outlook and summary





Silvia Crescioli, Hélène Kaplon, Lin Wang, Jyothsna Visweswaraiah, Vaishali Kapoor & Janice M. Reichert (2025) Antibodies to watch in 2025, mAbs, 17:1, 2443538, DOI: 10.1080/19420862.2024.2443538

Carter, Paul J. et al.
Cell, Volume 185, Issue 15, 2789 - 2805