Antibody Drug Discovery

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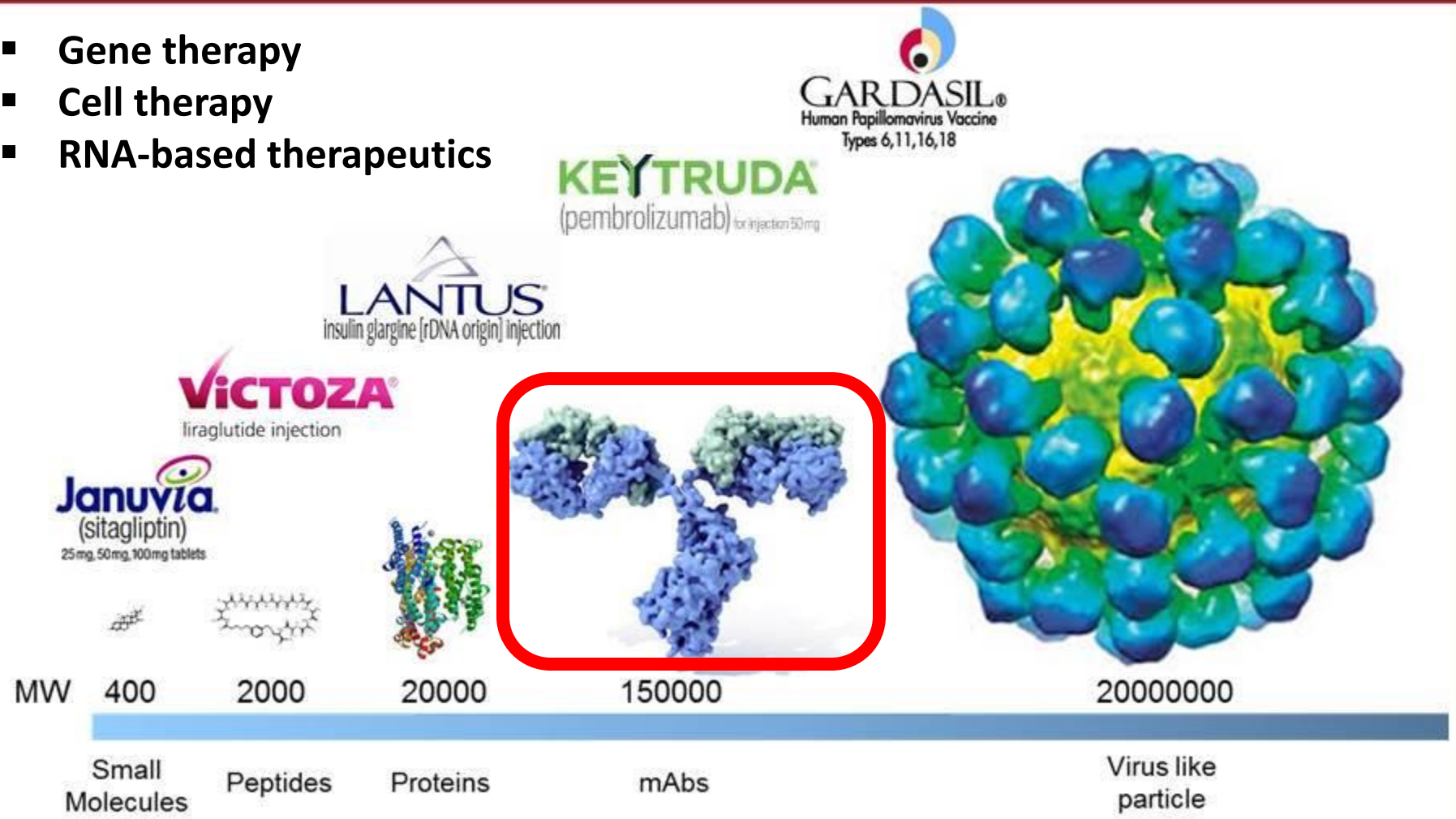
TIPS-CTTP Drug Discovery Course

The Brown Foundation
Institute of Molecular Medicine
for the Prevention of Human Diseases

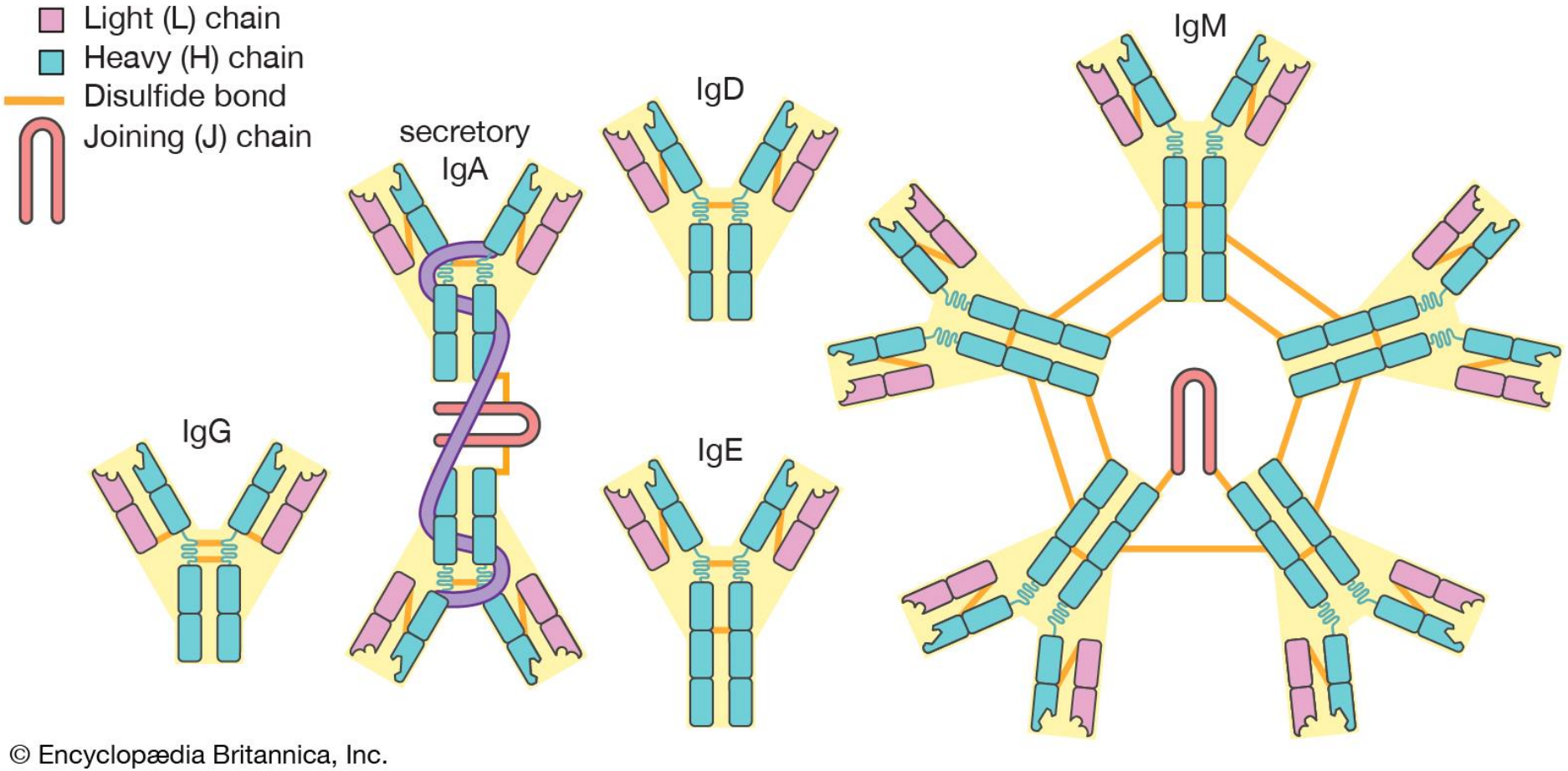
The University of Texas
Health Science Center at Houston

Drugs come as different Modalities

- **Gene therapy**
- **Cell therapy**
- **RNA-based therapeutics**



Antibody types and structures



Uses of antibodies in cell biology

Applications:

Western blotting (Immunoblotting)

- Identification of protein antigen following SDS-PAGE

Immunoprecipitation

- Isolation of specific proteins + binding partners

Immunofluorescence microscopy

- Localization of specific proteins in cells

ELISA (Enzyme-Linked Immunosorbent Assay)

- Detection of proteins in a sample

Antibody diversity

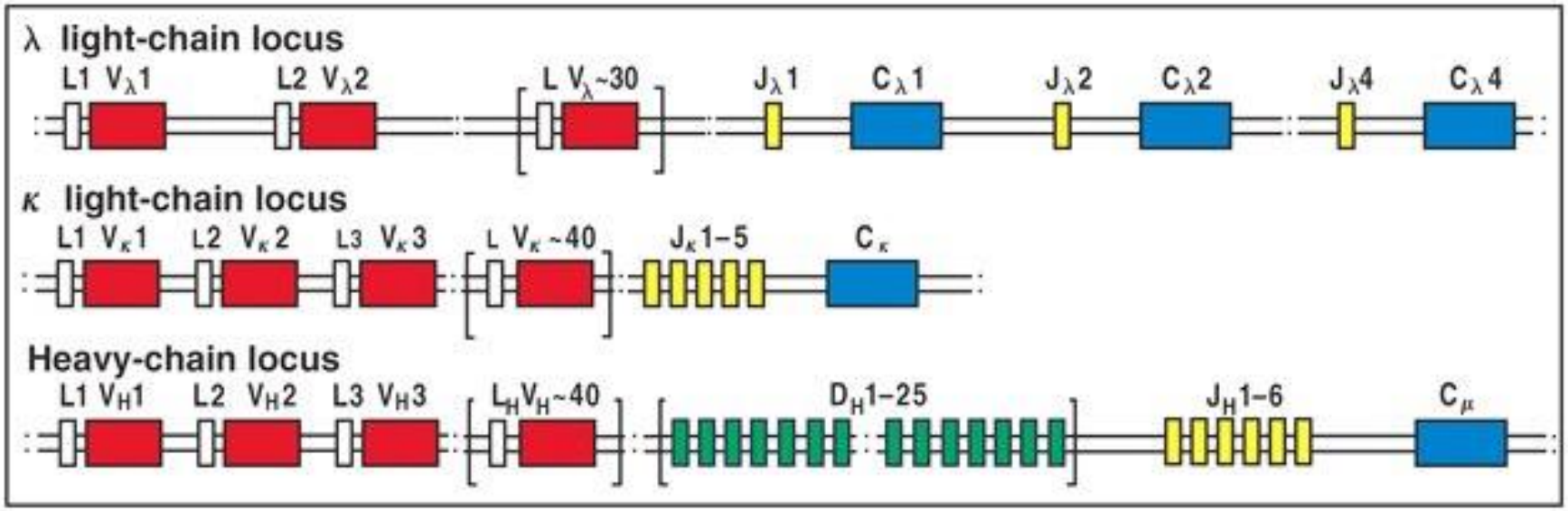


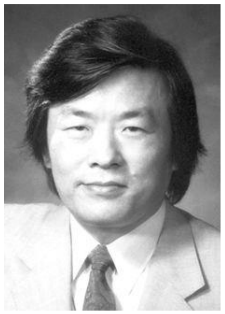
Figure 4-4 Immunobiology, 6/e. (© Garland Science 2005)

The Nobel Prize in Physiology or Medicine

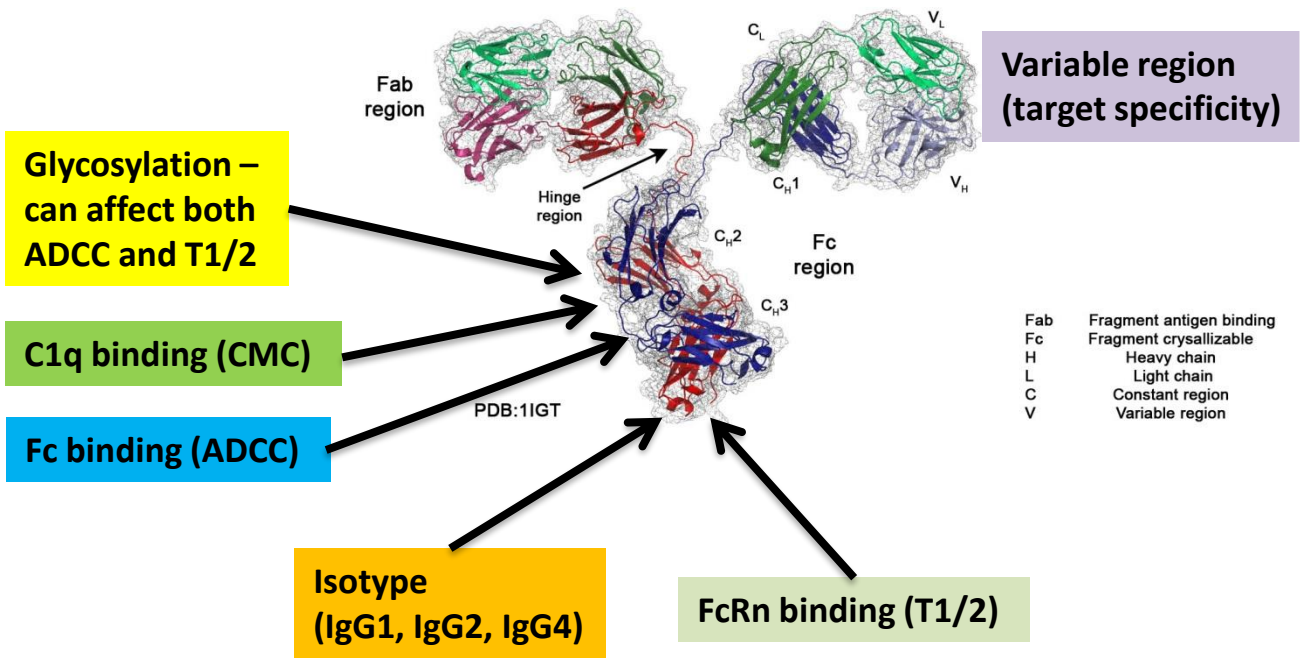
1987

"for his discovery of the genetic principle for generation of antibody diversity"

Susumu Tonegawa



Antibody functions



Variable region (target specificity)

Glycosylation – can affect both ADCC and T1/2

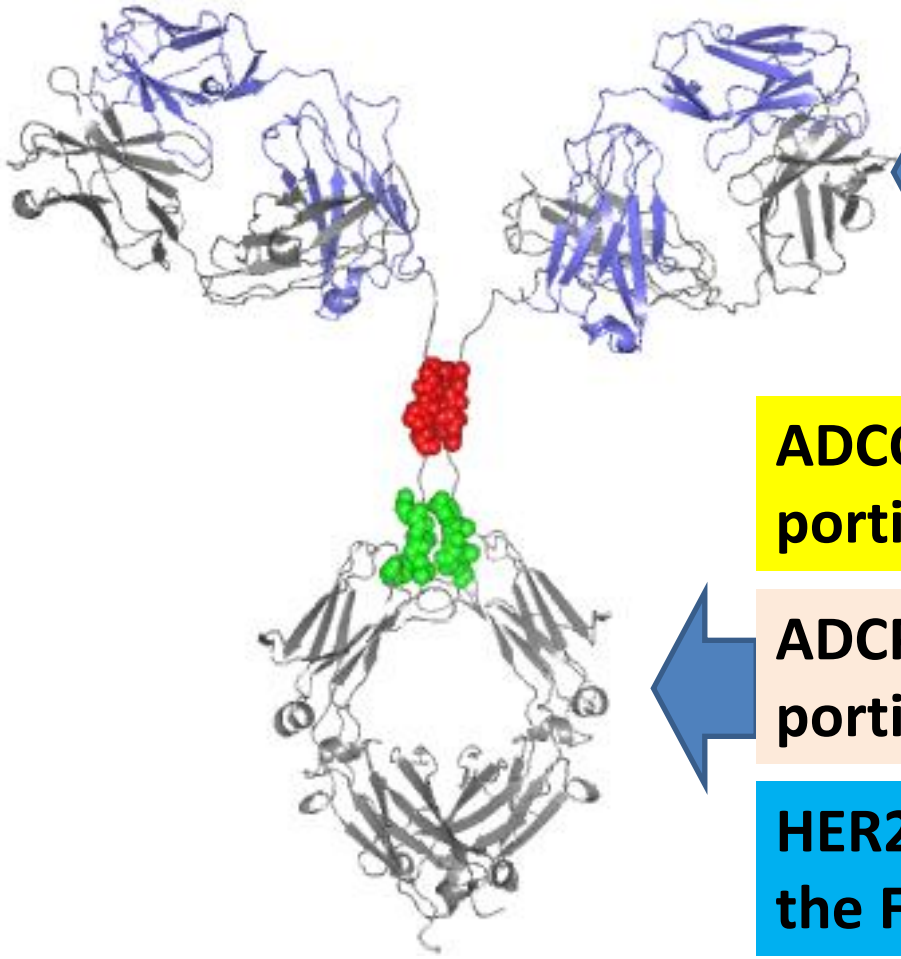
C1q binding (CMC)

Fc binding (ADCC)

Isotype (IgG1, IgG2, IgG4)

FcRn binding (T1/2)

Trastuzumab Mode of Actions



Inhibition of HER2 downstream signaling mediated by the Fab portion of the antibody

ADCC mediated by the Fc portion of the antibody

ADCP mediated by the Fc portion of the antibody

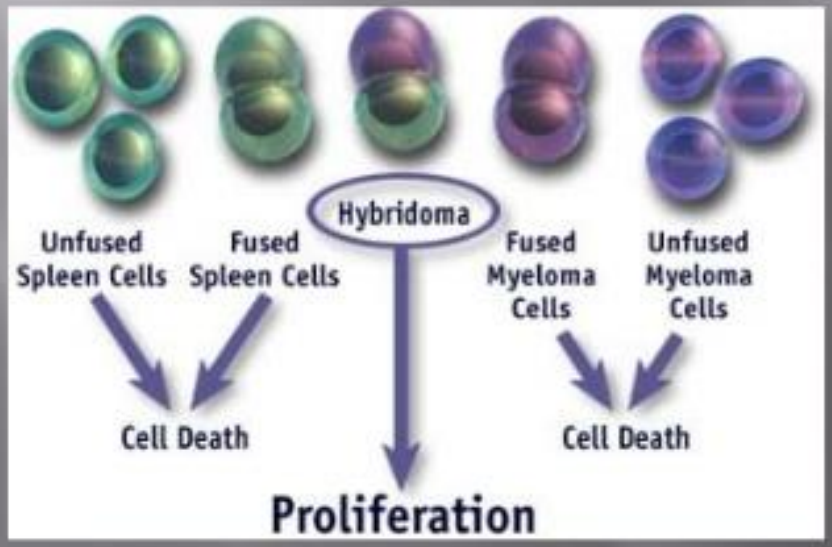
HER2 downregulation mediated by the Fc portion of the antibody

Sources of Antibody Genes

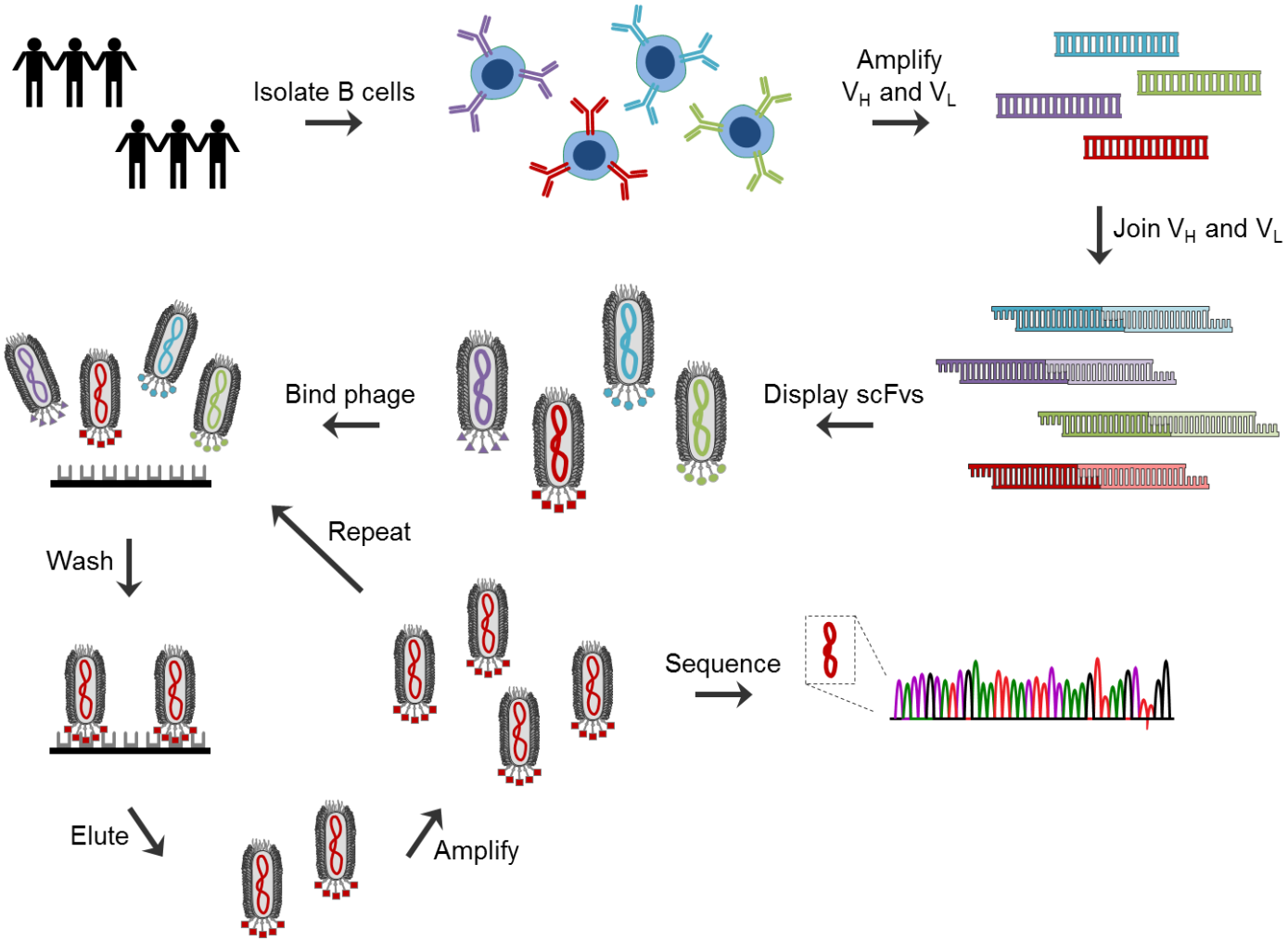
- **Mouse, rabbit, and other animal species**
  - **The old fashioned way...**
- **Humanized animals**
  - **Animals with human Ab genes (HuMAb-Mouse®, XenoMouse®, VelociMouse™, etc)**
- **Phage display libraries**
  - **Of affinity matured ab genes after immunization with desired target (Trans-Phage Technology®)**
  - **Of human Ab genes (CAT, Dyax, Morphosys, etc)**
- **Plasma antibody producing B-cells**
  - **Infectious diseases**
- **Memory B-cells**
  - **Autoantibodies for autoimmune diseases and cancer, and infectious diseases**

Hybridoma Technology

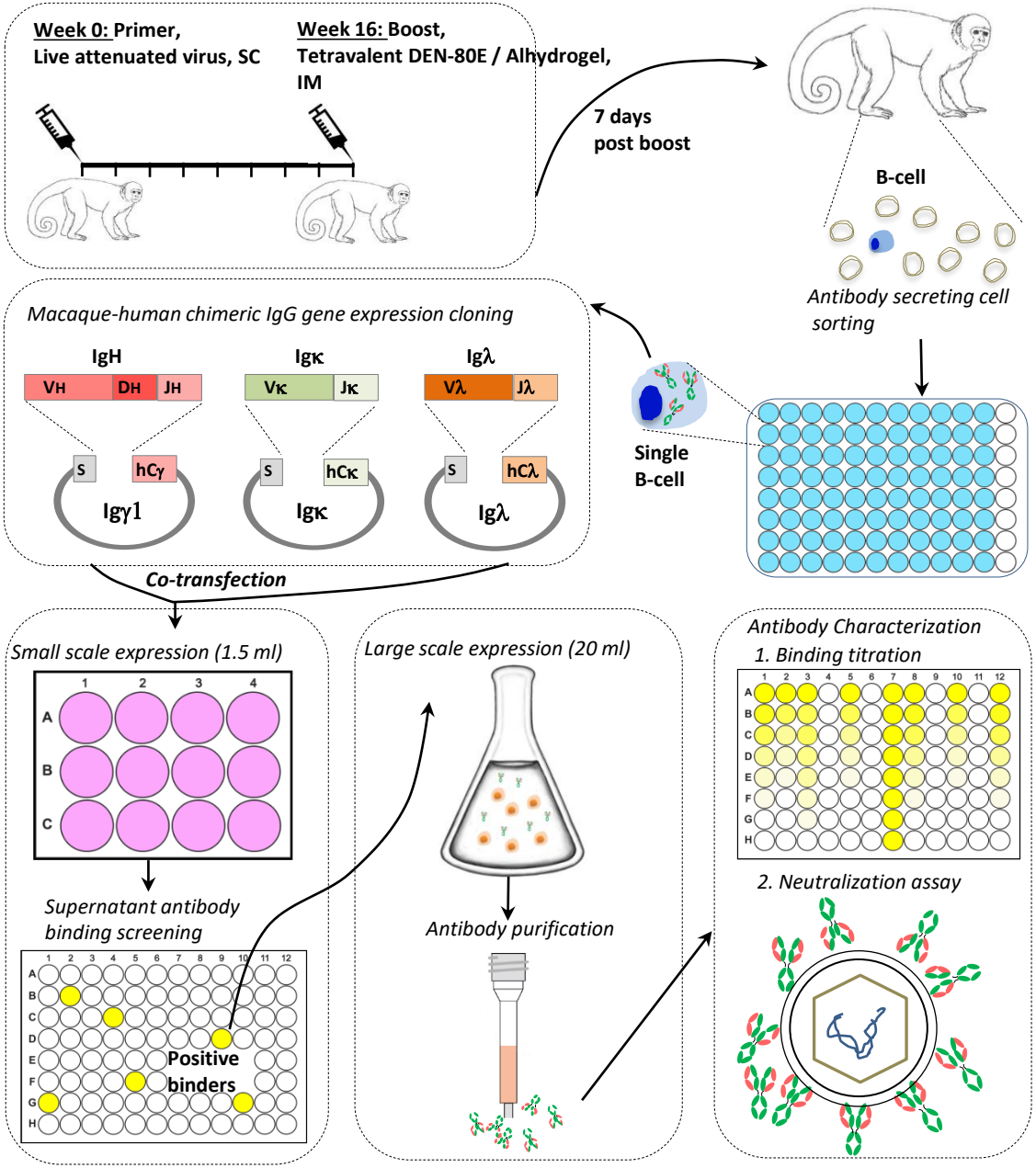
1975, Georges Köhler and Cesar Milstein - awarded Nobel Prize in1984



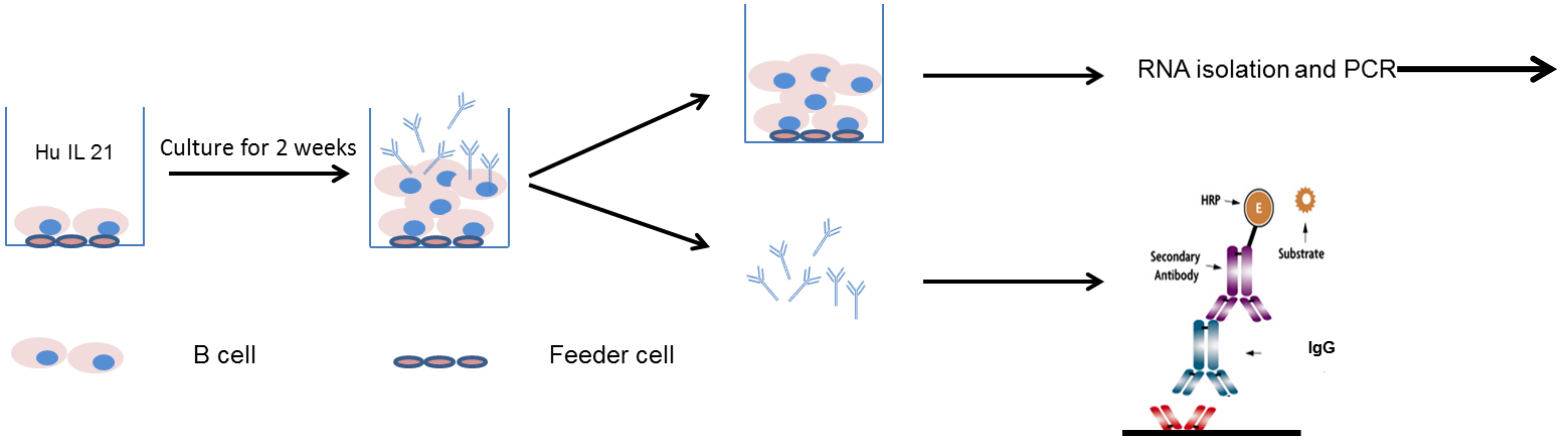
Human monoclonal antibodies using scFv phage display – 1 x10^{11}

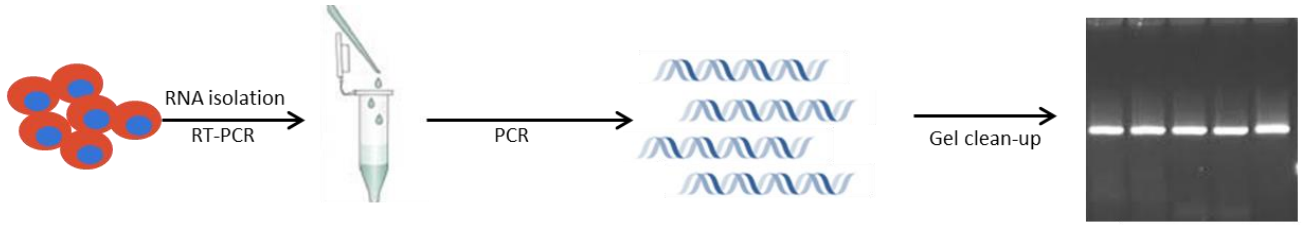


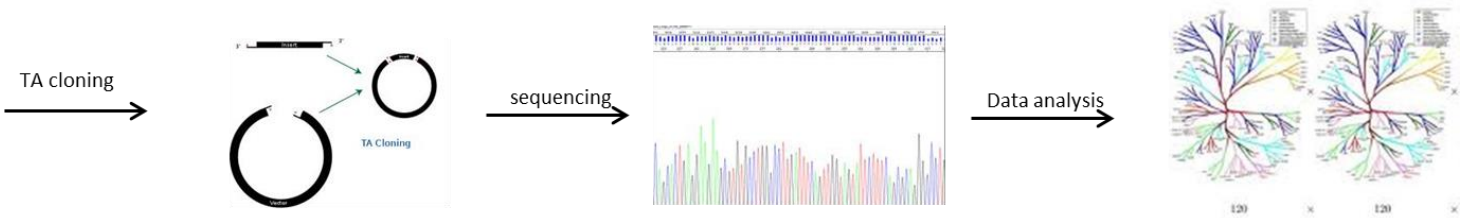
Cloning mAbs from plasma B cells



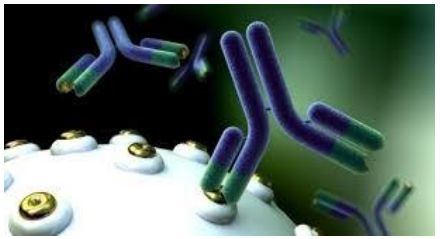
Cloning mAbs from memory B cells



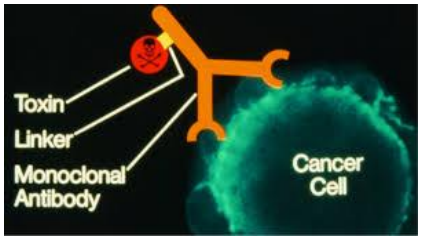




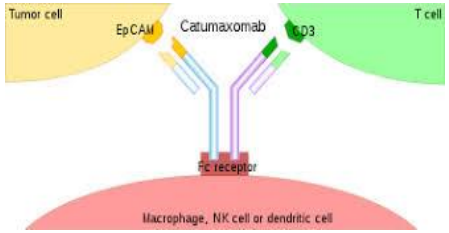
Antibody-based drug modalities



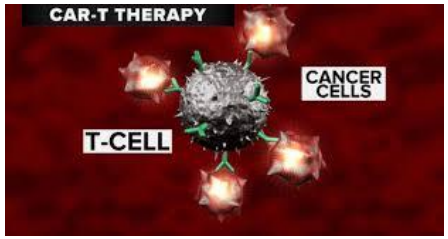
mAbs



ADCs, AACs, ARCs



Bispecific Antibodies



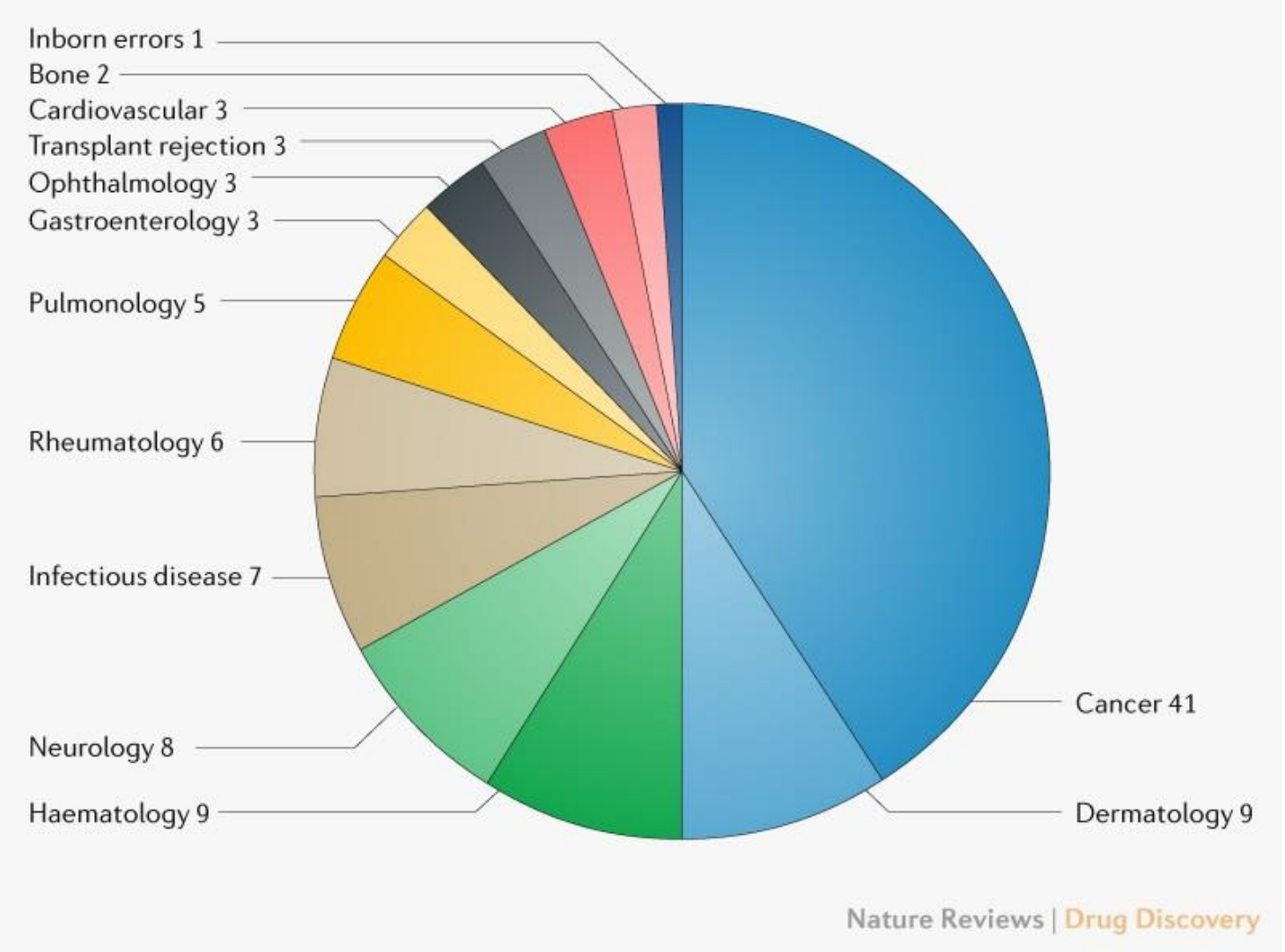
CAR-T Cell Therapies

Antibody isotypes: IgG1, IgG2, IgG4, IgGs with engineered Fcs, etc

Different sizes and formats: IgGs, fragments, nanobodies, ADCs, mAb-protein fusions, etc.

Origins: animal, humanized, human, synthetic, immunization, libraries, etc.

Mechanisms of action: agonist, antagonist, immune effector functions, T-cell engaging, receptor internalization, antigen depletion, etc.



The FDA's first 100 antibody approvals, by therapeutic area. Therapeutic areas are based on the indication of the first approval only. Cancer includes haematological malignancies. Sources: The Antibody Society, Drugs@FDA, *Nature Reviews Drug Discovery*.

Of the top 45 best selling drugs in 2021, 22 are antibody-based

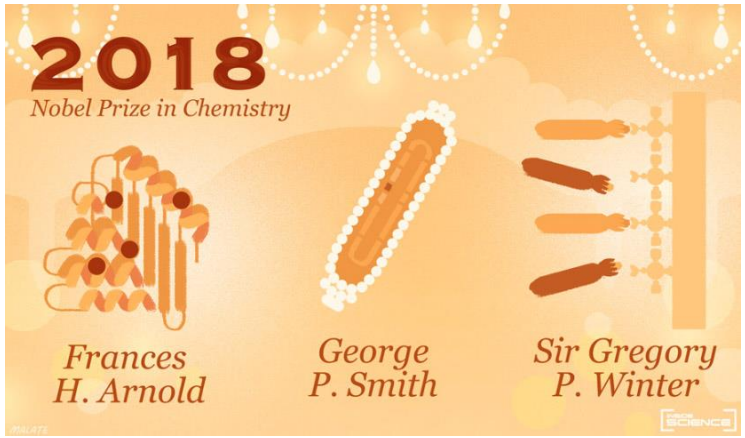
Rank	Product	Company	Target/indication	Sales ($ millions)
1	Humira (adalimumab)	AbbVie	Anti-TNFα, immune diseases	20,694
2	Keytruda (pembrolizumab)	Merck & Co.	Anti-PD1, cancer	17,186
6	Eylea (aflibercept)	Regeneron/Bayer	Anti-VEGF-Fc-fusion, AMD	9,244
7	Stelara (ustekinumab)	Johnson & Johnson	Anti-IL-12/IL-23, immune diseases	9,134
10	Opdivo (nivolumab)	Bristol-Myers Squibb	Anti-PD1, cancer	7,523
12	Dupixent (dupilumab)	Sanofi/Regeneron	IL-4Rα, immune diseases	6,209
13	Darzalex (daratumumab)	Johnson & Johnson	CD38, multiple myeloma, cancer	6,023
14	REGEN-COV	Regeneron/Bayer	SARS-CoV-2, COVID-19	5,828
20	Cosentyx (secukinumab)	Novartis	IL-17A, immune diseases	4,718
21	Ocrevus (ocrelizumab)	Roche	CD-20, multiple sclerosis	4,622
22	Enbrel (etanercept)	Amgen	TNFα-trap-Fc-fusion, immune diseases	4,465
25	Entyvio (vedolizumab)	Tekeda	Integrin $\alpha_4\beta_7$, immune diseases	3,908
27	Perjeta (pertuzumab)	Roche	Anti-HER2, Her2 positive breast cancer	3,616
30	Orencia (abatacept)	Bristol-Myers Squibb	CTLA-4 ECD Fc-fusion, immune diseases	3,306
31	Actemra (tocilizumab)	Roche	IL-6R, immune diseases	3,257
32	Prolia (denosumab)	Roach	RANKL, immune diseases	3,248
33	Remicade (infliximab)	Johnson & Johnson	Anti-TNFα, immune diseases	3,190
34	Tecentriq (atezolizumab)	Roche	PD-L1, cancer	3,031
35	Skyrizi (Risankizumab)	AbbVie	IL-23A, plaque psoriasis	2,939
39	Avastin (bevacizumab)	Roche	Anti-VEGF	2,794
41	Hemlibra (Emicizumab)	Roche	Coagulation factor IX&X, haemophilia A	2,787
44	Herceptin (trastuzumab)	Roche	Anti-HER2, Her2 positive breast cancer	2,463

Brian Buntz, March 29, 2022, Drug Discovery & Development

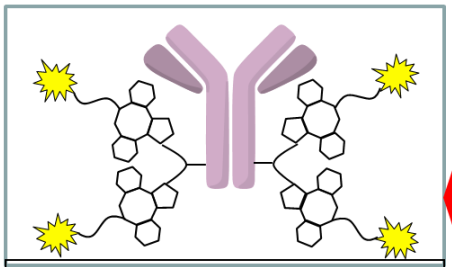
- **Antibody based drugs including cancer immunotherapies is the most active field in drug discovery and development**

- **The 2018 Nobel Prize in Chemistry were awarded to three scientists who pioneered protein engineering strategies which in part enabled antibody drug discovery**

- **The 2018 Nobel Prize in Physiology and Medicine were awarded to two scientists who developed immune check point inhibitors for cancer immunotherapy**



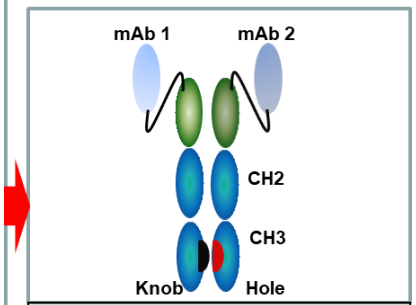
CPRIT Antibody Drug Discovery Cores



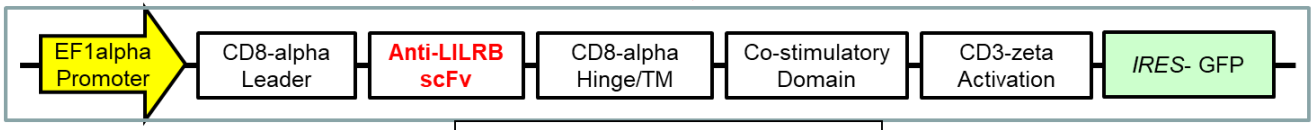
Antibody-Drug Conjugates (ADCs)

Monoclonal Antibody Generation, Engineering and Production

- Gene cloning and express recombinant antibody in HEK293 cells
- Binding & Functional assays
- Humanization
- Affinity maturation
- IgG conversion and lead selection
- Antibody drug conjugation (ADCs)
- Bi-specific antibodies
- IgG isotype and Fc engineering
- Antibody fragments
- IgG expression *E. coli* (scFvs/Fabs) Hybridoma HEK293 transient
- Immunize mice
- Generate and analyze hybridomas
- Immunize rabbits or monkeys
- Antibody memory and/or antibody producing plasma B cell sorting
- Screening phage displayed human antibody libraries
- Analysis of lead antibodies by ELISA and sequencing
- Binding & Functional assays
- HEK293 transient antibody expression
- CHO production cell line



Bispecific Antibodies (BsAbs)



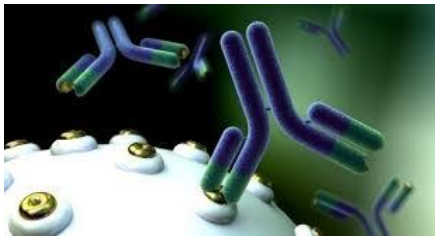
CAR-T Constructs

Preclinical development

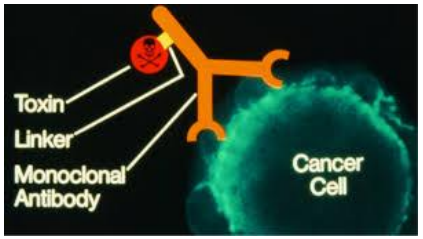
Antibody technologies

- **mAbs from immunized animals**
  - **Rabbits, mice, rat**
- **mAbs from plasma B cells**
- **mAbs from memory B cells**
- **mAbs from phage libraries**
- **Bispecific mabs**
- **ADCs**
- **CAR-T**
- **Stable CHO cell lines for antibody expression**
- **Antibodies crossing the BBB**
- **Generation of synthetic nanobody library using phage display**
- **Antibodies targeting complex membrane proteins**

Antibody-based drug modalities



mAbs



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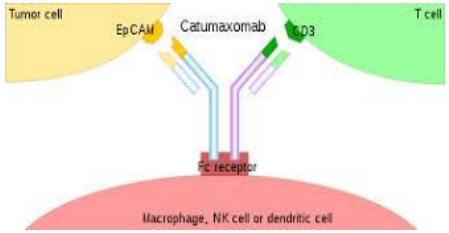

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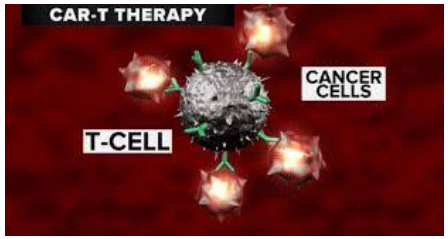

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Origins: animal, humanized, human, synthetic, immunization, libraries, etc.

Mechanisms of action: agonist, antagonist, immune effector functions, T-cell engaging, receptor internalization, antigen depletion, etc.

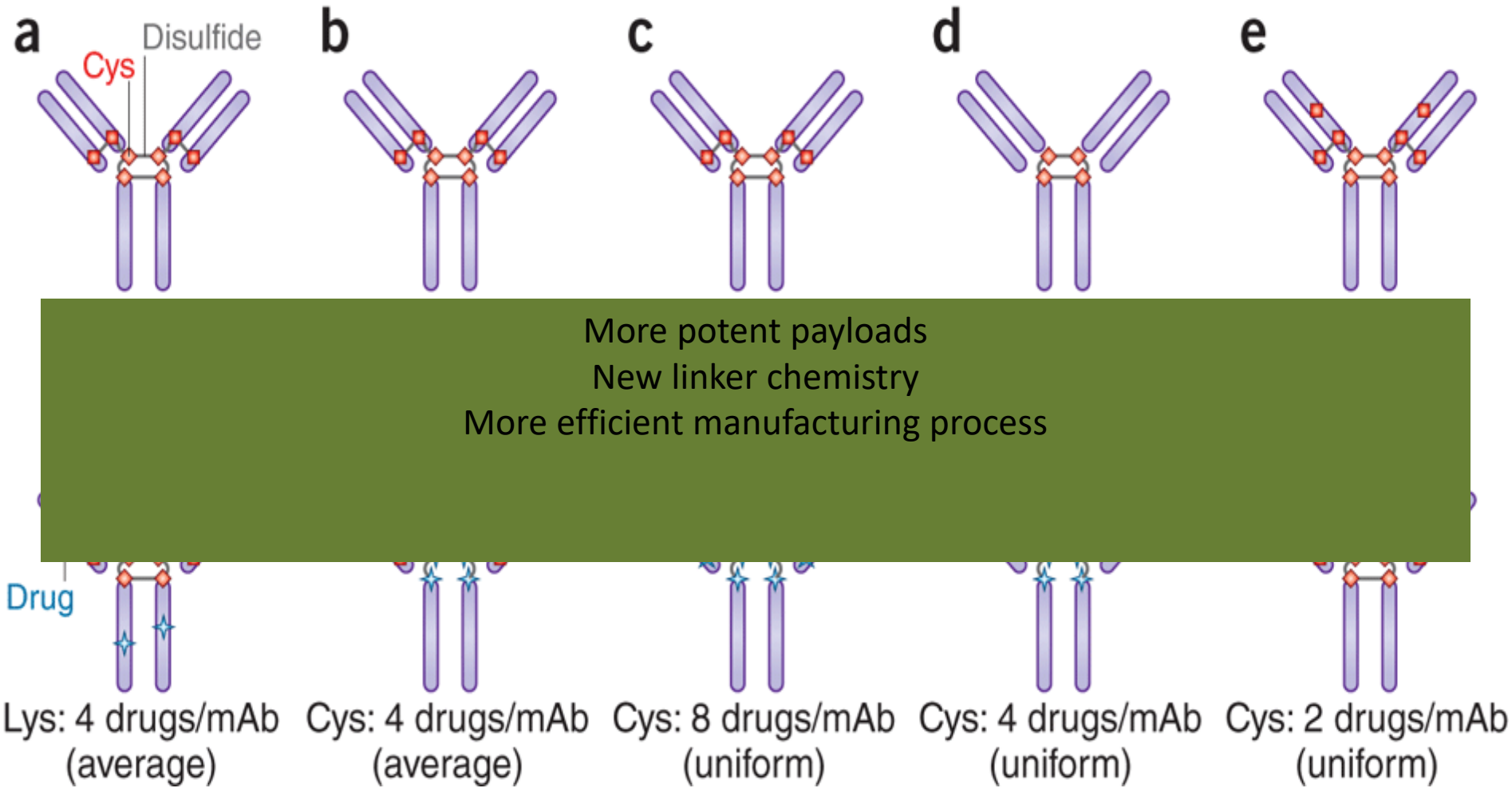
Clinical Stage and Approved Antibody-based Protein and Cellular Candidates

Format	Phase of development			Totals
	Approved	Phase IIb/III	Phase I/II	
Protein-based antibody-based therapeutics	97	96	735	928
Cell-based antibody therapeutics	2	6	330	338
Total antibody/TCR based therapeutics/candidates	99	102	1065	1266*

* Targeting 357 unique targets, 101 of which have been clinically validated

Database lock 12/8/19 –

Antibody-drug conjugates (ADC)



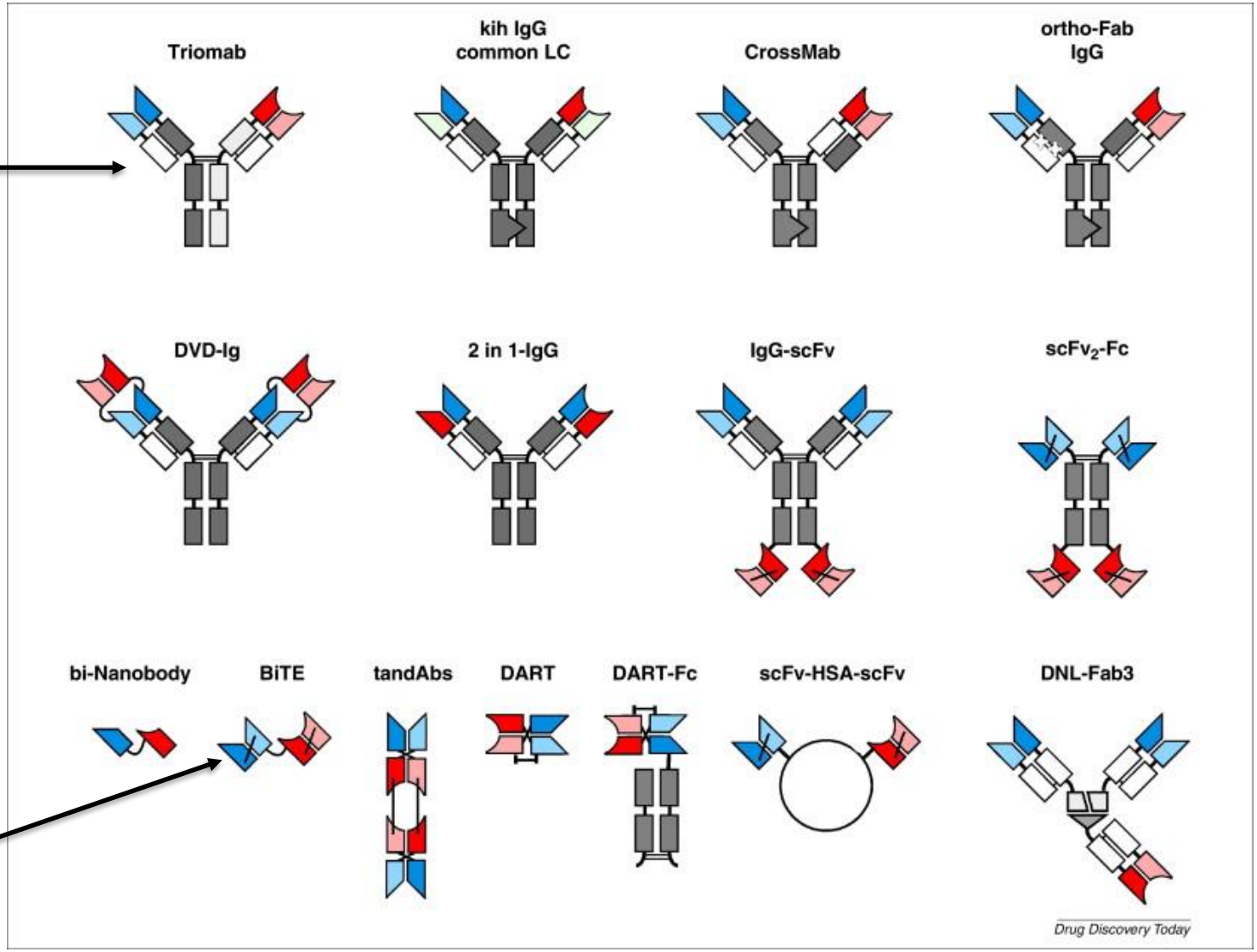
More potent payloads
New linker chemistry
More efficient manufacturing process

Nature Biotechnology 30: 631–637 (2012)

Bispecific antibodies (bsAbs)

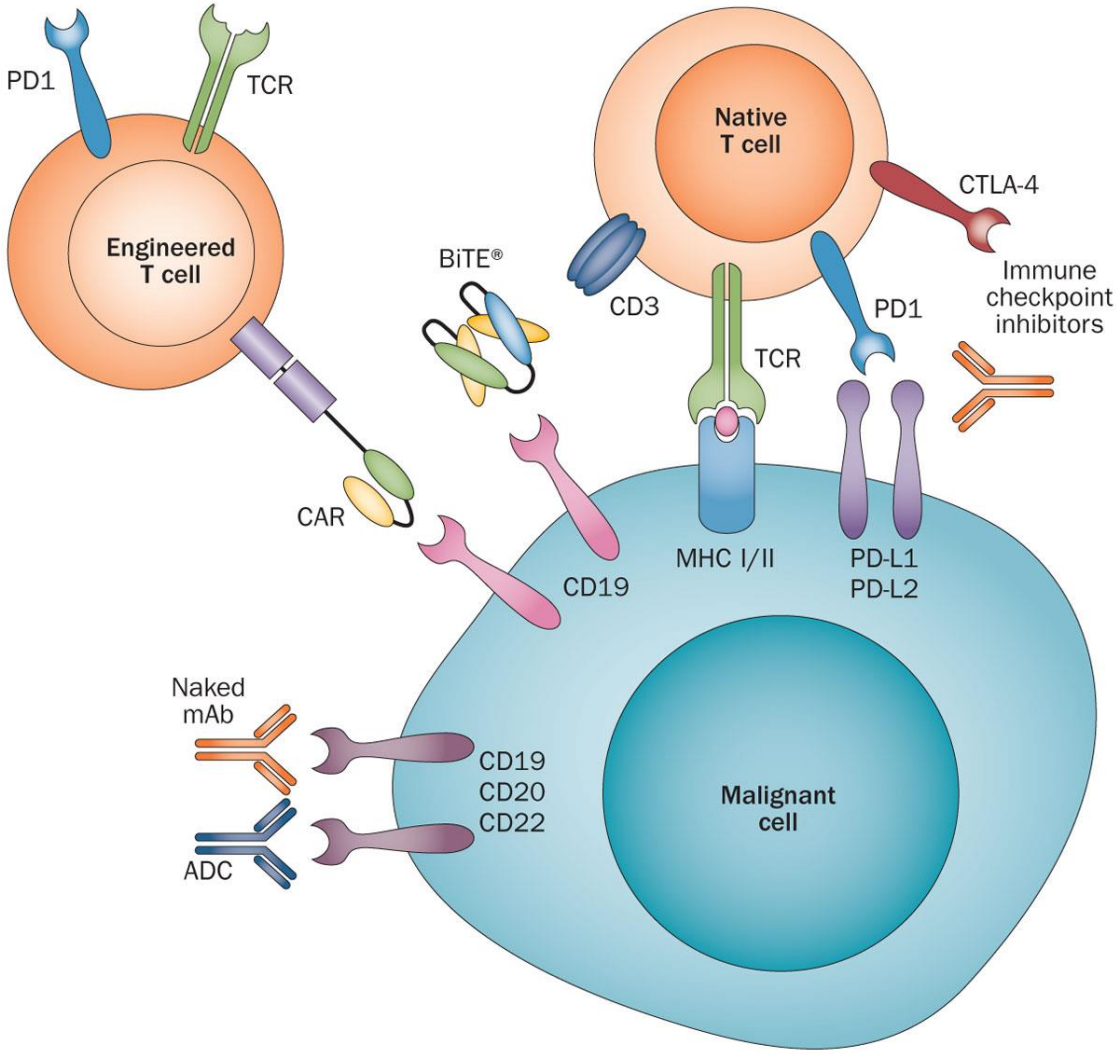
Catumaxomab
CD3/EpCAM
Malignant ascites

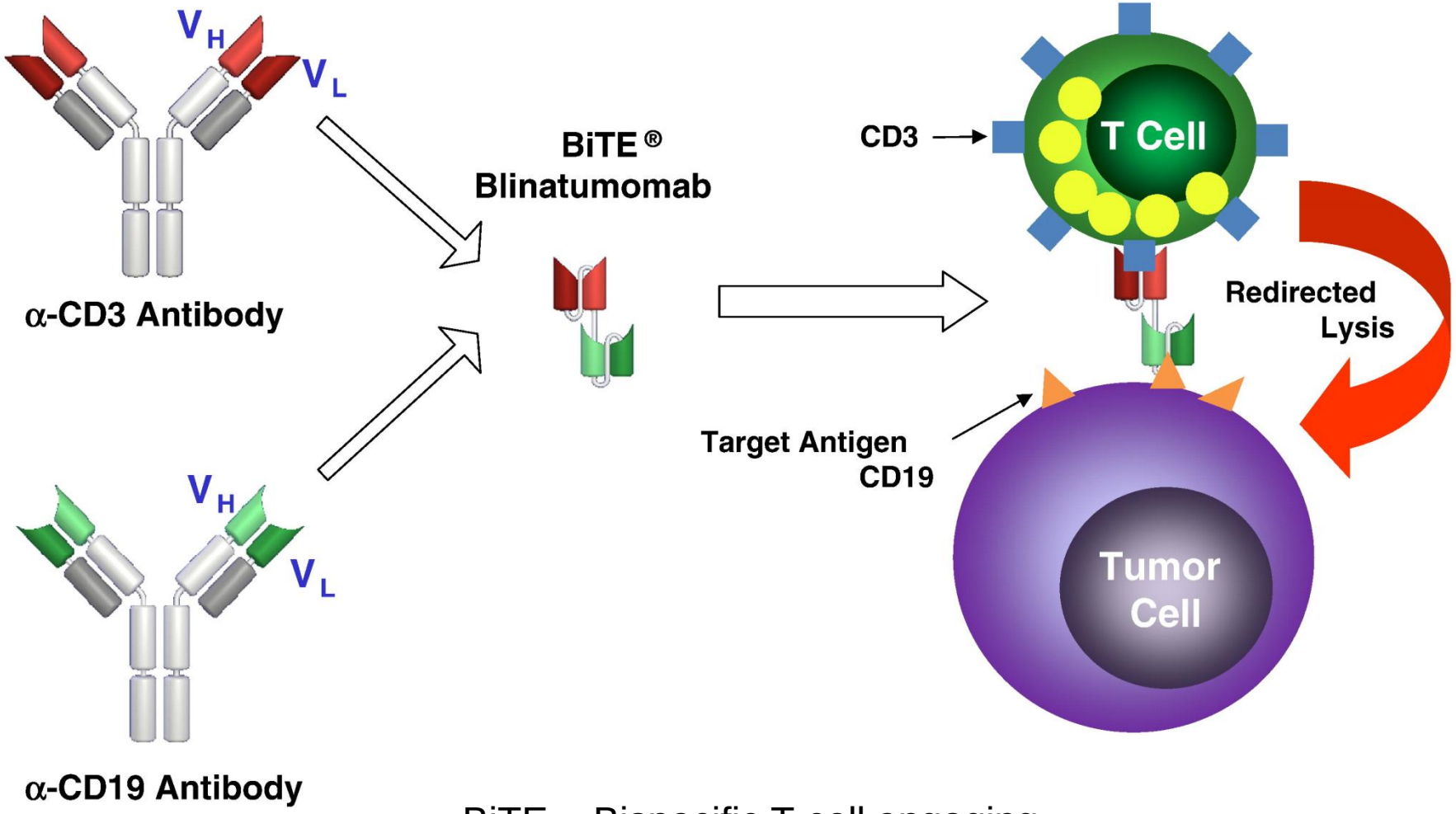
Blinatumomab
CD3/CD19
Acute lymphoid leukemia (**ALL**)



Kontermann & Brinkmann. **Bispecific antibodies**Drug Discovery Today, 2015

Mechanisms of action of immunotherapy modalities



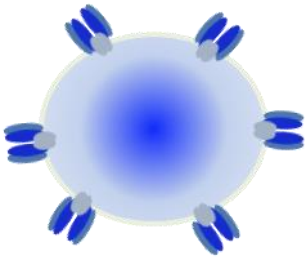


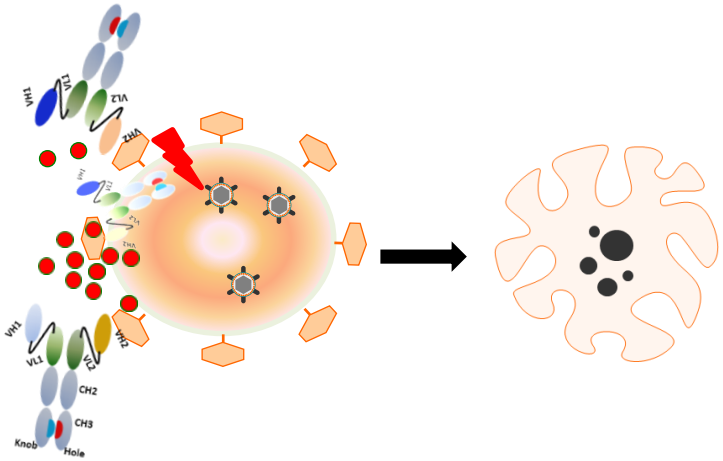
BiTE = Bispecific T-cell engaging

B-cell non-Hodgkin's lymphoma (NHL)
B-precursor acute lymphocytic leukemia (ALL)

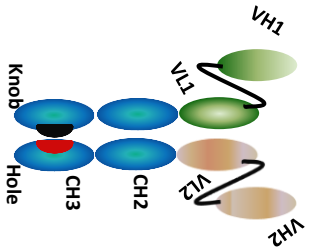
Nagorsen & Baeuerle, Experimental Cell Research. 317 (9), 2011, 1255-1260

Anti-CD3 / anti-HCMV bispecific antibodies (BsAb) for the elimination of latent infection - concept





Anti-CMV



Anti-CD3

Bind to cells infected with CMV and expressing CMV related proteins on the cell membrane

Recruit any T cells and activate T cells in the presence of CMV-infected cells

Meng et al., 2018. Antimicrobial Agents and Chemotherapy

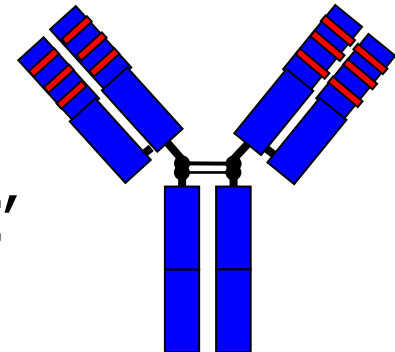
Humanization - CDR Grafting

Donor V gene of animal monoclonal antibody

Acceptor human V gene

CDR-grafted V gene

Final CDR-grafted V gene

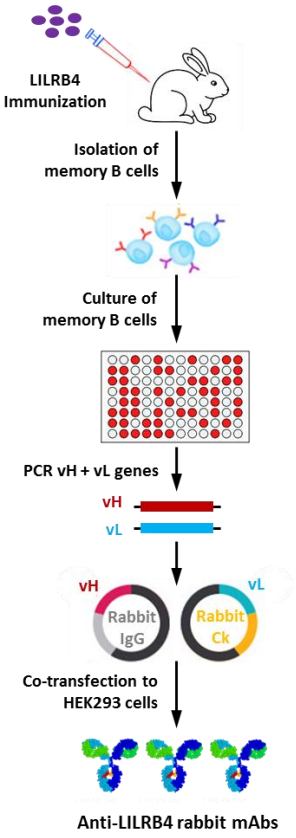


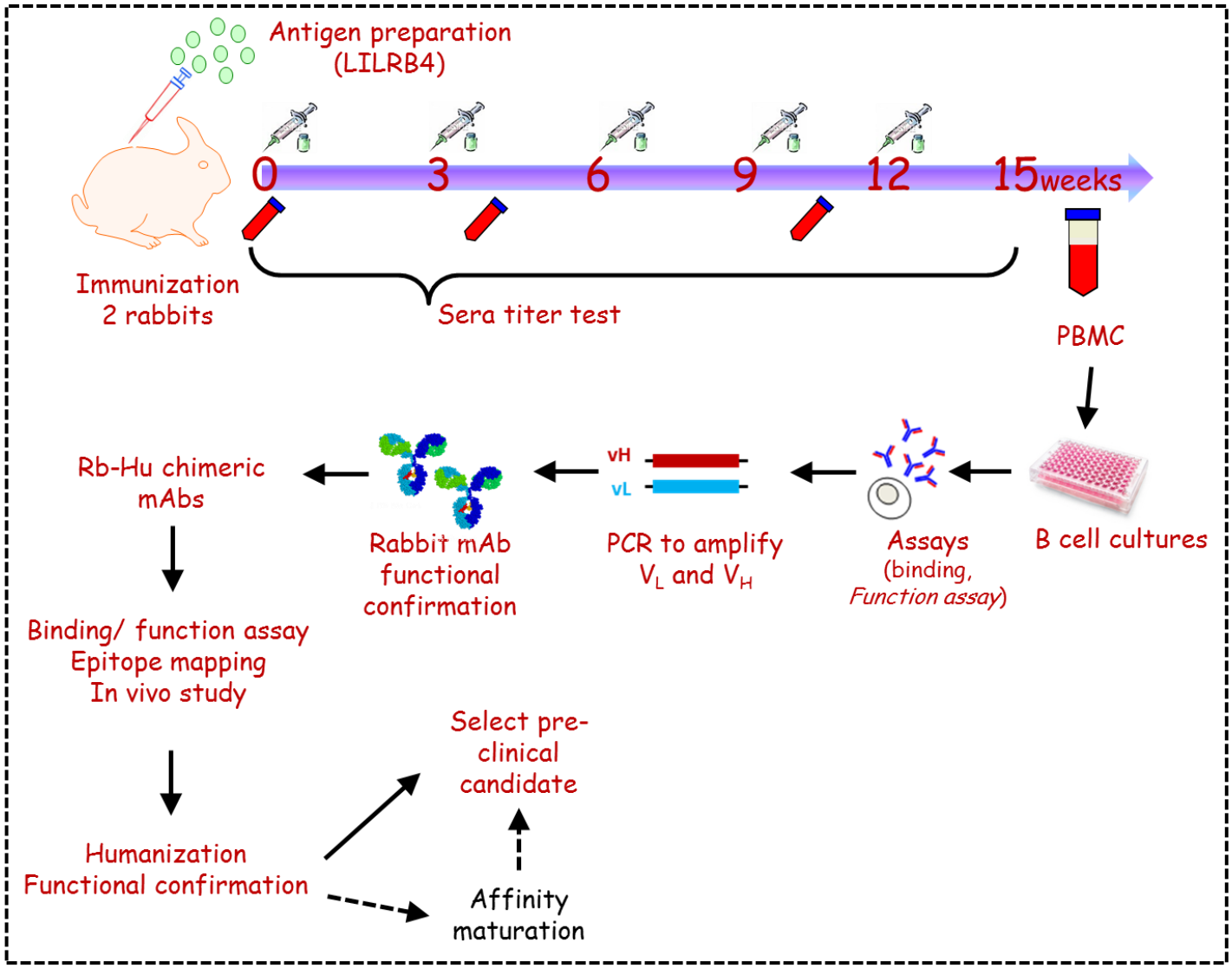
Affinity maturation of anti-IL-13Rα1 mAbs

IgG	CDR	Kd (Kinexa)
10G5wt	CDR H3: CAR FPNWGSFDY CDR L3: QQYET	861pM
10G5H6	CDR H3: CAR MPNWGSFDY CDR L3: QQYET	99.43pM
10G5-2	CDR H3: CVR MPNWGSLDH CDR L3: QQYAS	31.44pM
10G5-4	CDR H3: CVR MPNWGSLDH / T120I CDR L3: QQYAS	20.35pM
10G5-6	CDR H3: MPNWGSLDH CDR L3: QQYAS	26.8pM
8B4wt	CDR L3: HQSSSLPYT	480 pM
8B4-78M	CDR L3: MSSMGLPYT	30.03pM
178C05	-----	5.7 pM

REDPATH et al., Biochem. J. (2013) 451, 165–175

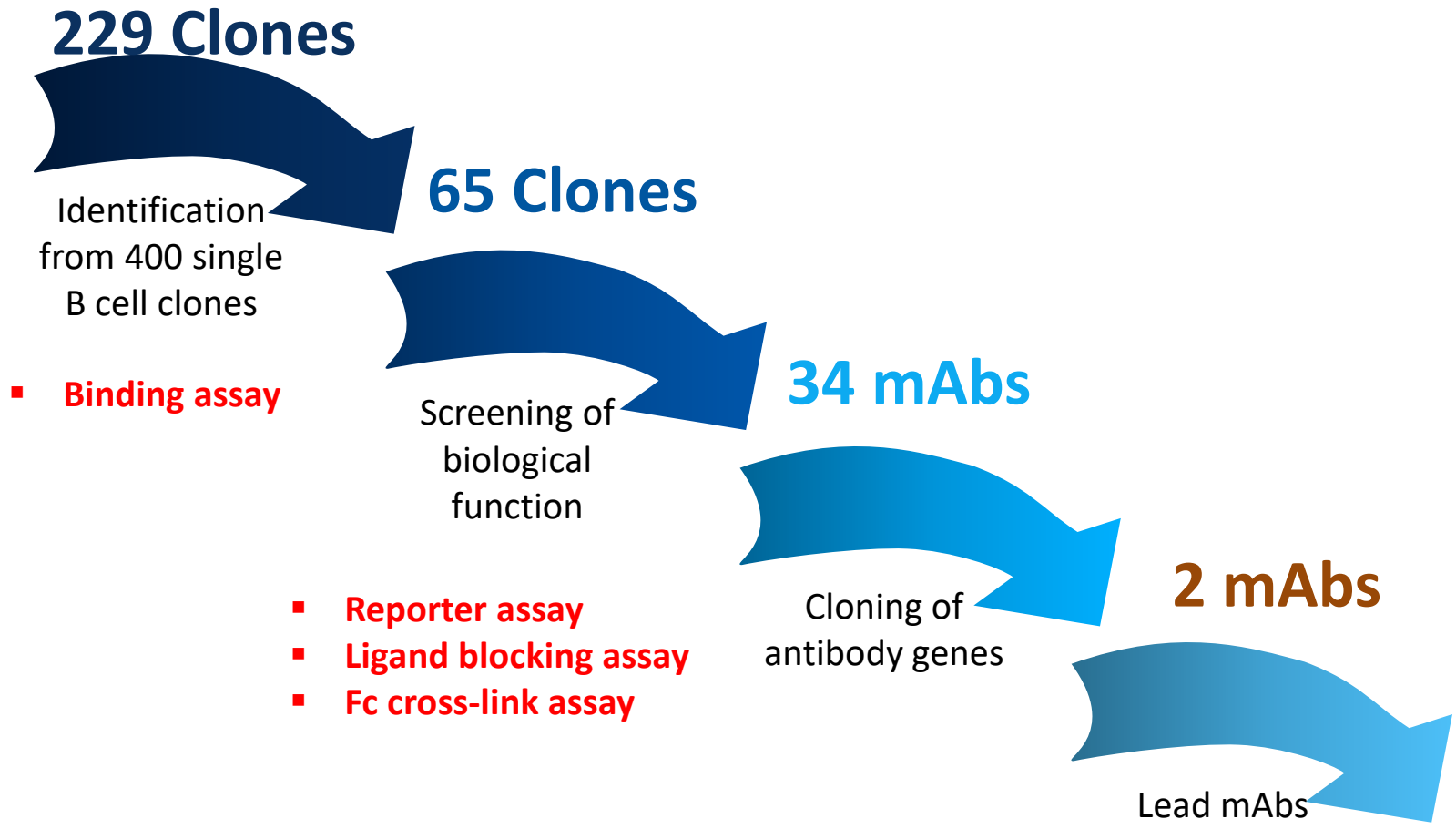
Strategy for LILRB4 antibody generation





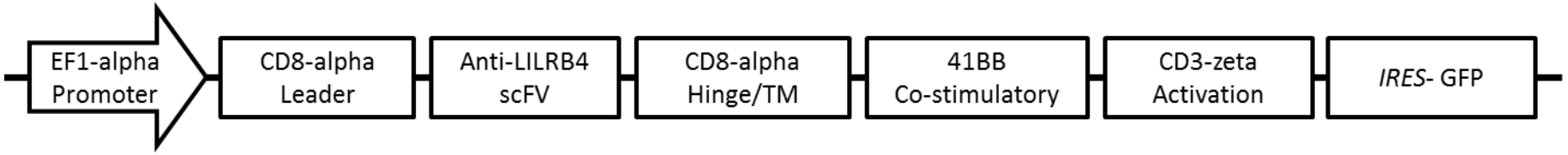
Gui & Deng et al 2019 Cancer Immunology Res 7:1244–57.

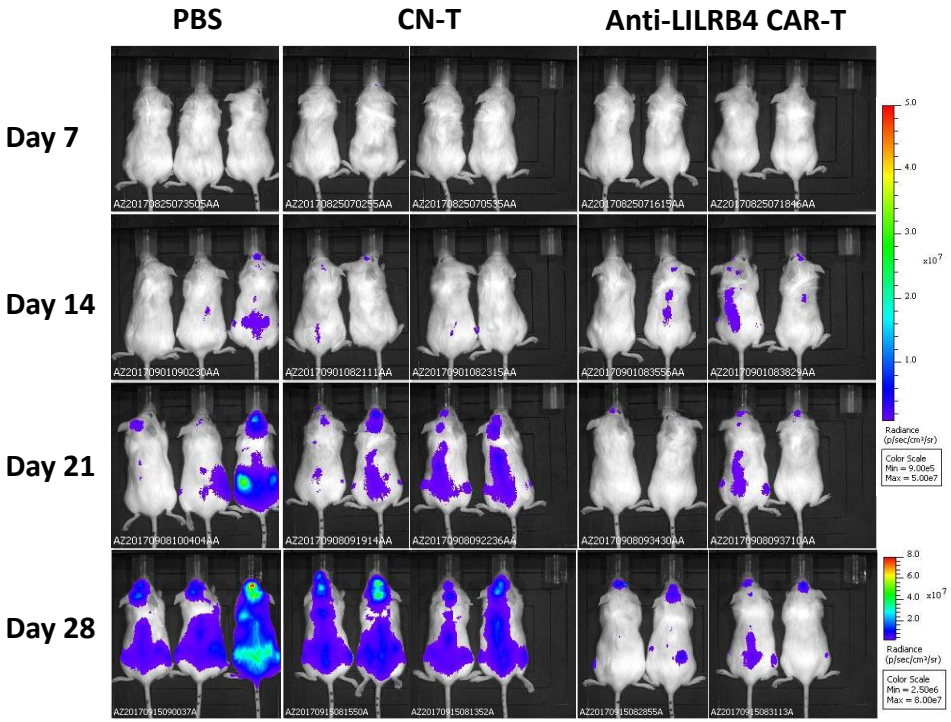
Steps for mAbs generation



Gui & Deng et al 2019 Cancer Immunology Res 7:1244–57.
Deng & Gui et al 2018 Nature https://doi.org/10.1038/s41586-018-0615-z

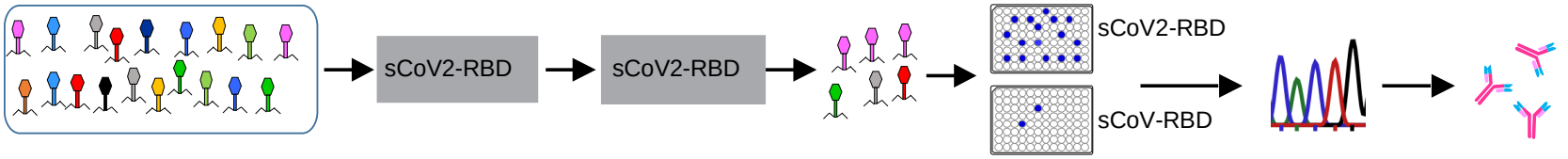
Anti-LILRB4 CAR-T cells display efficient *in vitro* cytotoxicity and specific cytokine release when stimulated by LILRB4^{+} AML cells





Samuel John et al., 2018 Molecular Therapy

Isolation of mAbs targeting the RBD of the SARS-CoV-2 spike protein



Phage-displayed human antibody library (~10^{12})

Round 1 phage panning

Round 2 phage panning

Output phages

Phage ELISA

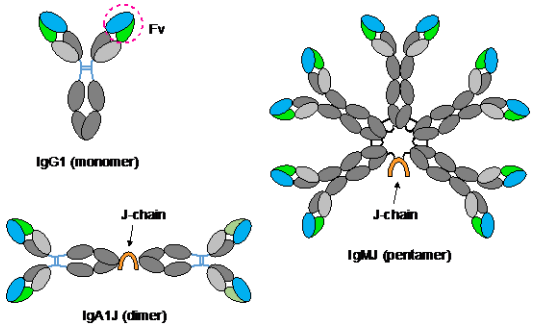
(149)

Sequencing

35 scFvs

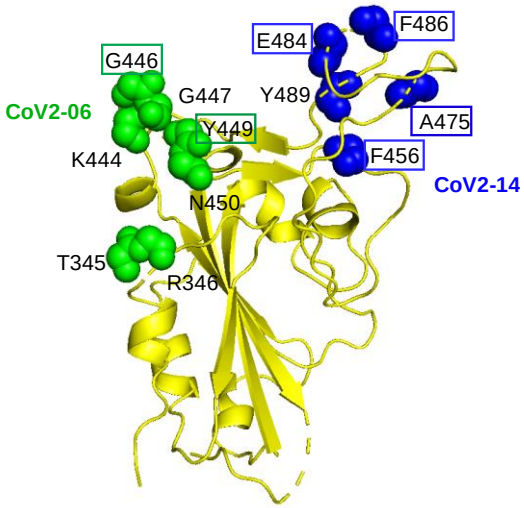
IgG1 expressior

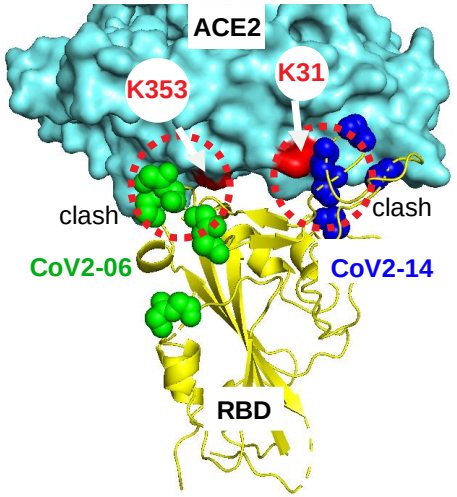
(30)

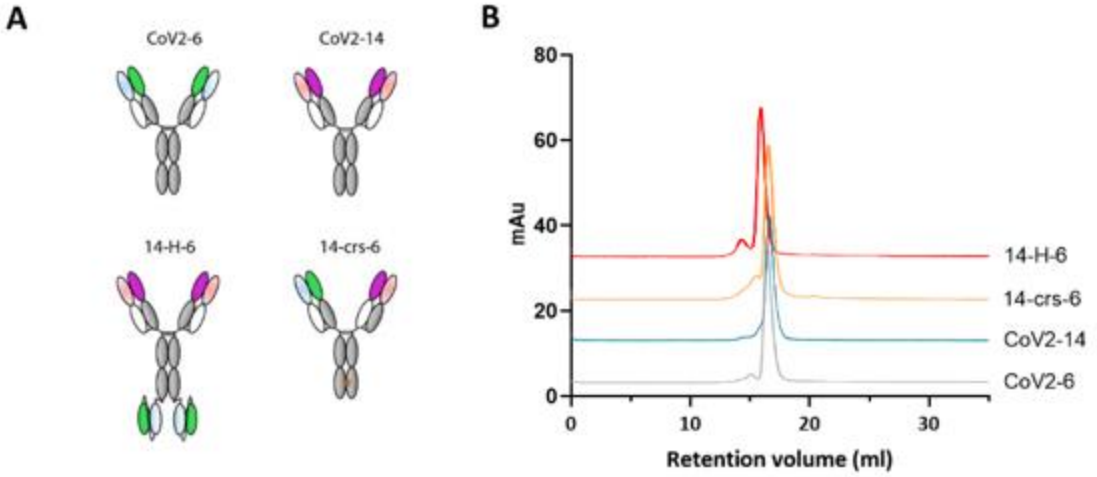


Ku et al. Nature Communications 2021
Ku et al. Nature 2021

Broader RBD epitope coverage by the tetravalent bsAb 14-H-6 prevents viral escape

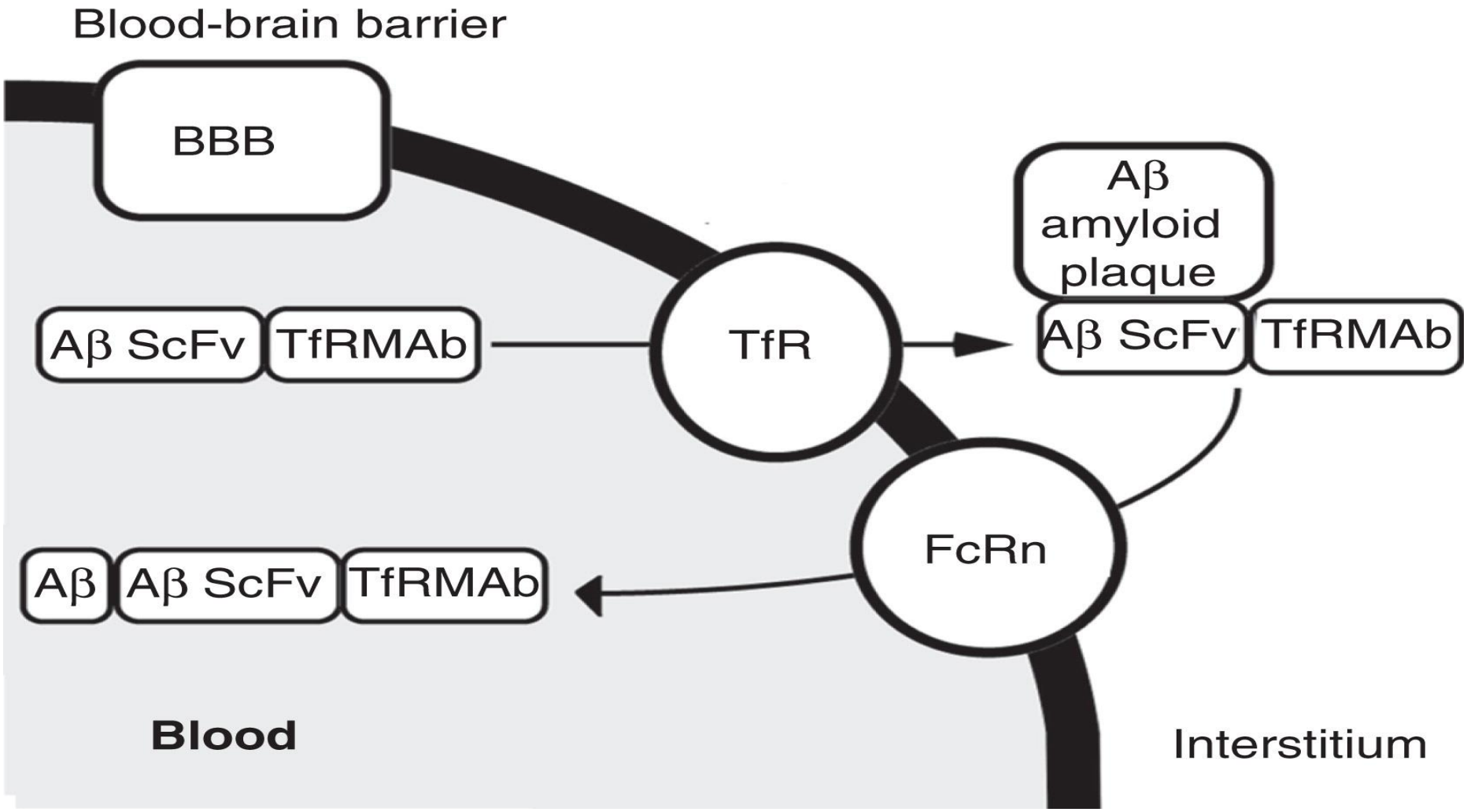






Gao et al. manuscript submitted

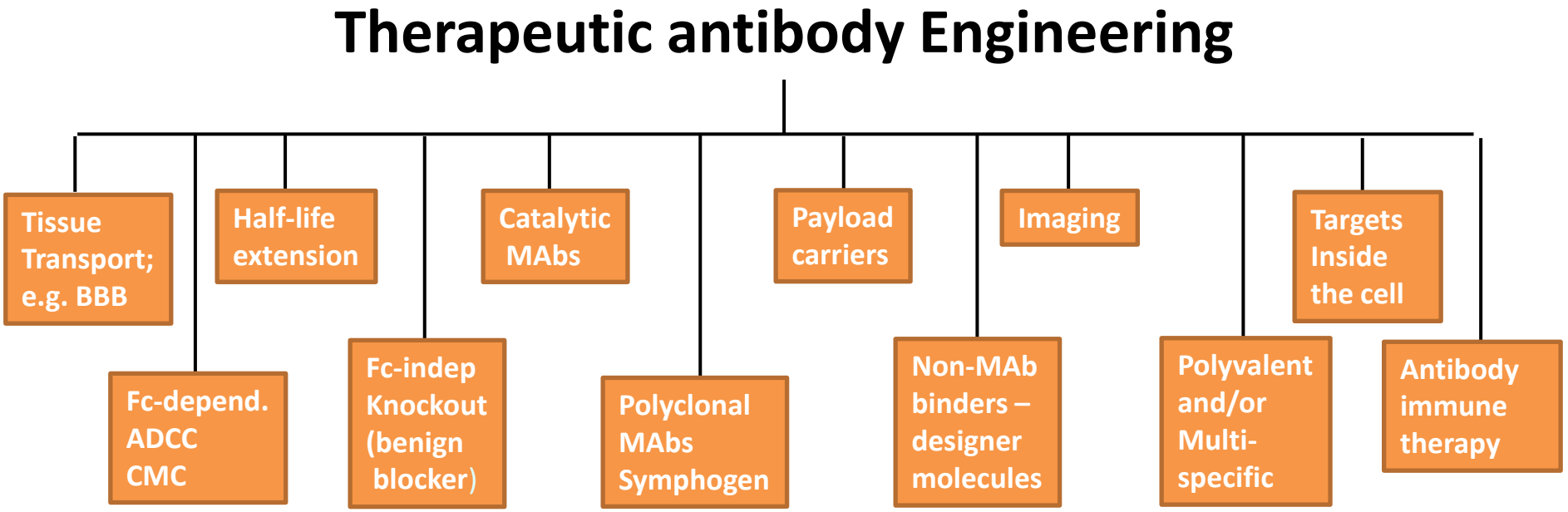
The cTfRMAb–ScFv fusion protein clears amyloid from brain



William M Pardridge; *Expert Opinion on Drug Delivery* **2015,** 12, 207-222.

Major challenges in antibody drug development

- **Lack of novel antibody drug targets**
  - Of the 37 antibodies for oncology indication
    - CD20, EGFR, HER2, VEGF, CTLA-4, PD-1, PD-L1, CD38, SLAMF7, GD2, CD19/CD3, and VEGFR-2
    - 6 are targeting CD20
    - 6 are targeting EGFR/HER2
    - 6 targeting PD1/PD-L1
  - Of the 156 entered clinical trials in 2018-2019
    - 15 are targeting Her2
    - 16 are targeting CD3
    - 21 targeting PD1/PD-L1
- **Lack of biomarkers**
  - IGFR1
  - HER3
  - CTLA4/PD-1/PD-L1
- **Drug resistance to antibody therapies**
  - Combination therapies
  - Bispecific
  - ADCs
- **Technology breakthroughs**
  - Targeting intracellular proteins
  - Crossing the BBB



Novel targets
Biomarkers
Better designed clinical trials

The reproducibility crisis in drug discovery

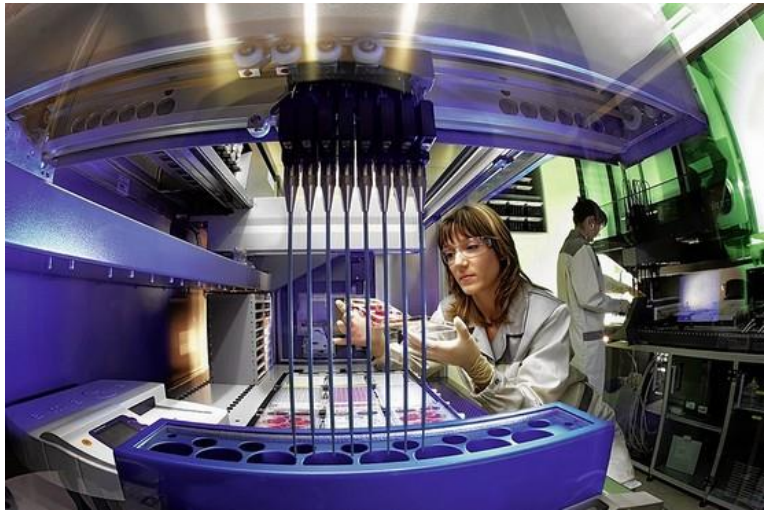
- **Between 10-50% of scientific research believed to be reproducible**
- **$28.2 billion dollars spent in the US annually on preclinical research believed to be irreproducible**
- **Four causes of irreproduciblility**
  - 36.1%, biological reagents & reference materials
  - 27.6%, study design
  - 25.5%, data analysis & reporting
  - 10.8%, laboratory protocols

Nature 2017

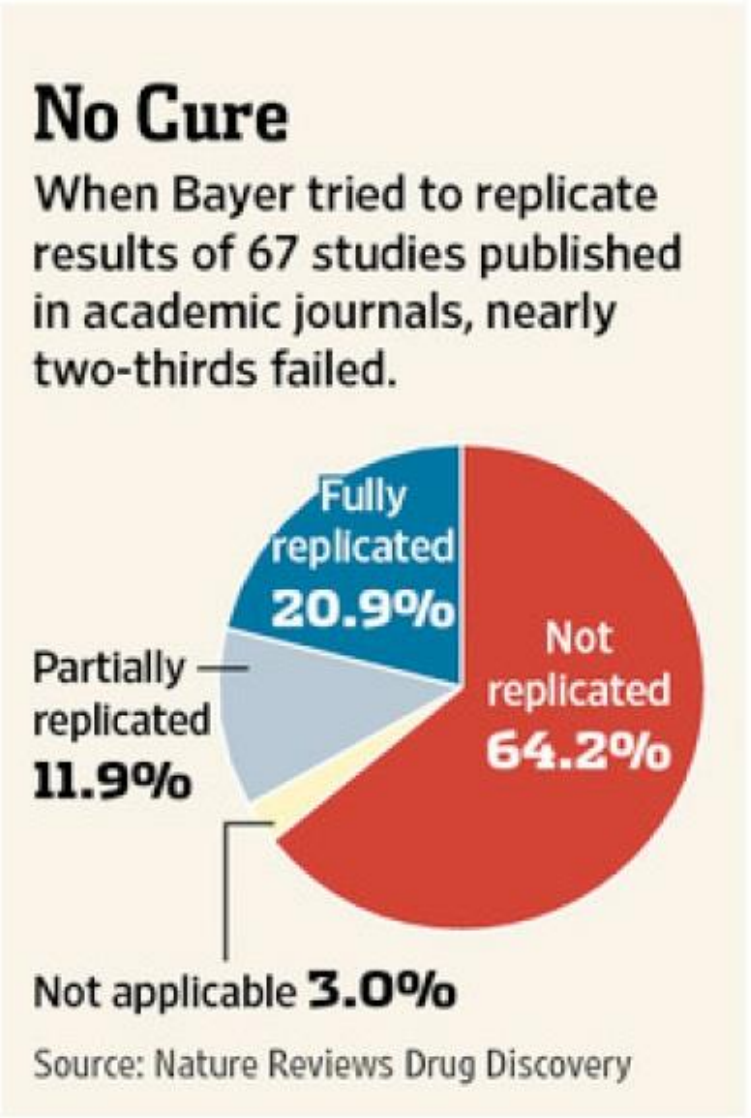
Scientists' Elusive Goal: Reproducing Study Results

By **GAUTAM NAIK**

December 2, 2011



THE WALL STREET JOURNAL.
WSJ



THE WALL STREET JOURNAL, April 8-9, 2017

Harold Bloom on Falstaff's joy and the current woes of the humanities C17

REVIEW

Spring into books on owls, Prince Charles and dystopias. A special section C5-C16

BOOKS | CULTURE | SCIENCE | COMMERCE | HUMOR | POLITICS | LANGUAGE | TECHNOLOGY | ART | IDEAS

Dismal Science

In the Search for Cures

BY RICHARD HARRIS

LATER THIS MONTH, HBO will air a movie starring Oprah Winfrey about the story of Henrietta Lacks, an African-American woman who died of cervical cancer in 1951 but whose cells live on today in laboratories around the world. The film, based on Rebecca Skloot's best-selling book "The Immortal Life of Henrietta Lacks," explores the Lacks family's struggle to get recognition for the crucial contribution that the Maryland woman inadvertently made to science. Her cancerous cells, dubbed HeLa, were extracted and cultured at the Johns Hopkins Hospital in Baltimore and became the first perpetual supply of cancer cells to be used in medical research.

But there is more to the story of HeLa than this compelling personal angle. The cells neatly illustrate a serious problem in biomedical research: Because they reproduce so quickly and have been mishandled

Such contamination is just one of the many problems now confronting biomedical research. Scientists point to what they call the "reproducibility crisis"—that is, studies whose results can't be duplicated and are untrustworthy if not invalid. The issue isn't just wasted time and money. Many observers now think that biomedical research worldwide has been so compromised that it is slowing and

Contaminated samples, faulty studies and poor training have created a crisis in labs and industry, slowing the quest for new treatments.

tives for professional advancement.

Failure is an essential part of science, and no one expects researchers to get everything right on the first try. Scientific discovery is usually self-correcting in the long run, with useful information, treatments and drugs emerging even from experiments that don't work out. But false starts can slow progress.

How much of biomedical research is actually wrong? John Ioannidis, an epidemiologist and health-policy researcher at Stanford, was among the first to sound the alarm with a 2005 article in the journal PLOS Medicine. He showed that small sample sizes and bias in study design were chronic problems in the field and served to grossly overestimate positive results. His dramatic bottom line was that "most published research findings are false."

The problem is especially acute in laboratory studies with animals, in which scientists often use just a few animals and fail to select them randomly. Such errors inevitably introduce bias. Large-scale human studies, of the sort used in drug testing, are less likely to be compromised in this way, but they have their own

1. **ICLAC has reported 450 misidentified cell lines, HeLa cell is the contaminant in 113 of these cases**

2. **MDA-MB-435 isolated in 1976 at MDACC from a women with breast cancer. In 2000, it was confirmed to be a melanoma. , after that, more than 900 breast cancer reports involved the cell line**

ICLAC. The International Cell Line Authentication Committee

Thank you for your attention!