Antibody Drug Discovery

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Texas Therapeutics Institute

August 19, 2021

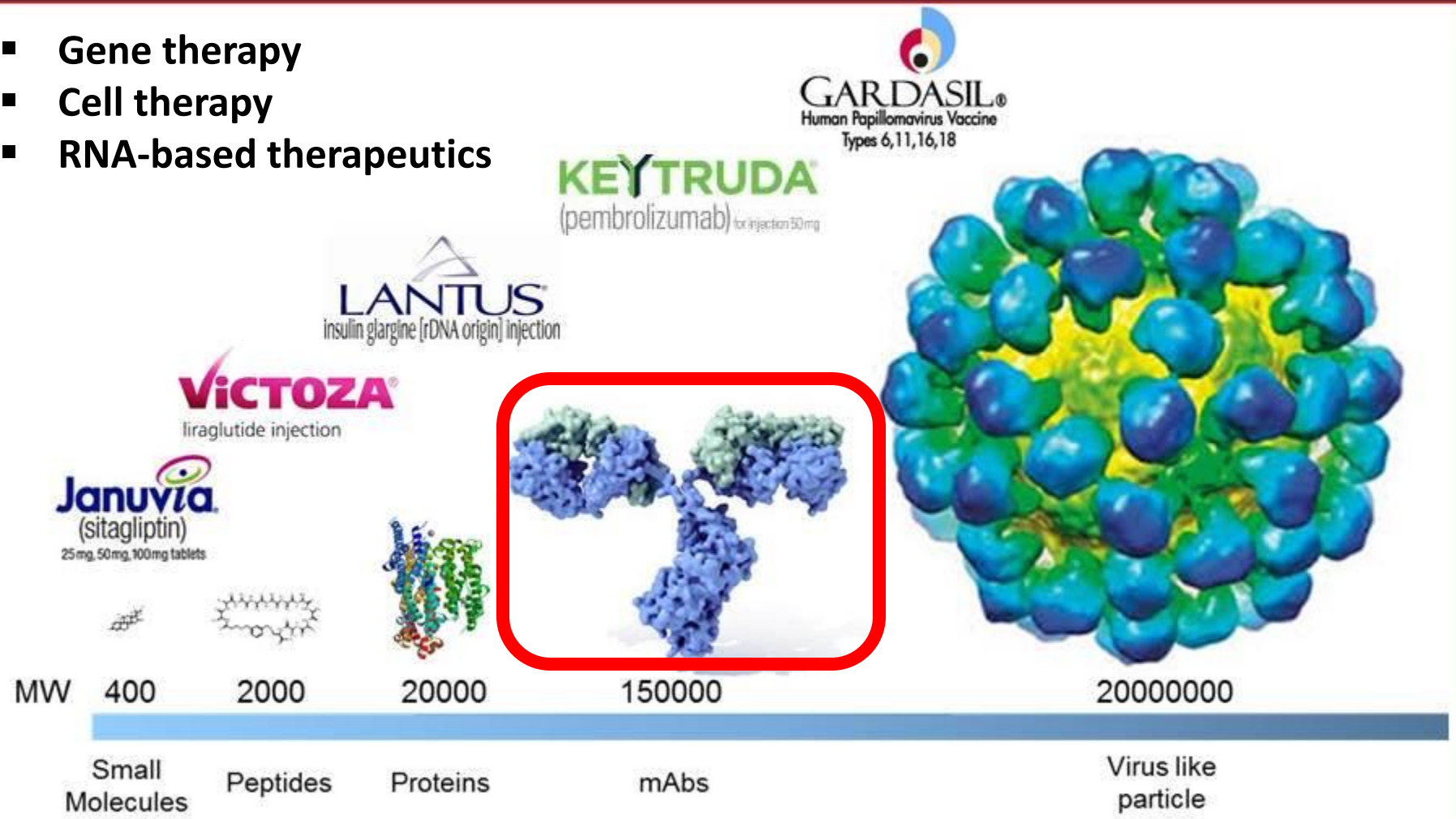
TIPS-CTTP Drug Discovery Course

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

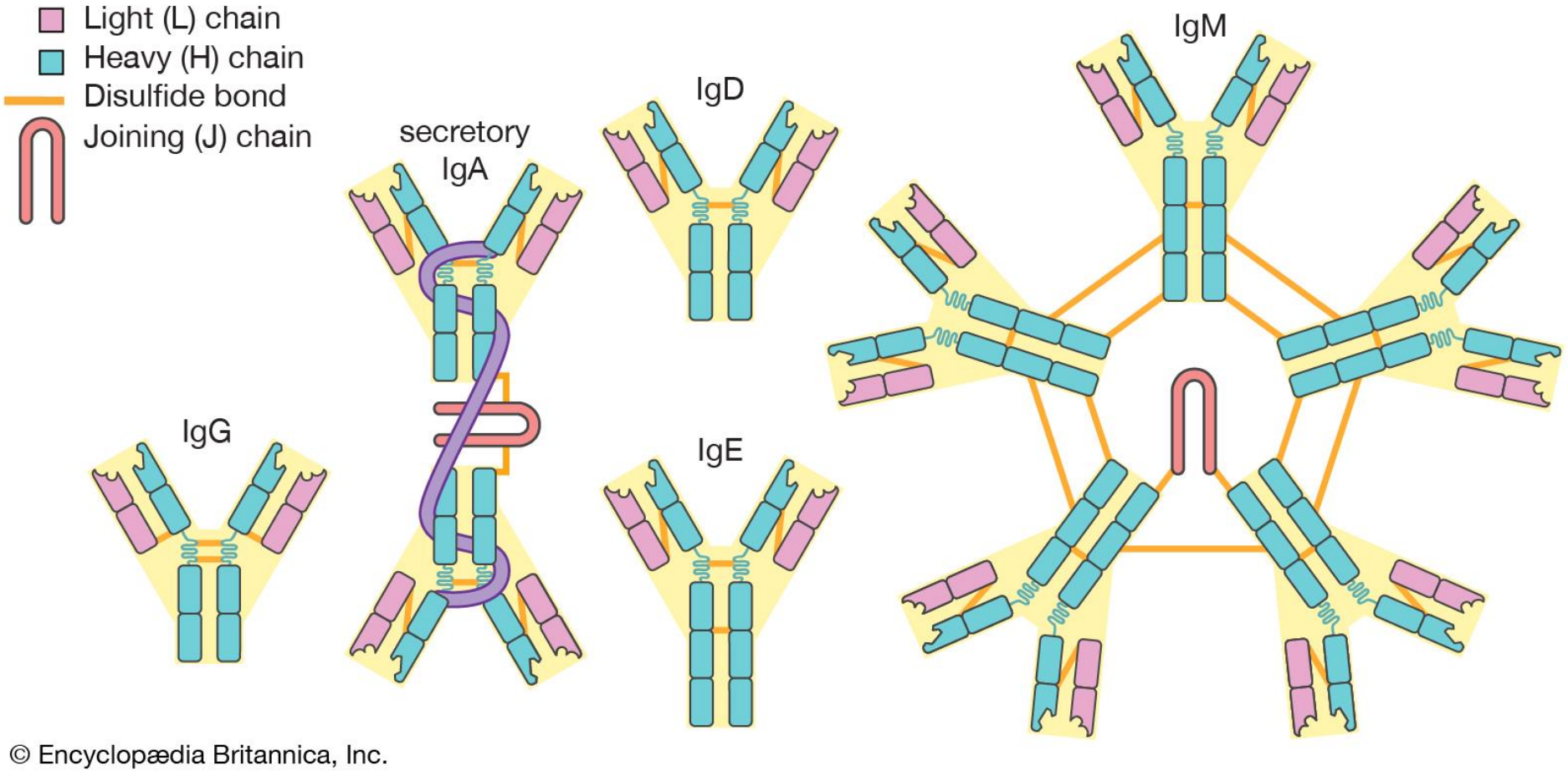
The University of Texas
Health Science Center at Houston

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

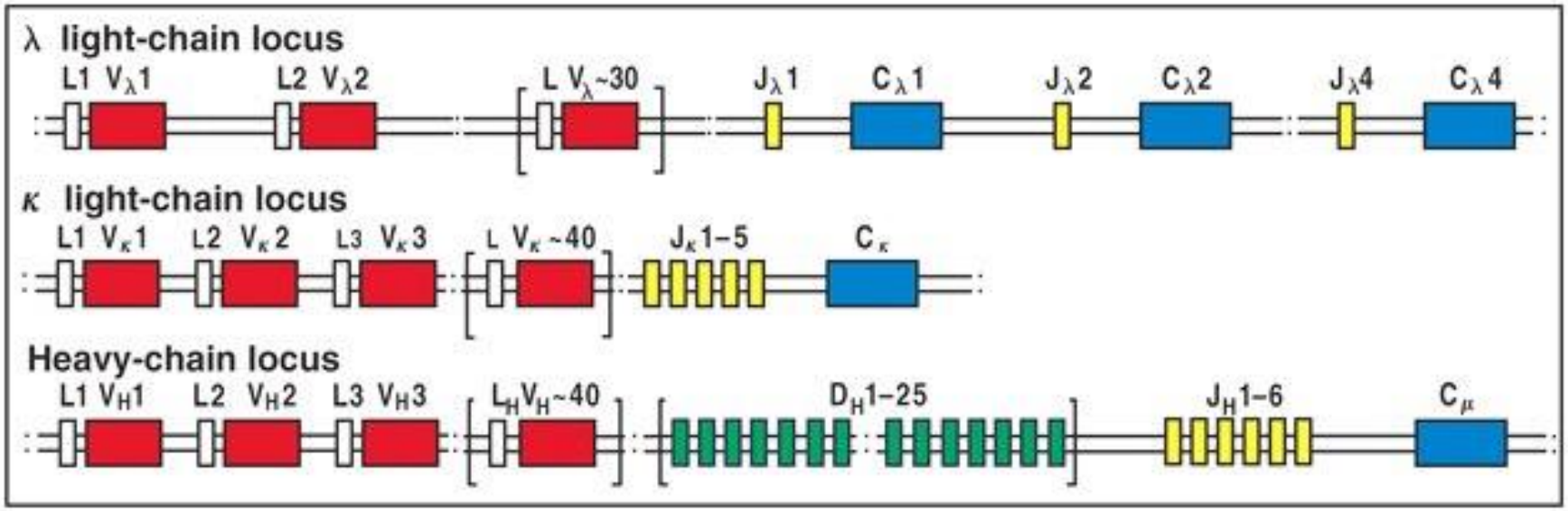


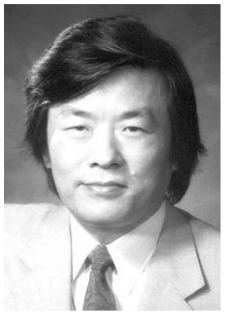
Figure 4-4 Immunobiology, 6/e. (© Garland Science 2005)

The Nobel Prize in Physiology or Medicine

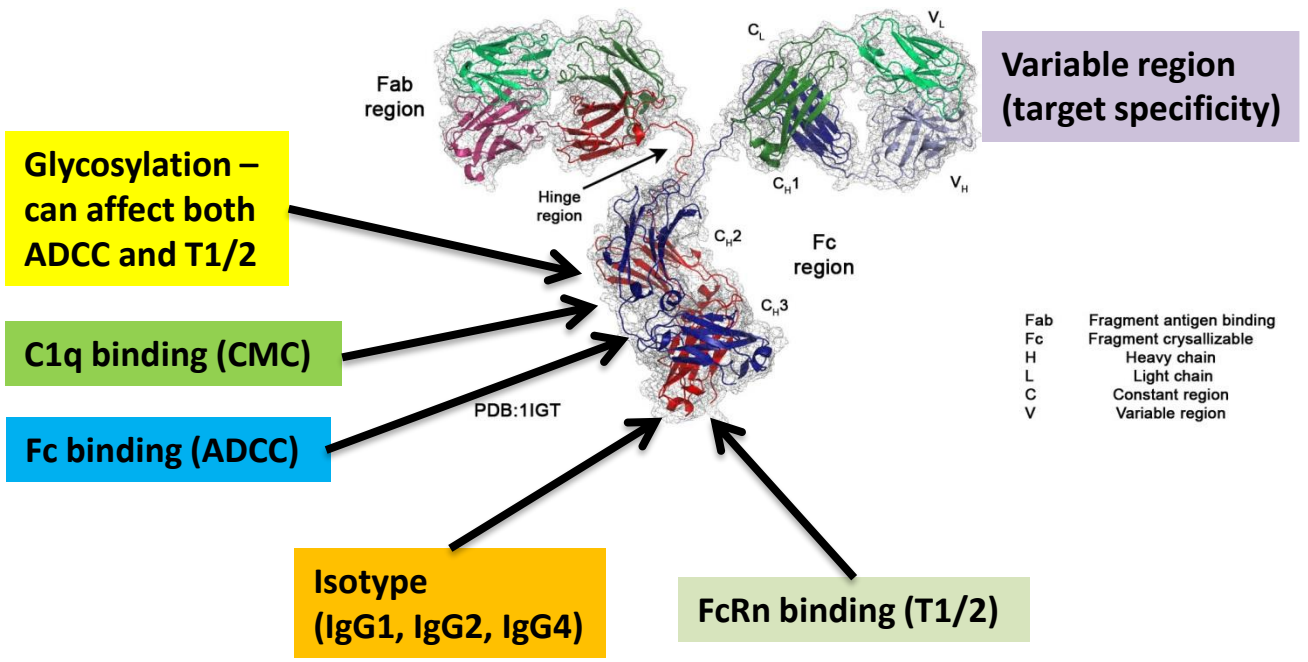
1987

"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

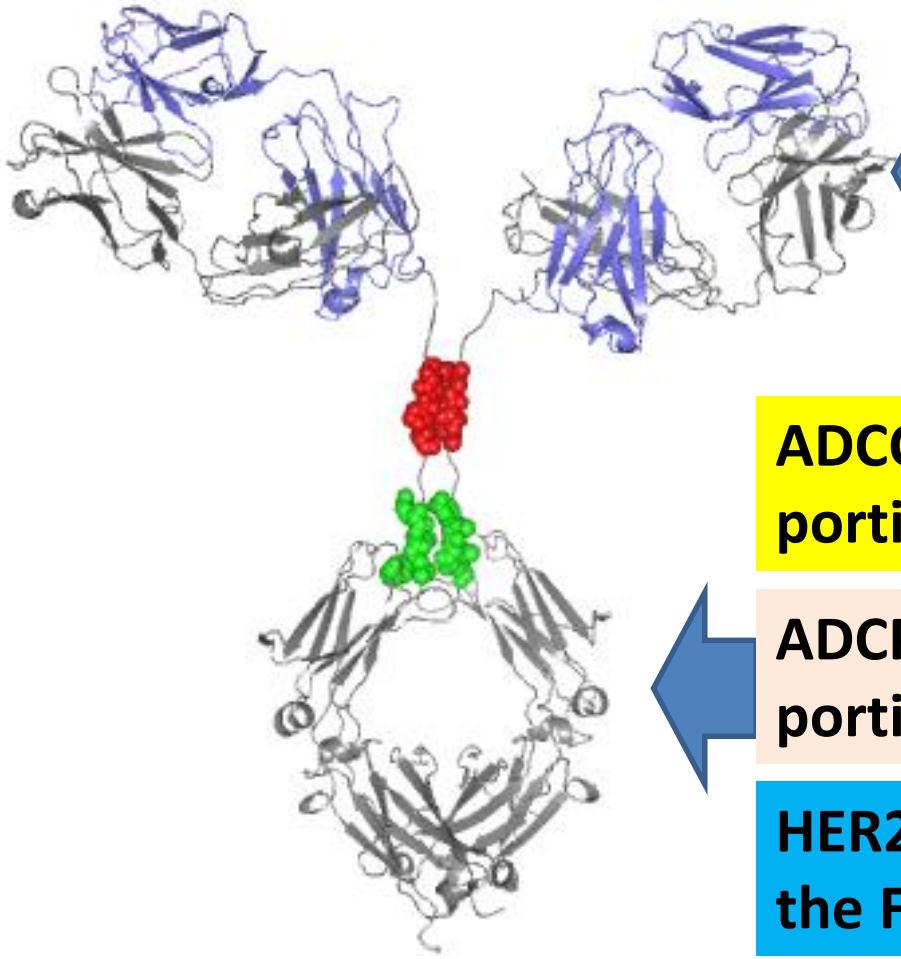
C1q binding (CMC)

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

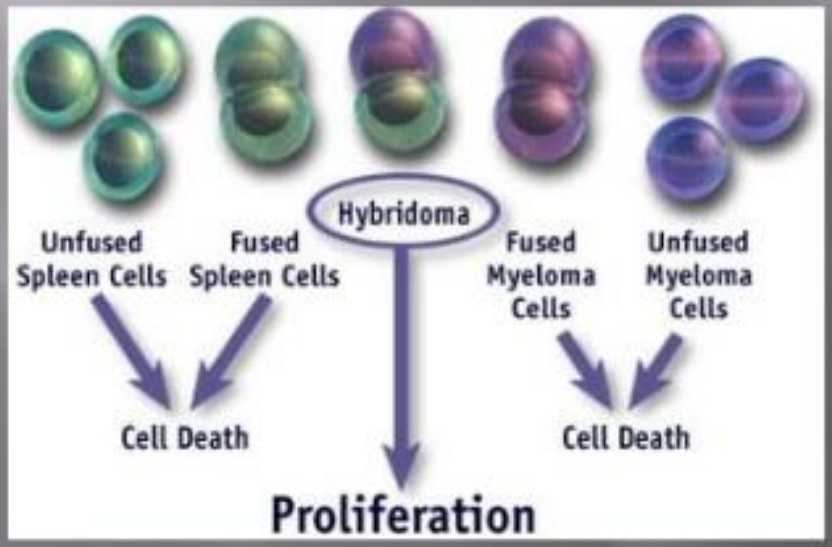
HER2 downregulation mediated by the Fc portion of the antibody

Sources of Antibody Genes

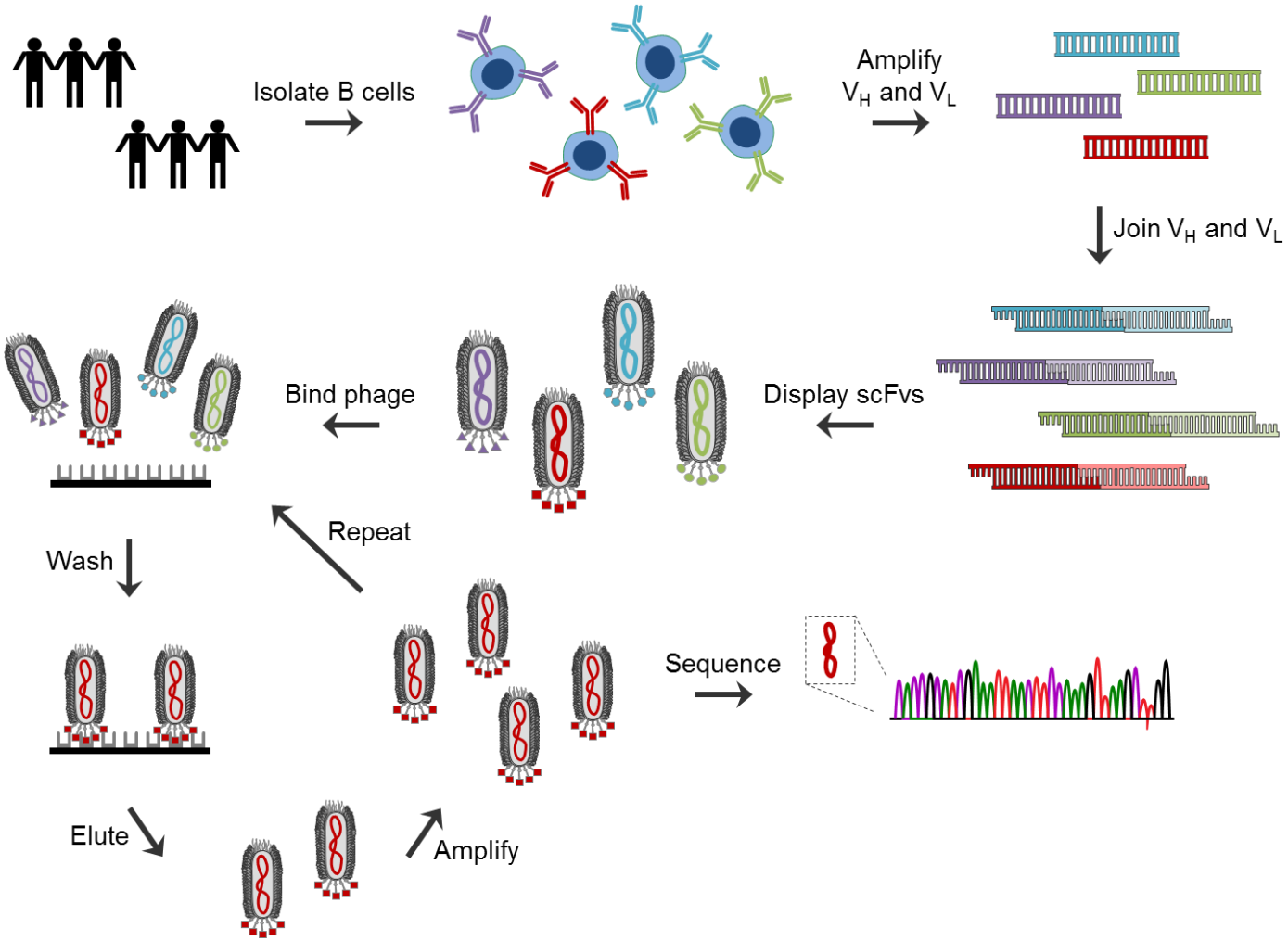
- **Mouse, rabbit, and other animal species**
  - **The old fashioned way...**
- **Humanized animals**
  - **Animals with human Ab genes (HuMAb-Mouse®, XenoMouse®, VelociMouse™, etc)**
- **Phage display libraries**
  - **Of affinity matured ab genes after immunization with desired target (Trans-Phage 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

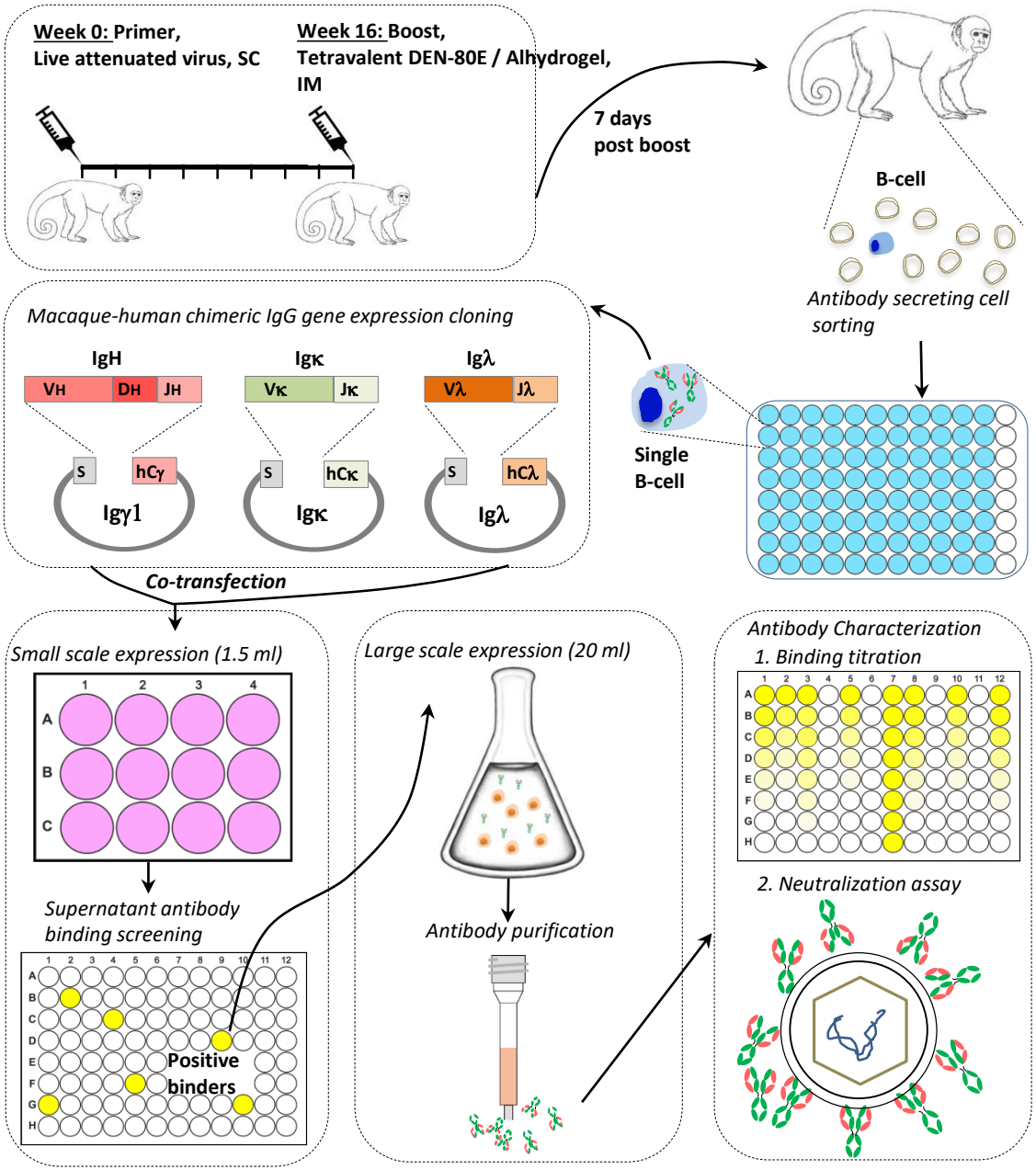
1975, Georges Köhler and Cesar Milstein - awarded Nobel Prize in1984



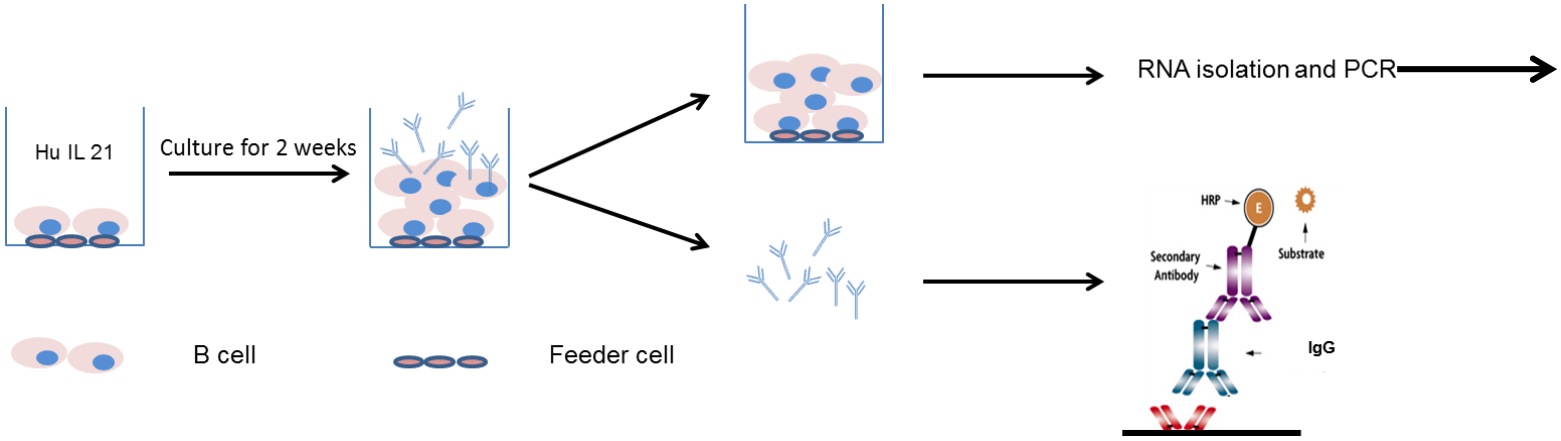
Human monoclonal antibodies using scFv phage display – 1 x10^{11}

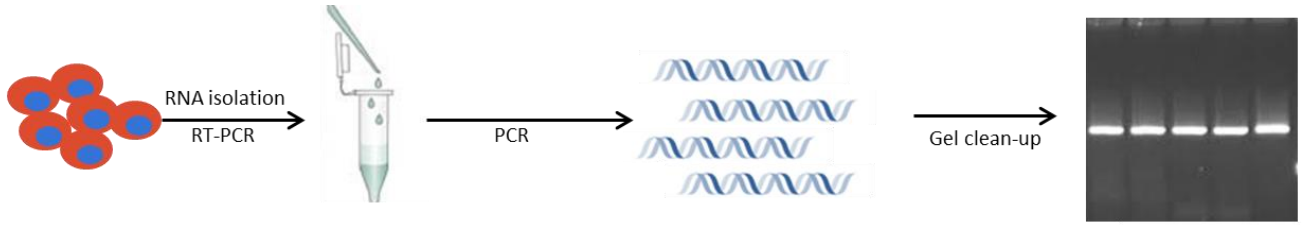


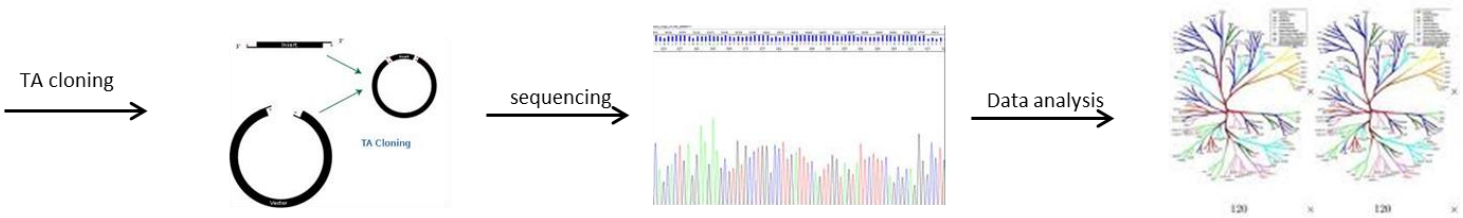
Cloning mAbs from plasma B cells



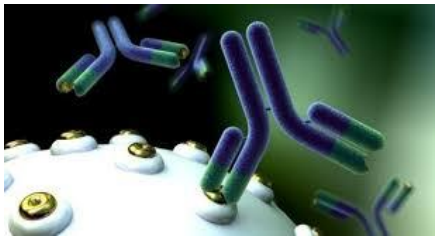
Cloning mAbs from memory B cells



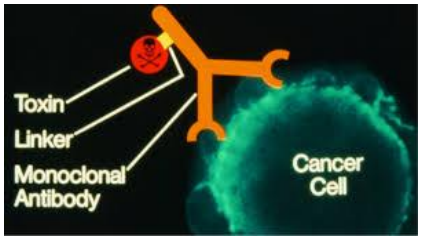




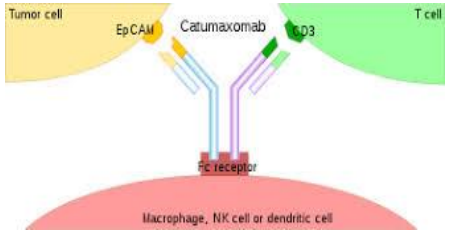
Antibody-based drug modalities



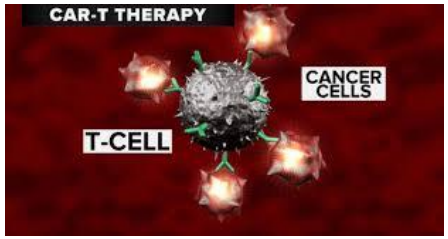
mAbs



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.

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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 –

Of the top 10 selling drugs in 2018, 7 are antibodies

Antibody	Rank	Drug	Manufacturer	Sale USD billion	Indication
√	1	Humira (Adalimumab)	AbbVie	19.9	Autoimmune diseases
	2	Revlimid (Lenalidomide)	Celgene	9.7	Multiple myeloma
√	3	Keytruda (Pembrolizumab)	Merck & Co	7.2	Cancer
√	4	Herceptin (Trastuzumab)	Roche	7.1	Cancer
√	5	Avastin (Bevacizumab)	Roche	7.0	Cancer
√	6	Rituxan (Rituximab)	Biogen, Roche	6.9	Cancer
√	7	Opdivo (Nivolumab)	BMS	6.7	Cancer
	8	Eliquis (Apixaban)	BMS, Pfizer	6.4	Atrial fibrillation, DVT
	9	Prevnar 13	Pfizer	5.8	Pneumococcal vaccine
√	10	Stelara (Ustekinumab)	J&J	5.7	Psoriasis, Crohn's disease

Three circulating protein targets (TNF-alpha, VEGF-A, p40 subunit of IL12/IL23)
Three membrane protein targets (PD-1, HER2, CD20)

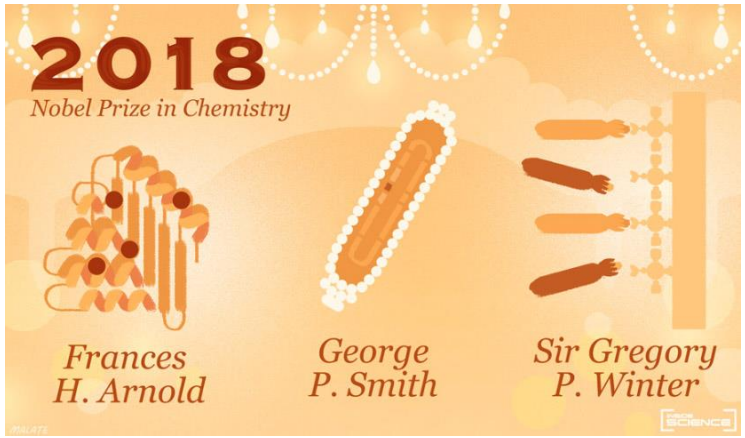
Of the top 20 selling drugs in 2020, 14 are antibody-based

Nature Review Drug Discovery 12 March 2019

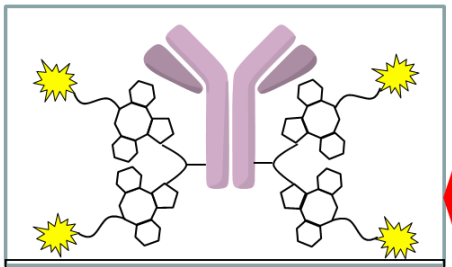
- **Antibody based drugs including cancer immunotherapies is the most active field in drug discovery and development**

- **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**



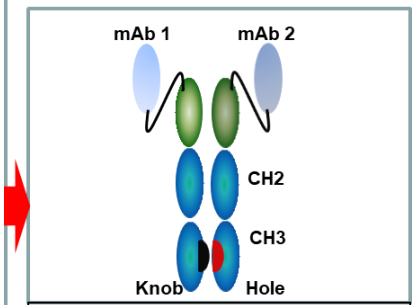
CPRIT Antibody Drug Discovery Cores



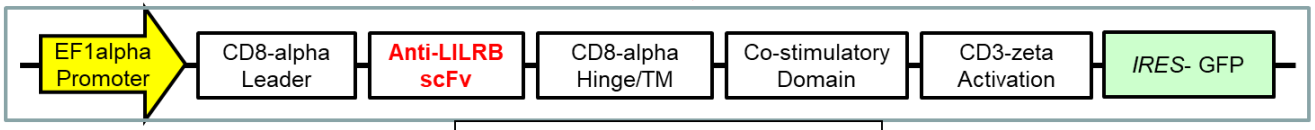
Antibody-Drug Conjugates (ADCs)

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)



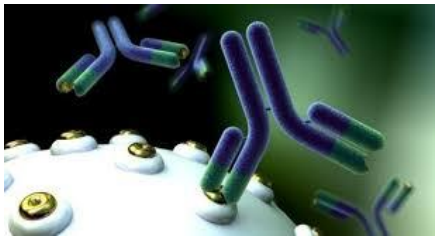
CAR-T Constructs

Preclinical development

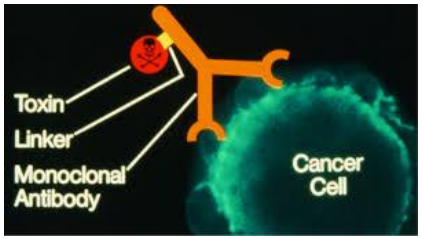
Antibody technologies

- **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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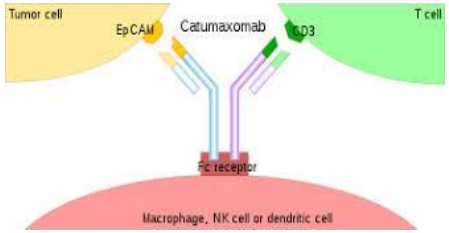

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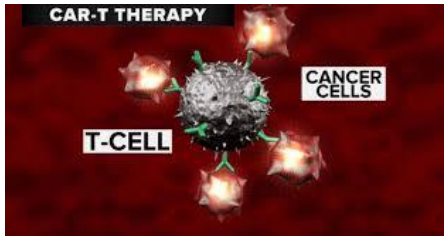

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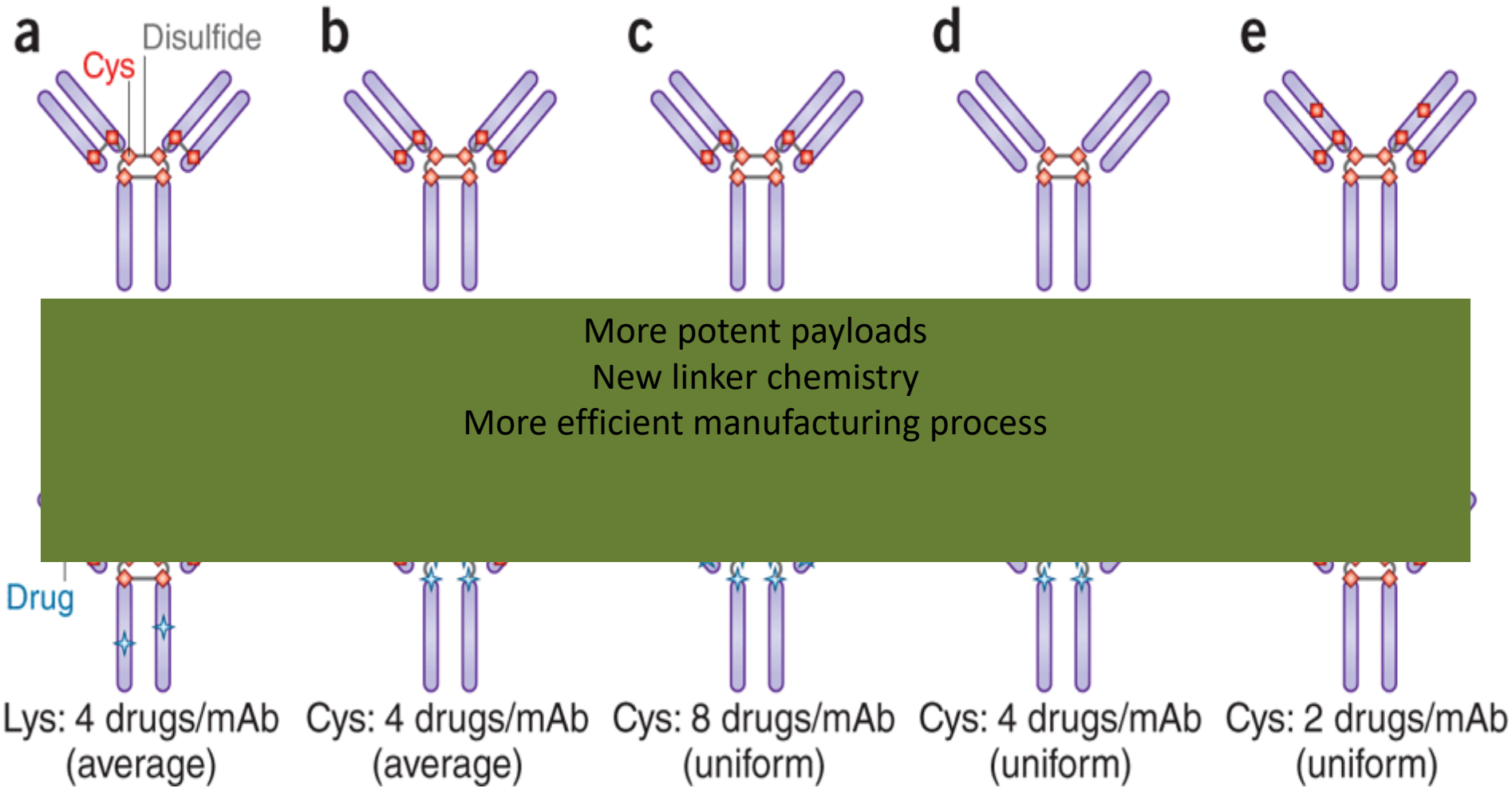
Database lock 12/8/19 –

Antibody-drug conjugates (ADC)

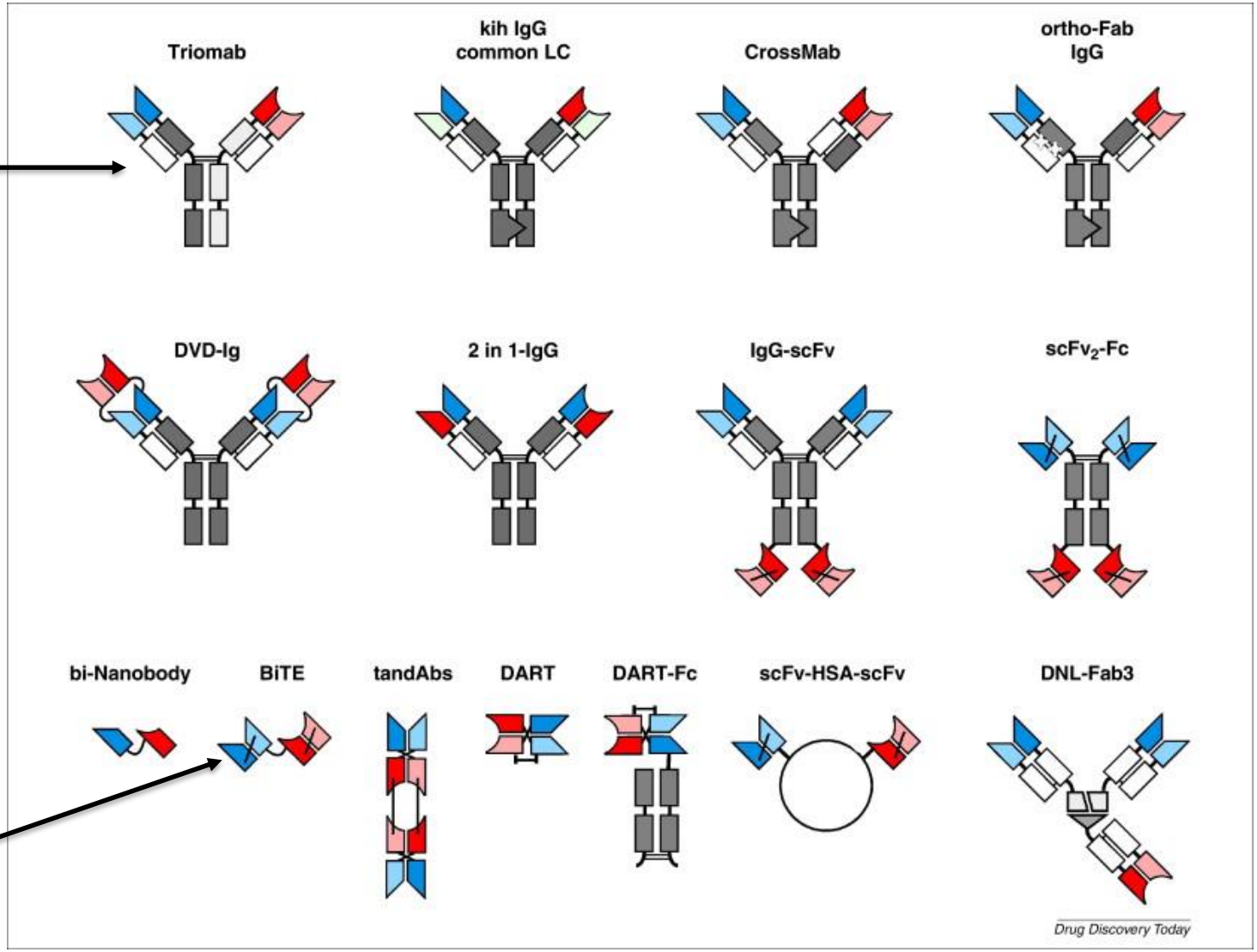


More potent payloads
New linker chemistry
More efficient manufacturing process

Nature Biotechnology 30: 631–637 (2012)

Bispecific antibodies (bsAbs)

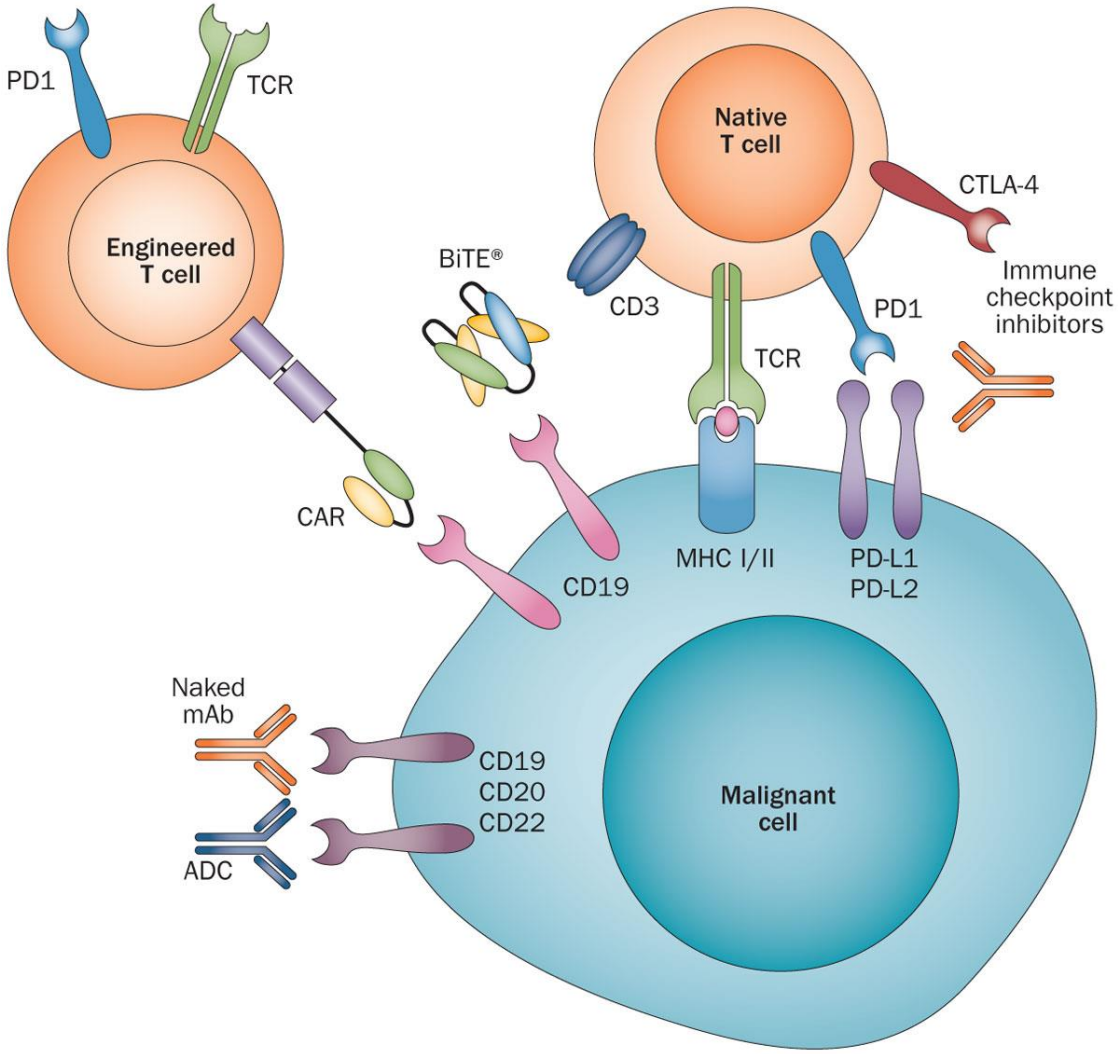
Catumaxomab
CD3/EpCAM
Malignant ascites

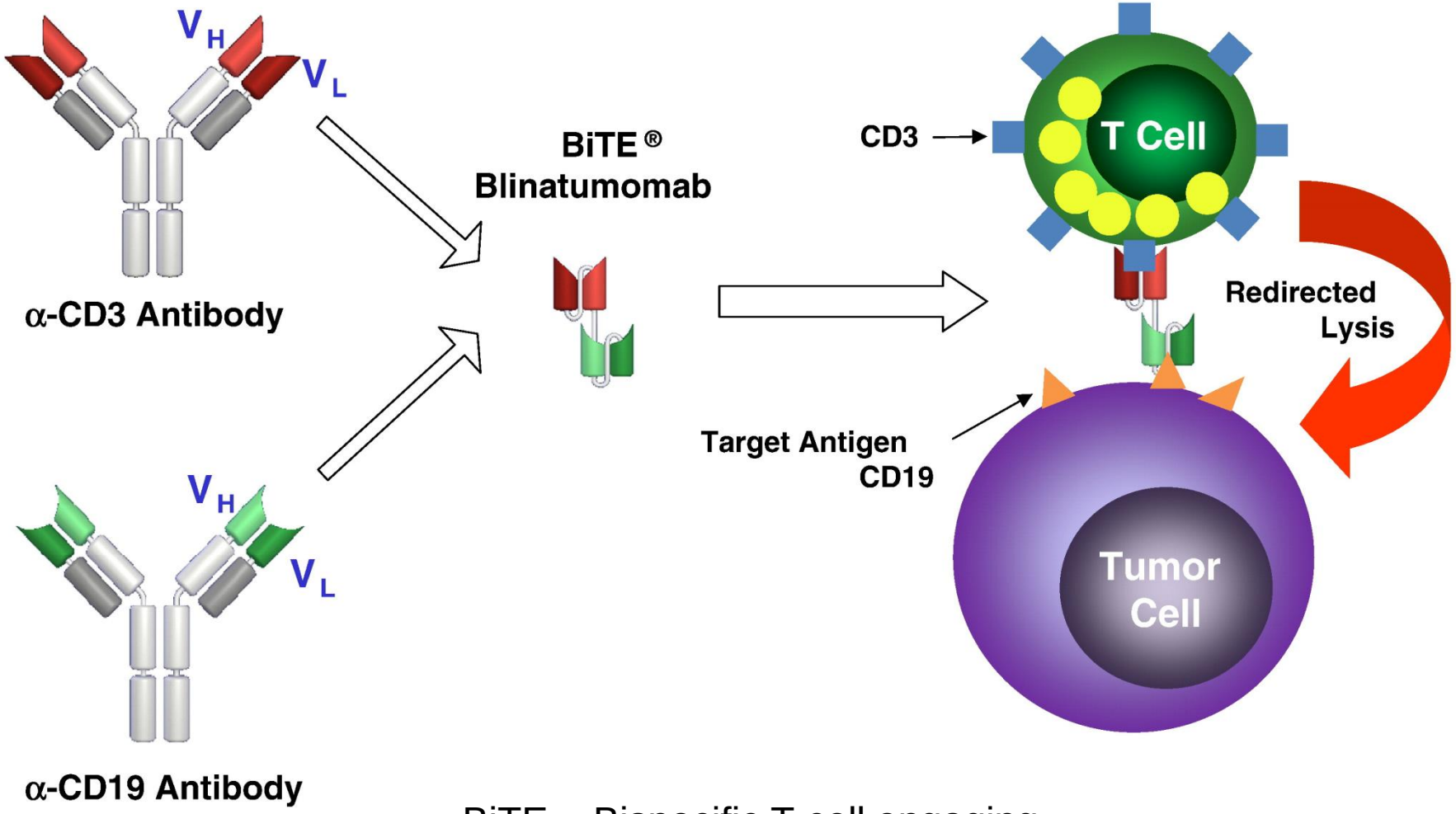


Blinatumomab
CD3/CD19
Acute lymphoid
leukemia (**ALL**)

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

Mechanisms of action of immunotherapy modalities



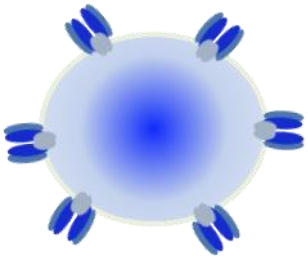


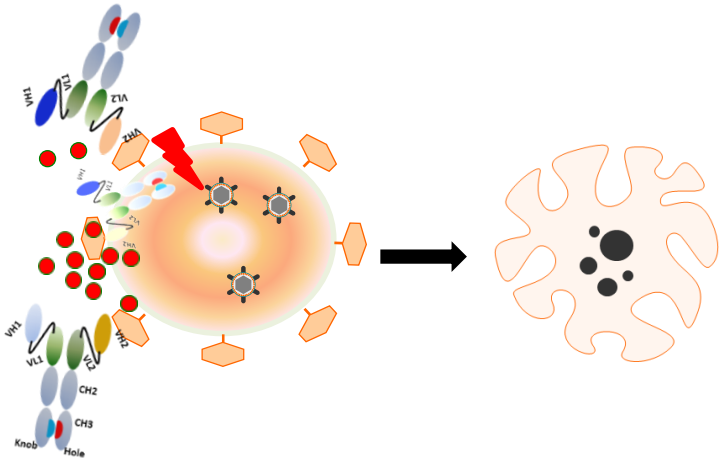
BiTE = Bispecific T-cell engaging

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

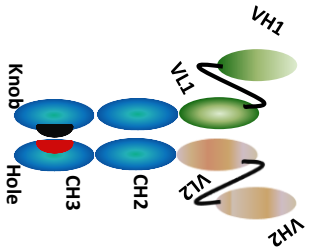
Nagorsen & Baeuerle, Experimental Cell Research. 317 (9), 2011, 1255-1260

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

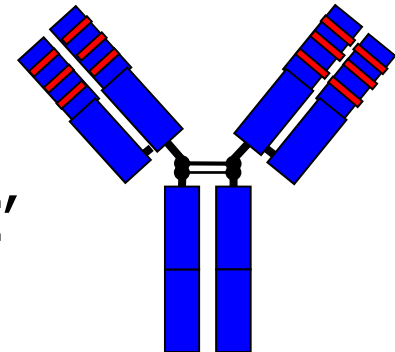
Humanization - CDR Grafting

Donor V gene of animal monoclonal antibody

Acceptor human V gene

CDR-grafted V gene

Final CDR-grafted V gene

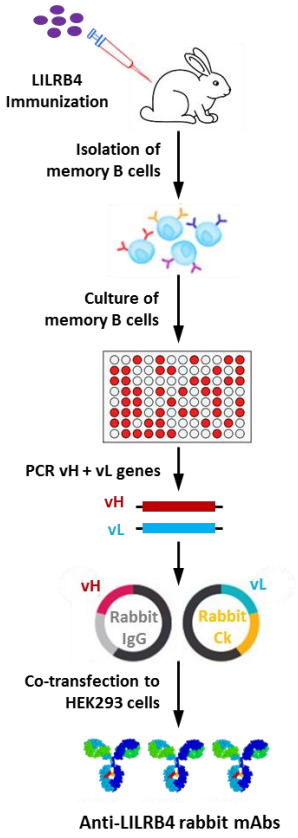


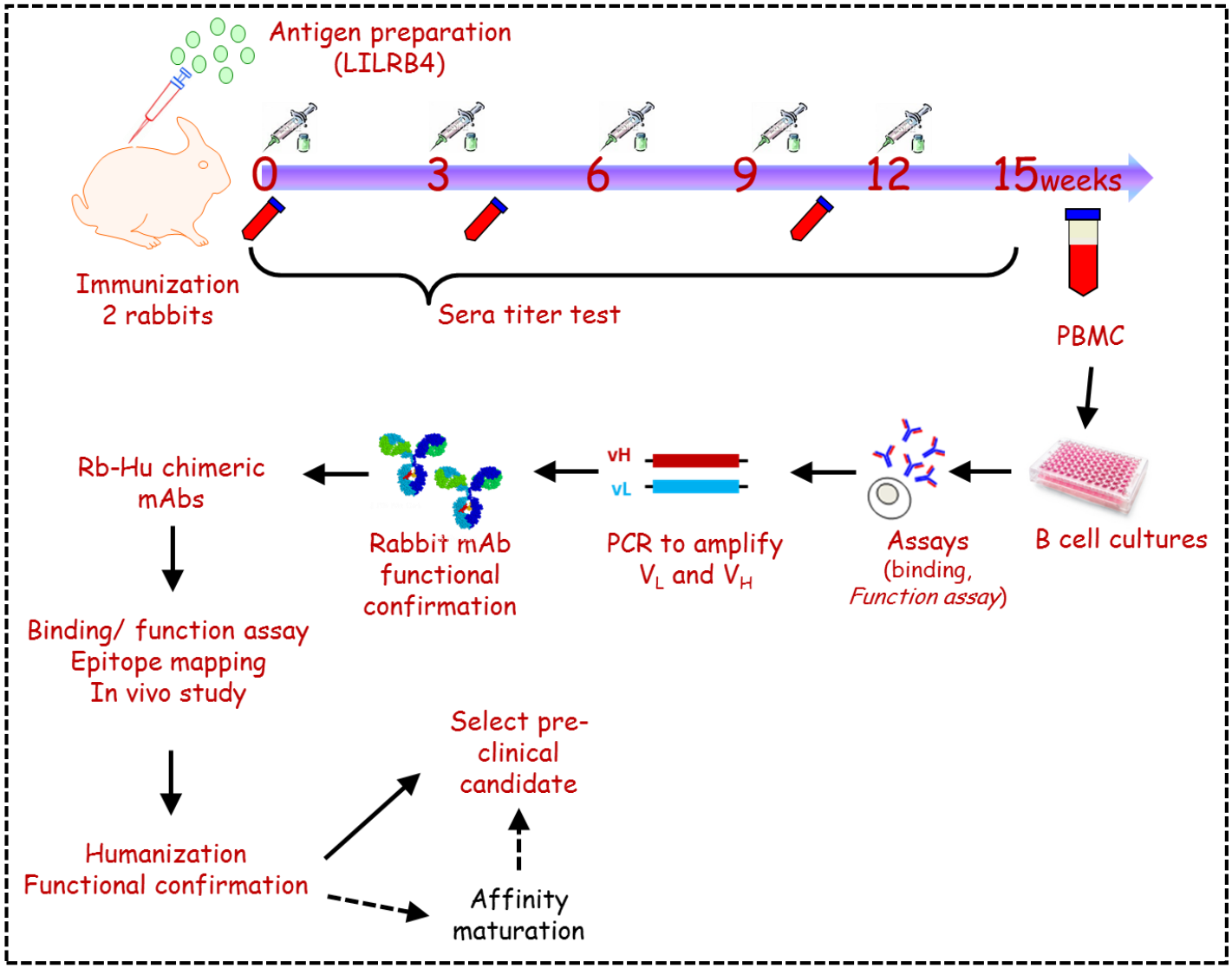
Affinity maturation of anti-IL-13Rα1 mAbs

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

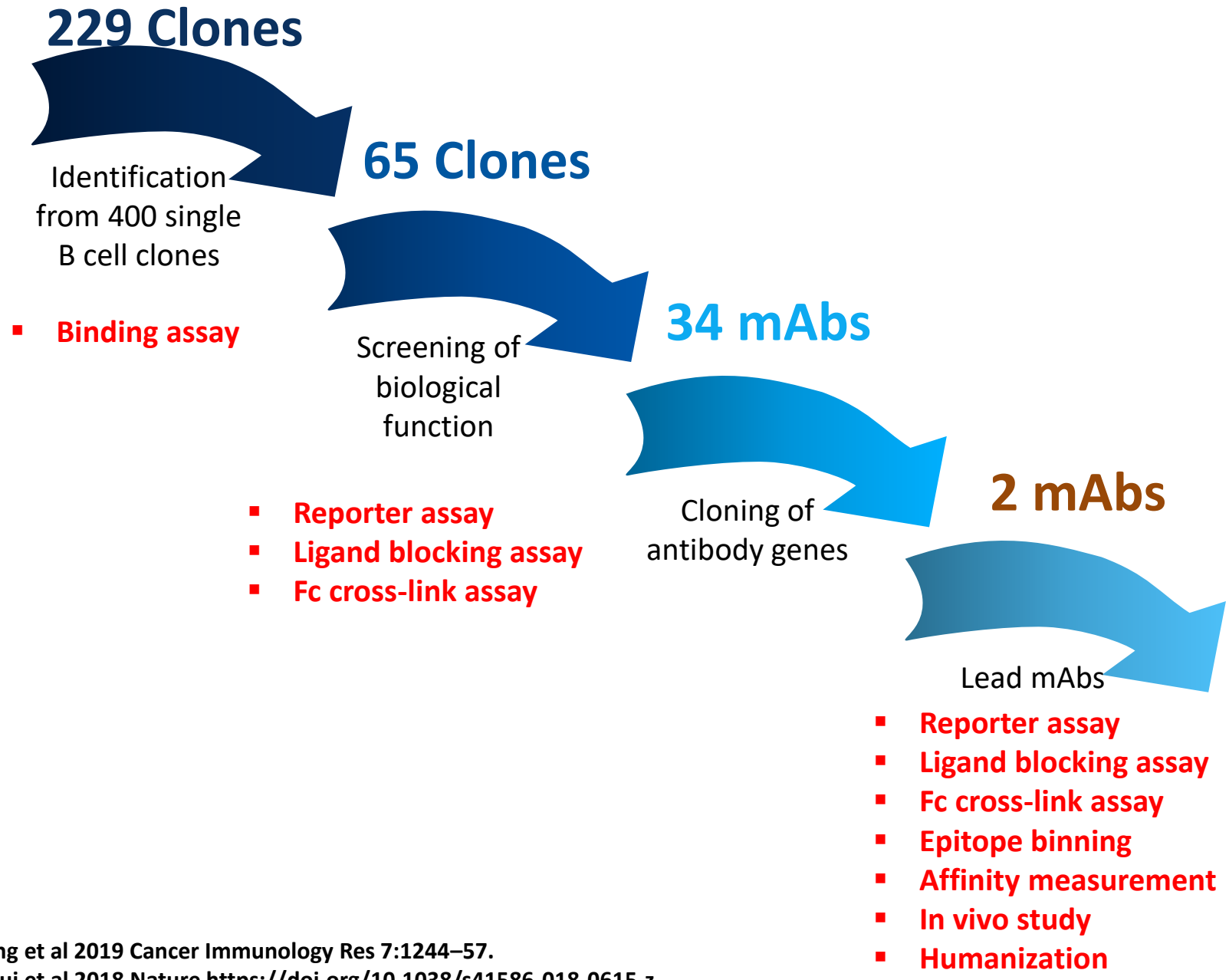
Strategy for LILRB4 antibody generation





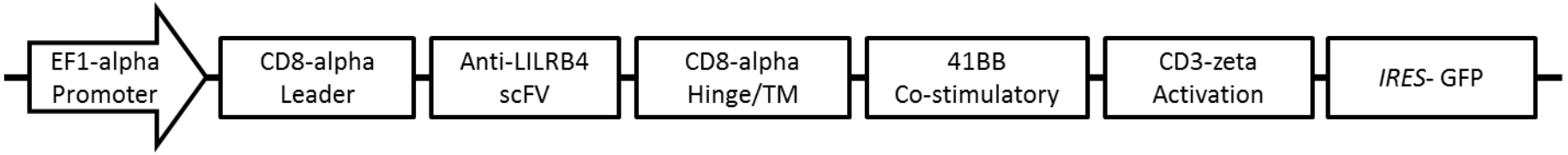
Gui & Deng et al 2019 Cancer Immunology Res 7:1244–57.

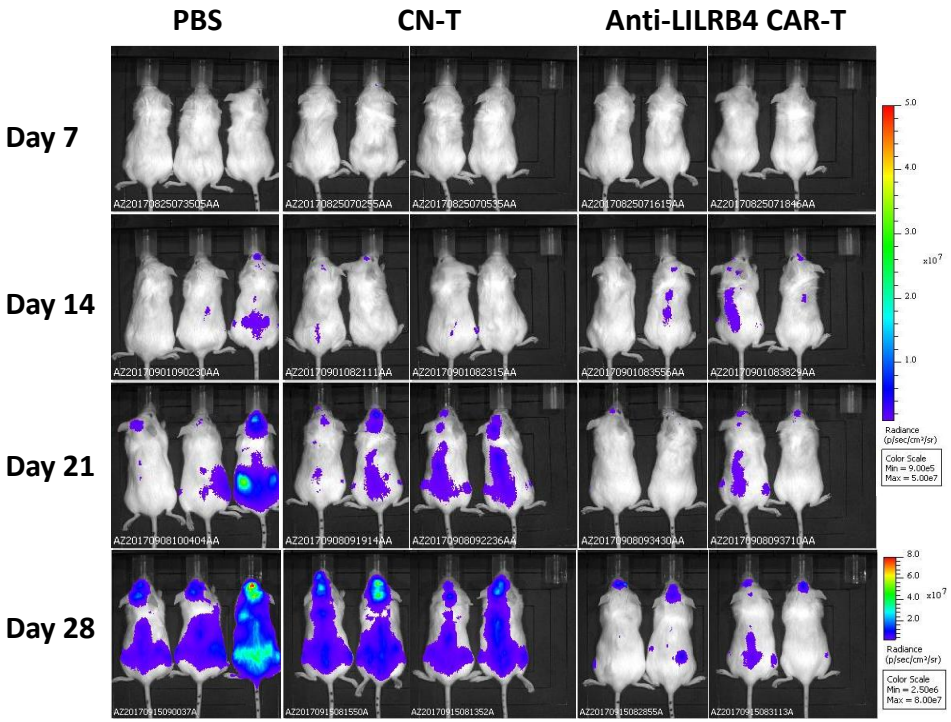
Steps for mAbs generation



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

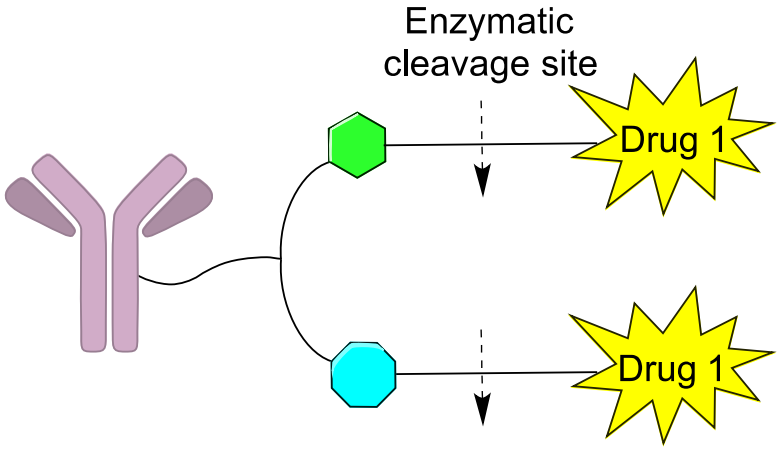
Anti-LILRB4 CAR-T cells display efficient *in vitro* cytotoxicity and specific cytokine release when stimulated by LILRB4^{+} AML cells



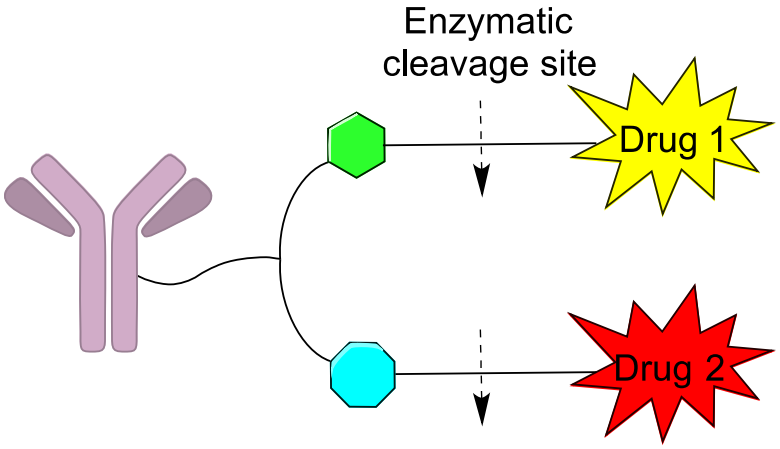


Samuel John et al., 2018 Molecular Therapy

Next-Generation Linker Platform



Loding two identical drug molecules
Improved potency



Loding two different drug molecules
Acts on two different target mechanisms

<u>mAb with two identical drugs</u>

- ✓ Enhanced potency
- ✓ Minimum mAb modification

<u>mAb with two **different** drugs</u>

- ✓ Dual modes of action
- ✓ May overcome drug resistance and cancer heterogeneity

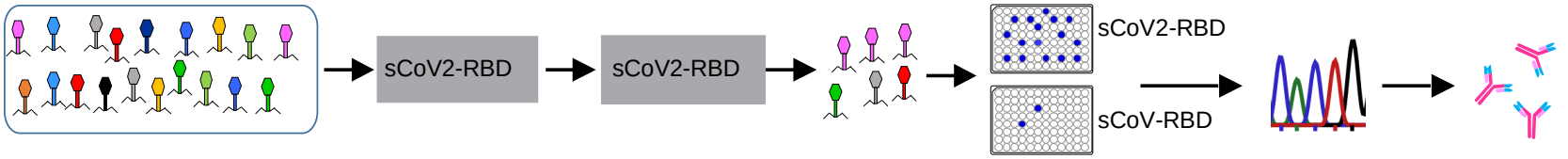
Our Strategy (Dr. Kyoji Tsuchikama)

Drug 1: hydrophilic payload

Drug 2: payload with bystander effect

1) Anami, Y., Xiong, W., Gui, X., Deng, M., Zhang, C. C., Zhang, N., An, Z., Tsuchikama, K. *Org. Biomol. Chem.* **2017**, *15*, 5635–5642.
2) Anami, Y., Yamazaki, C.M., Xiong, W., Gui, X., Zhang, N., An, Z., Tsuchikama, K. *Nat. Commun.* **2018**, 9:2512.
3) Anami et al, *Mol. Cancer Ther.* **2020**, 19:2330–2339.
4) Yamazaki et al., *Nat. Commun.* **2021,** doi.org/10.1038/s41467-021-23793-7

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

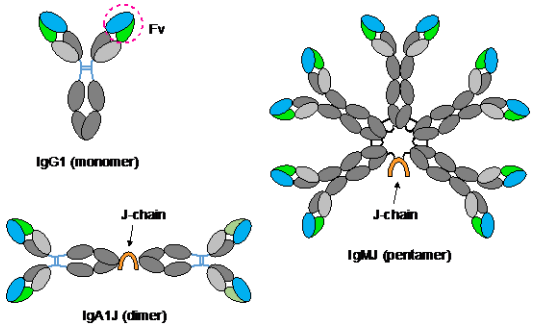
(149)

Sequencing

35 scFvs

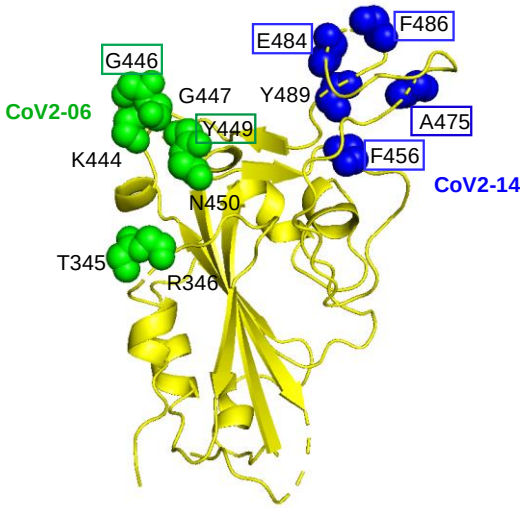
IgG1 expressior

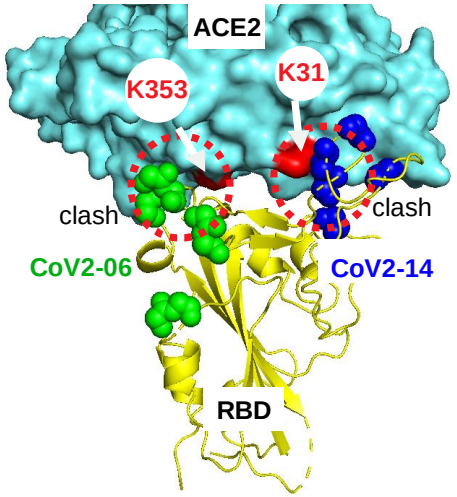
(30)

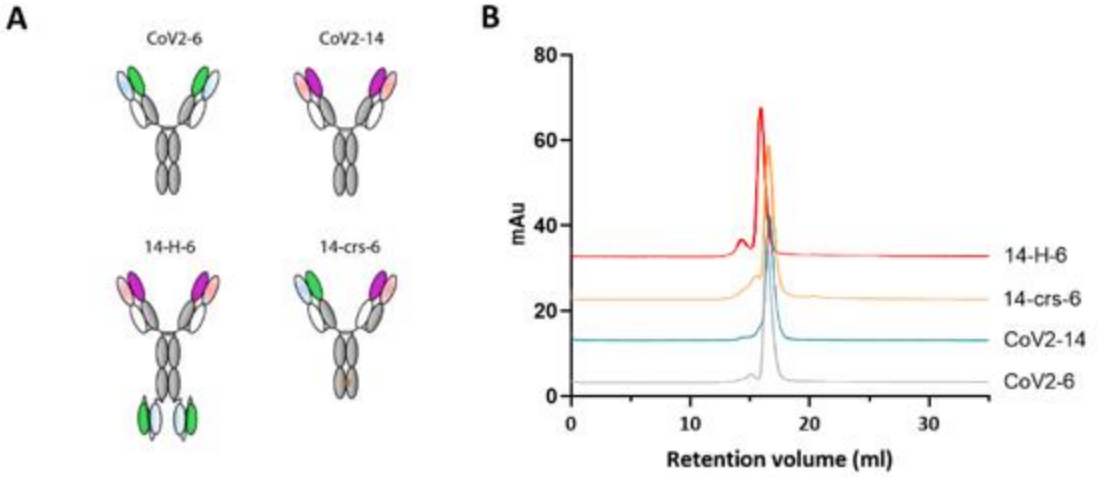


Ku et al. Nature Communications 2021
Ku et al. Nature 2021

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

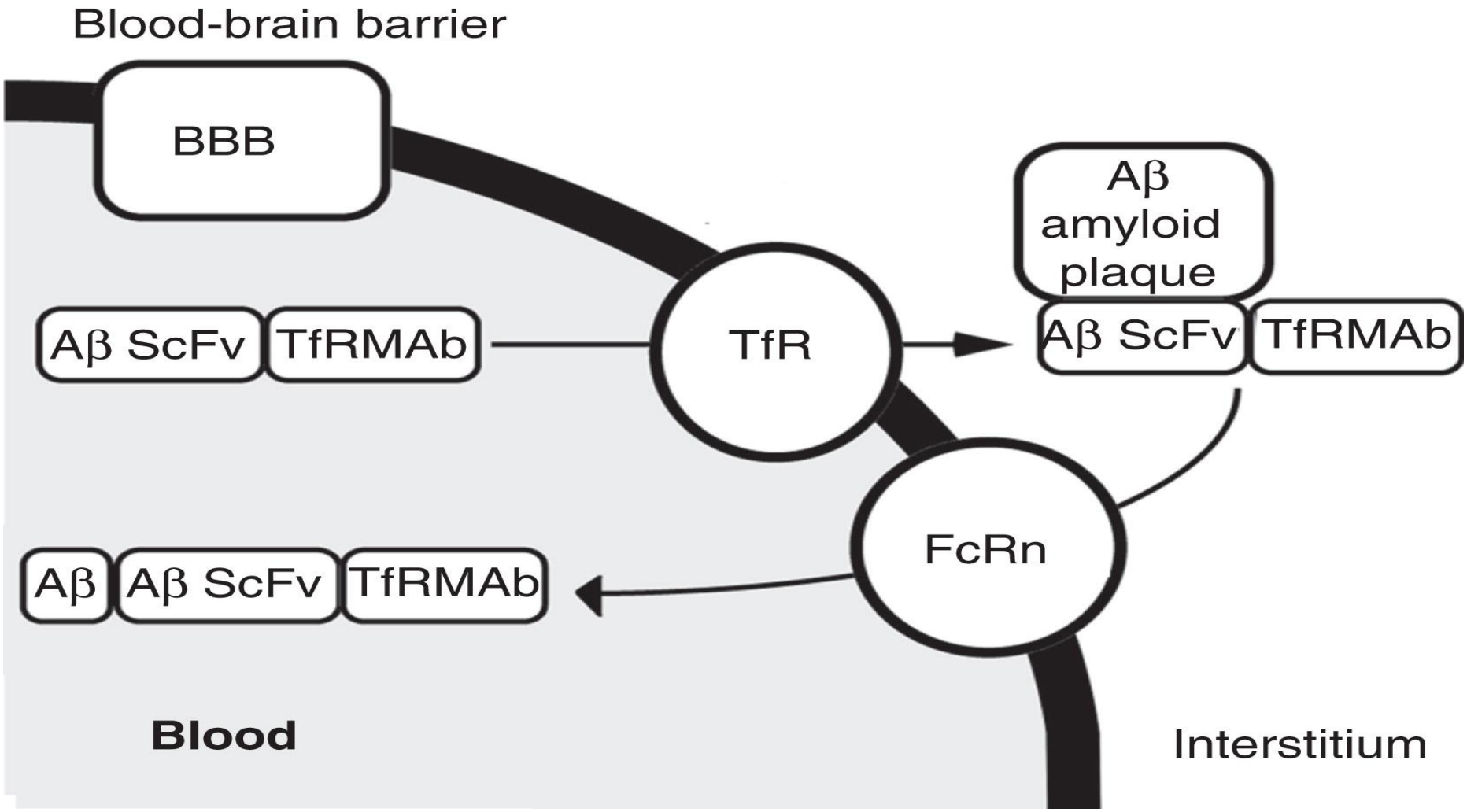






Gao et al. manuscript submitted

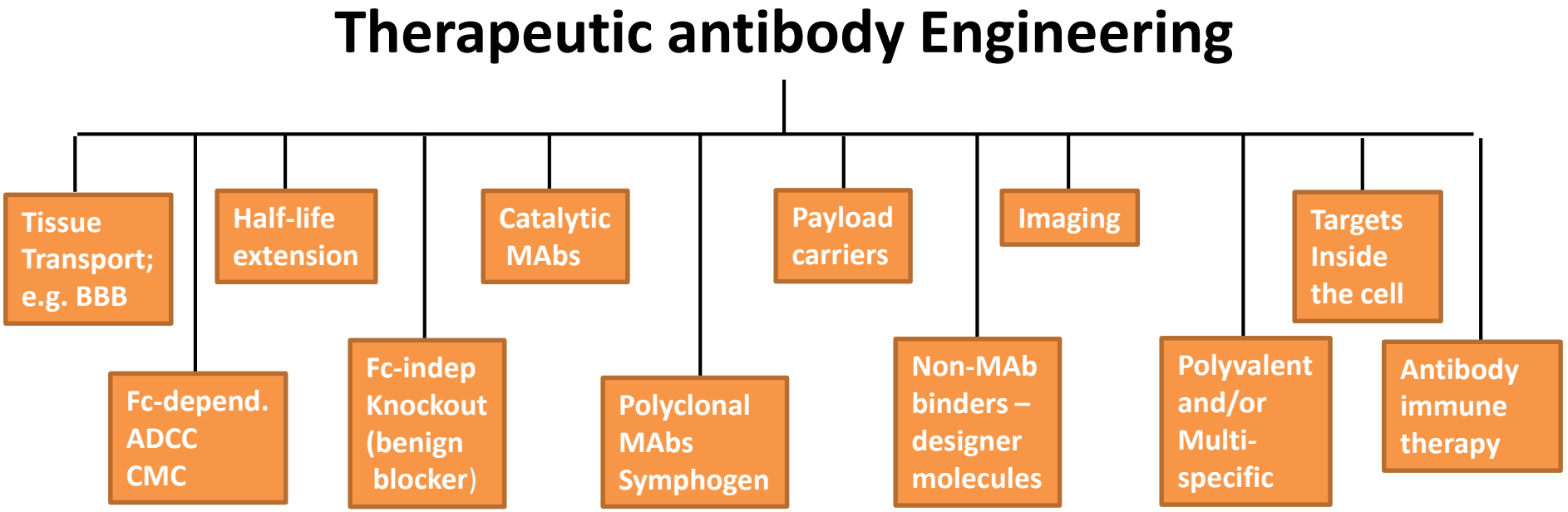
The cTfRMAb–ScFv fusion protein clears amyloid from brain



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

Thank you for your attention!