Development of Novel Therapeutic Antibodies via Computational Design

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Ab Studio Inc.

Unique feature of Ab Studio Inc.

A novel biotech platform company with promising therapeutic antibody programs

We have invented the following three novel platforms via computational design:

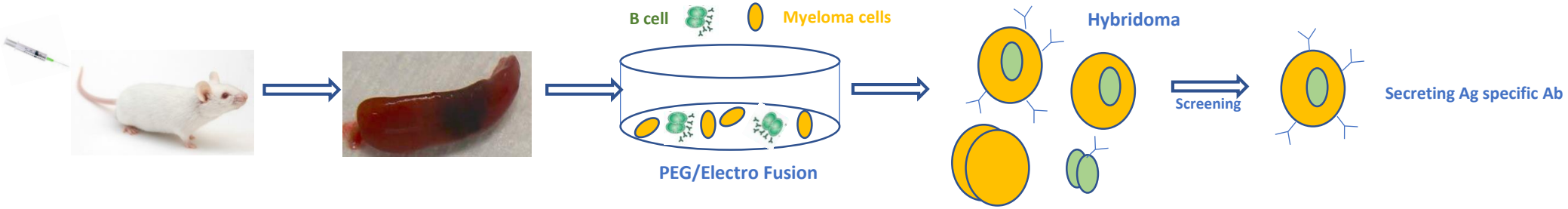
1) “Imbalanced” bispecific antibody platform
2) “Serial” internalization antibody platform
3) “Catalytic” antibody platform

We deeply believe in the “quality by design” concept and wish our work can help addressing unmet clinical needs.

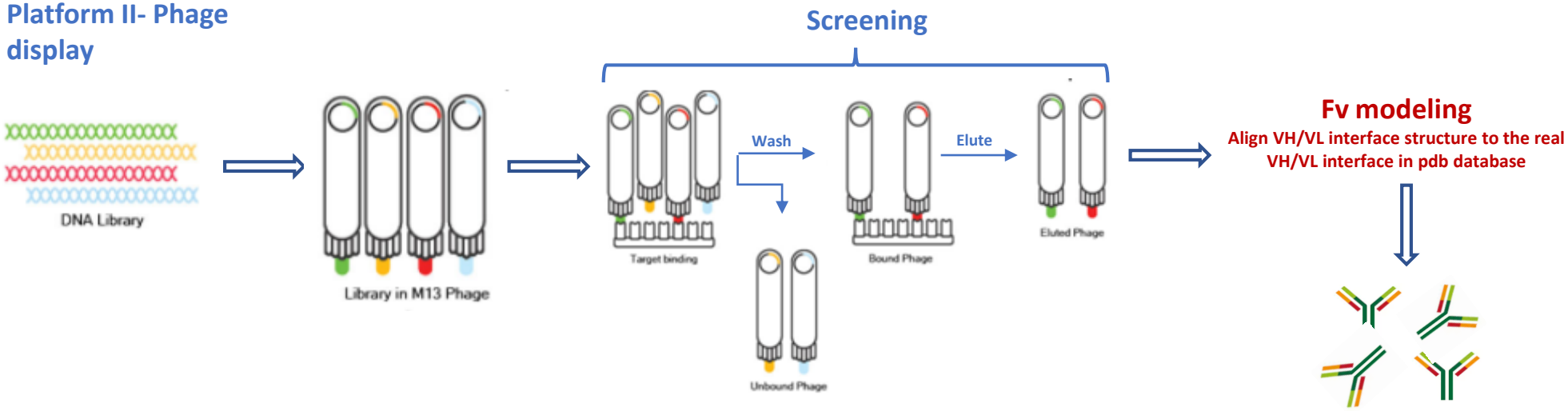
Introduction of Ab Studio's novel therapeutic antibody discovery platforms

Basic technologies:

Platform I- Hybridoma



Platform II- Phage display



Hybridoma + Computational Design:

1) Apply computer aided modeling to identify epitopes exposed on 3D surface of antigen
2) Based on target biology, 3D information and bioinformatics (such as cross-species homology), design and apply appropriate strategy for immunization
3) Based on target biology and target protein structure, design and apply high throughput binding assay, functional assay and epitope bin assay to screen the best functional antibody
4) Align the sequences of several “best functional antibodies” to get “best developable antibodies” by screening candidates with: high “human score” + less immunogenicity epitopes + less PTMs

Unique features for phage display technology:

1) In house developed 10e9 human naïve ScFV phage library with a restriction enzyme site between VH and VL. Therefore, VH and VL can be replaced separately (suitable for common light chain screening)
2) Leading phage screening not only based on antigen binding ability pre and post heating, but also based on ScFV seqs' other develop-ability features. After building up a Fv model structure, we study

- Aggregation surface on Fv model
- PTMs on Fv model
- Immunogenecity epitopes inside ScFV sequence
- "Naturality" of VH/VL interface by aligning the query ScFV's VH/VL interface to that of real 3D antibody structures in the pdb database

Antibody Humanization and Optimization

We balance:

Humanization percentage

CDR Immunogenicity

Antigen binding affinity

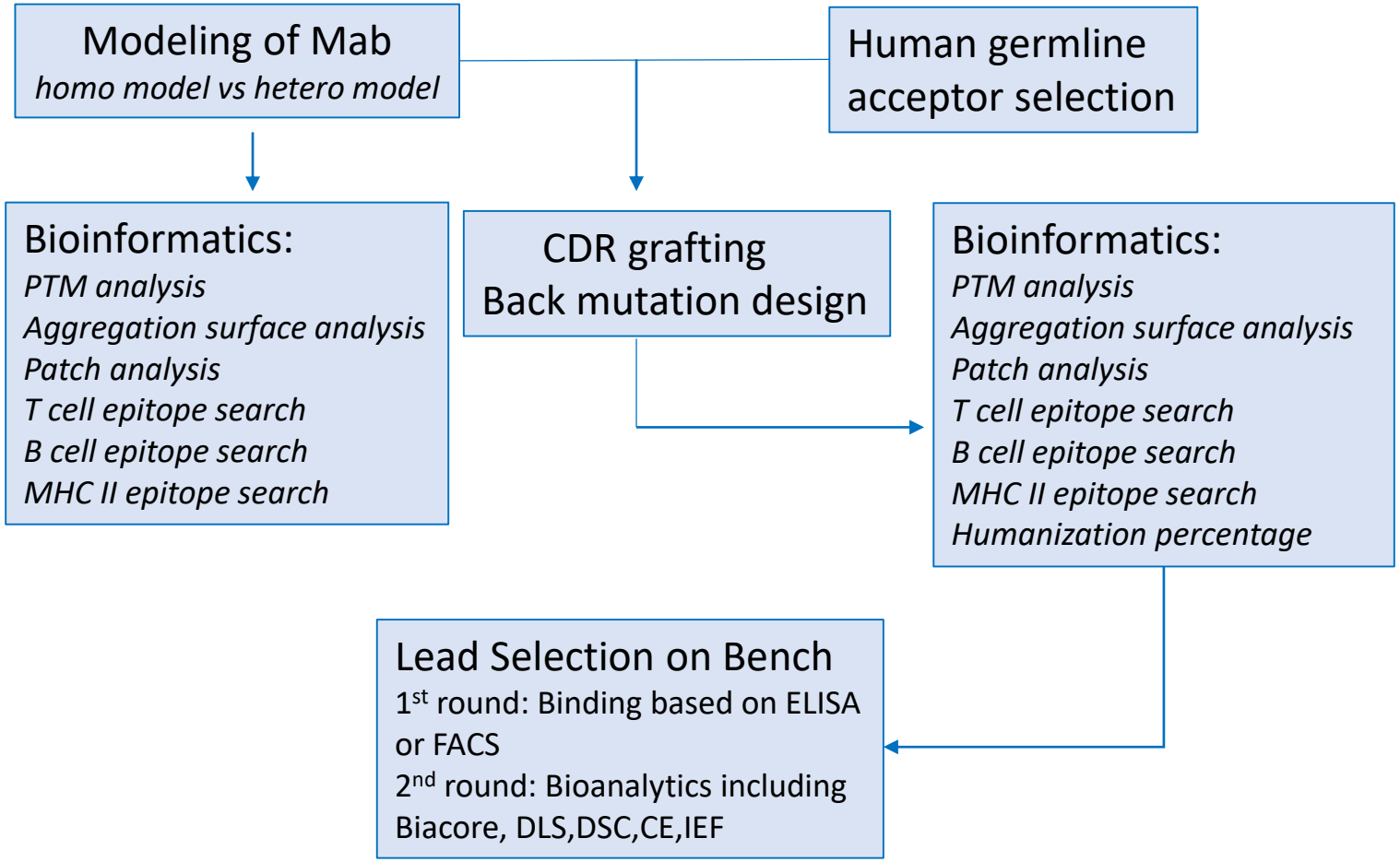
Antibody expression level

Aggregation potential

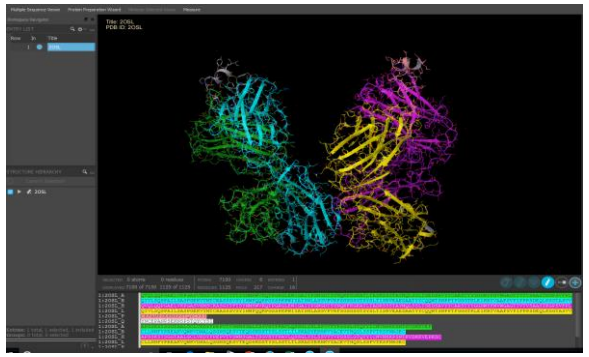
Antibody stability

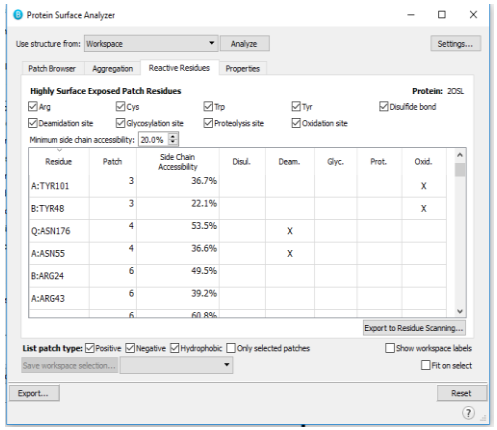
Heterogenicity

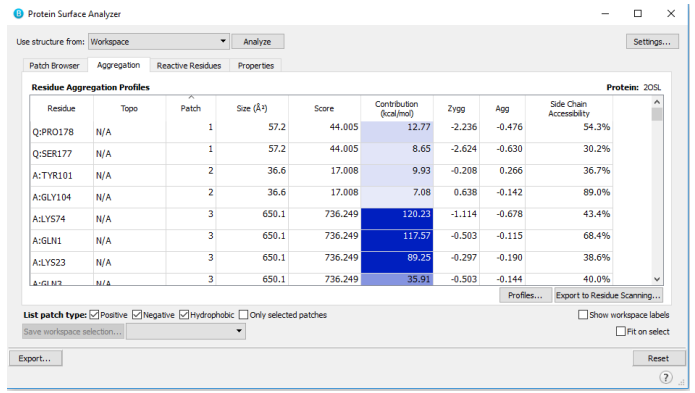
Workflow of Antibody Humanization and Optimization

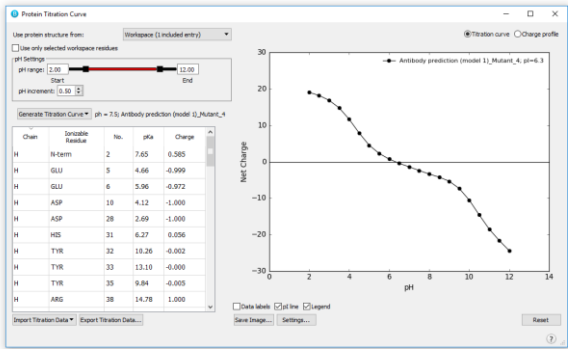


Molecular Modeling and 3D Surface Analysis

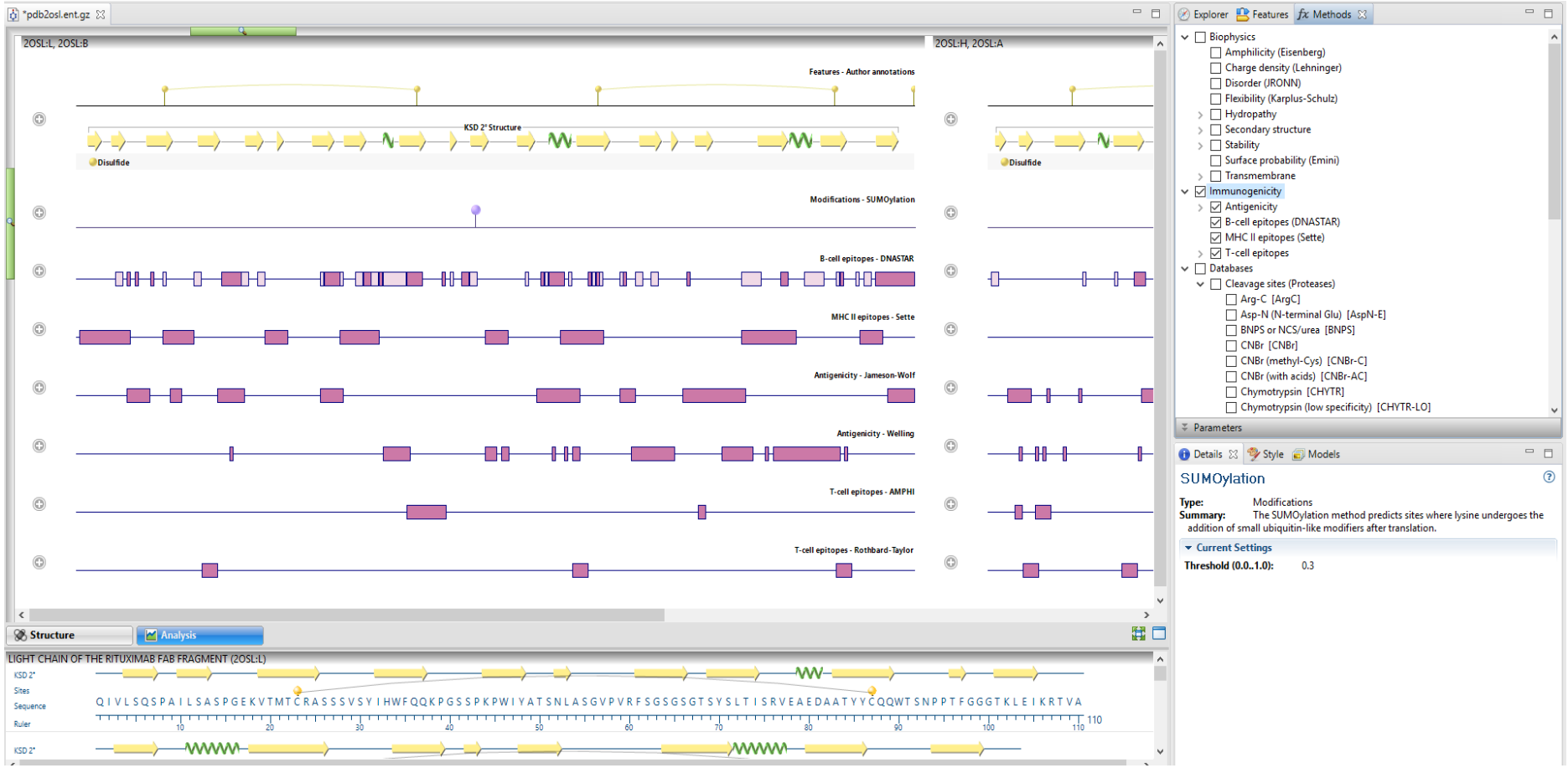






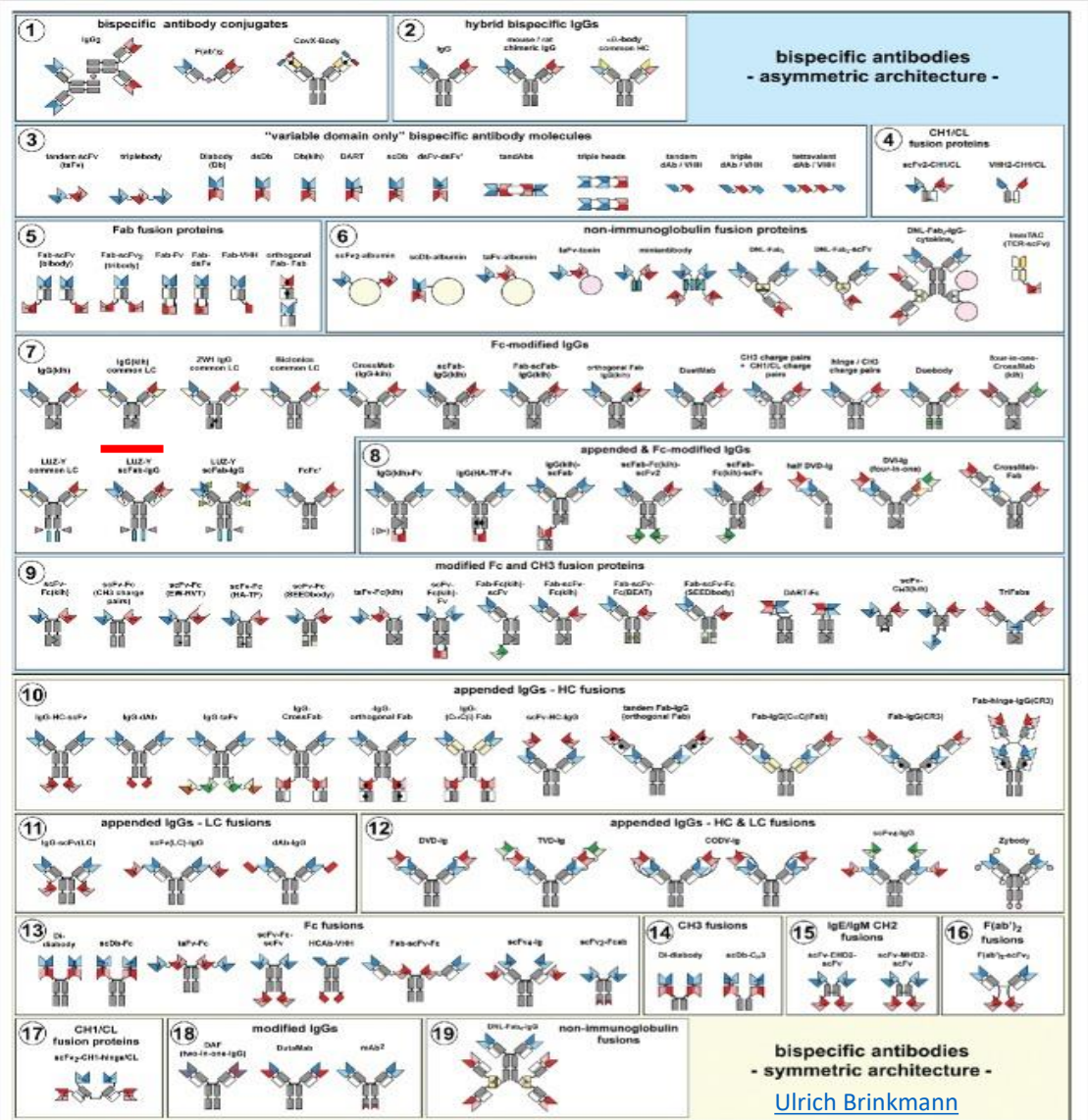


Immunogenecity Epitope Scan



Novel platform 1:

Imbalanced Bispecific Antibody Technology Platform



The Zoo of Bispecific Antibody Formats

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5297537/

Only a few platforms meet the following criterias:

1) Free to operate
2) No extra immunogenicity and developability concerns
3) No PKPD concerns

"Knob into hole" + Common LC format is an one meets the above criterias

Imbalanced Bispecific Antibody Technology Platform

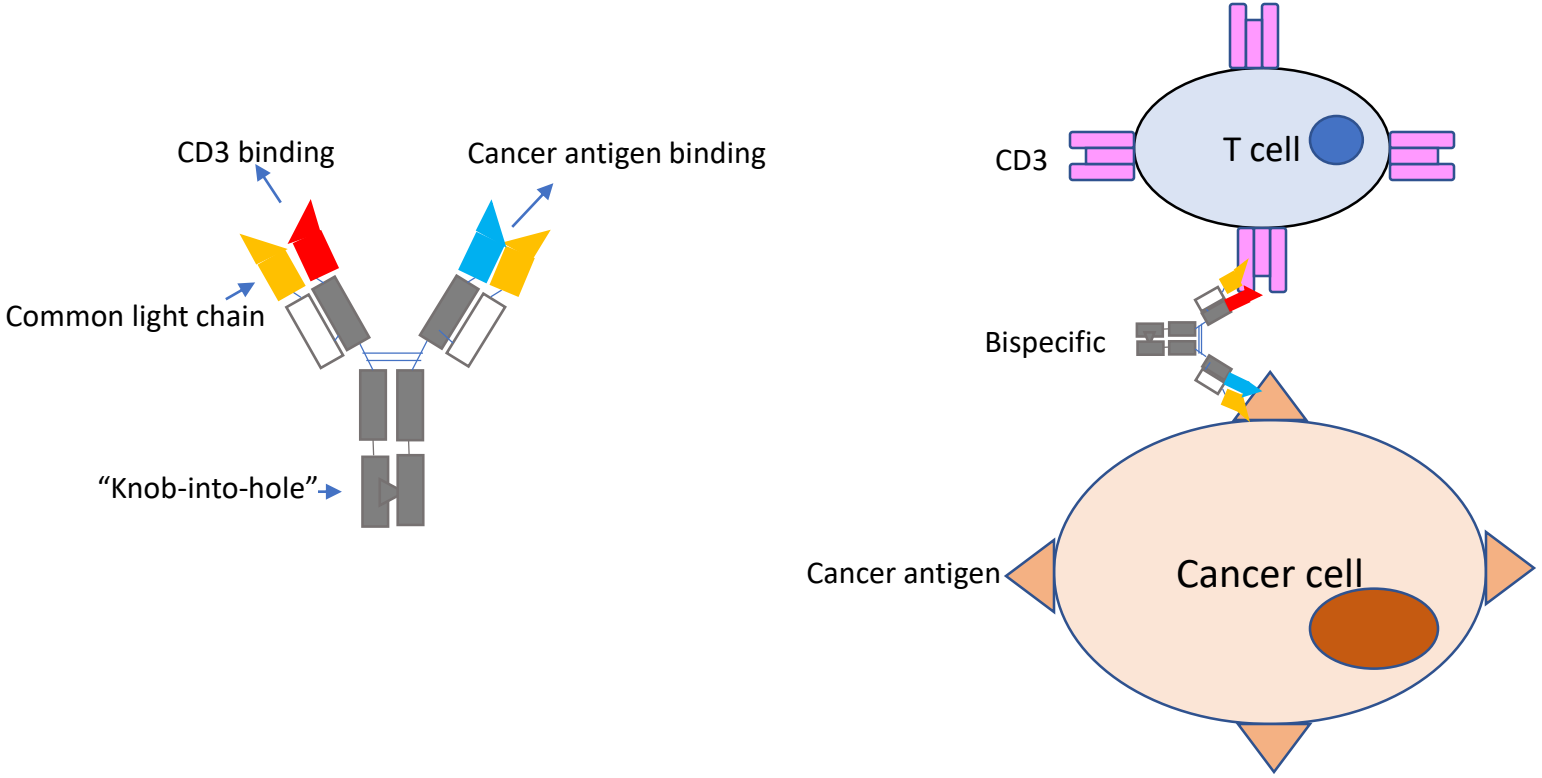
What is an imbalanced bispecific antibody?

- A “Knob-into-hole plus common LC” version allowing two arms to have very different binding affinity to each one’s own antigen (natural IgG structure and half life).

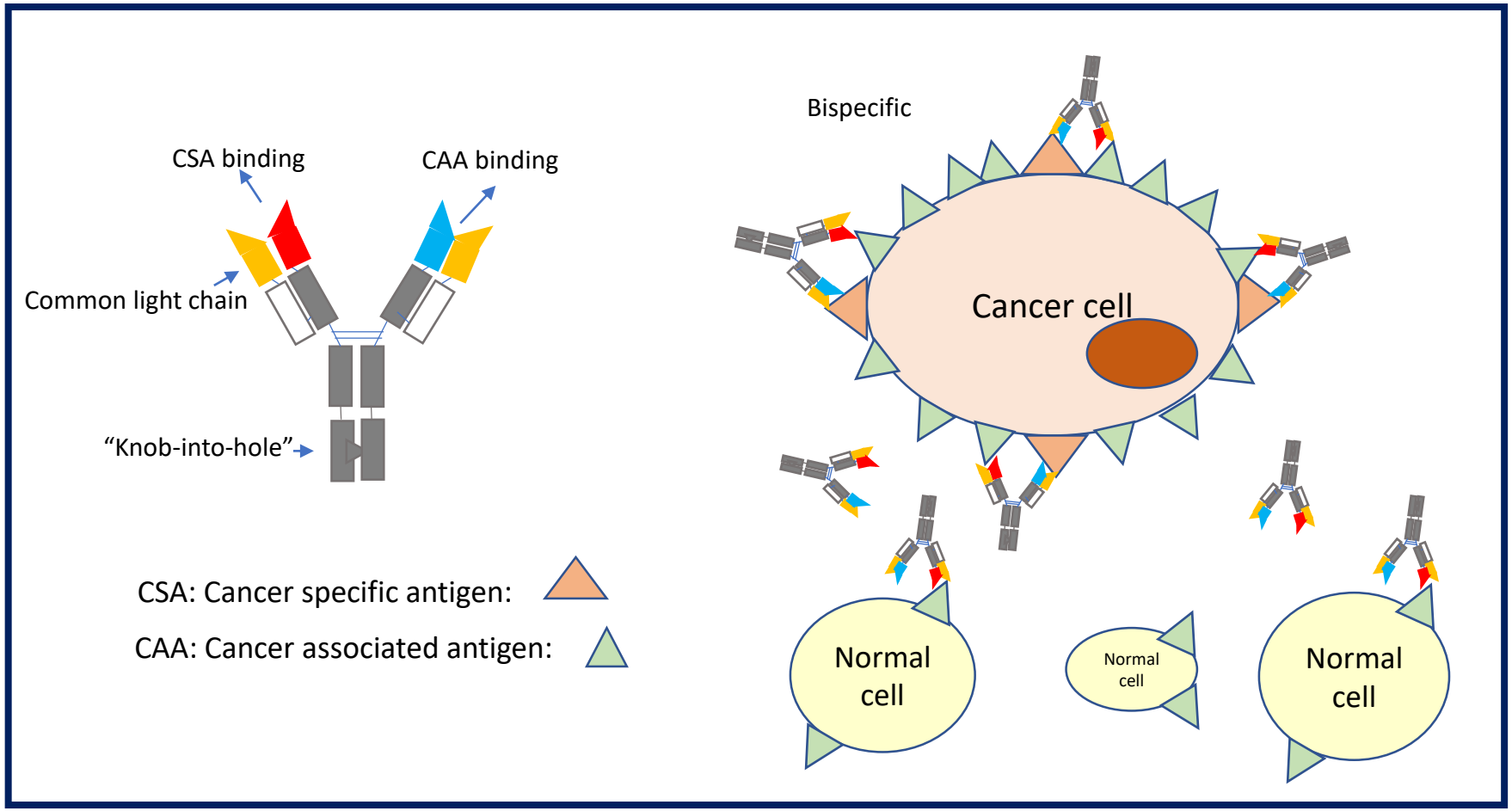
Why “imbalanced”?

- Imbalanced binding affinity is required for some bispecific therapeutic antibodies’ Mechanism of Action.

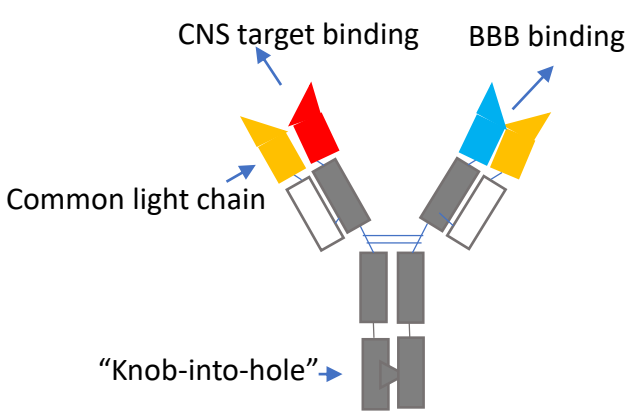
Bispecific involves a CD3 binder

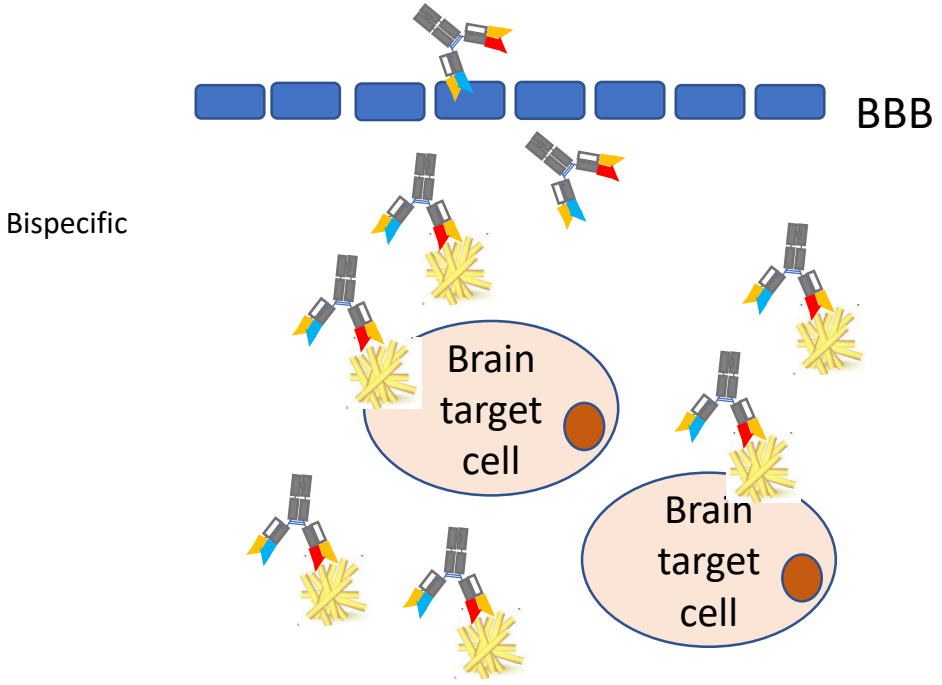


Bispecific against a CSA (cancer specific antigen) and a CAA (cancer associated antigen)



Bispecific against one target for CNS and another target on BBB





How “imbalanced bispecific antibodies” are made at Ab Studio?

(‘A’ is the corresponding high affinity arm and ‘B’ is the corresponding low affinity arm)

VL Design:

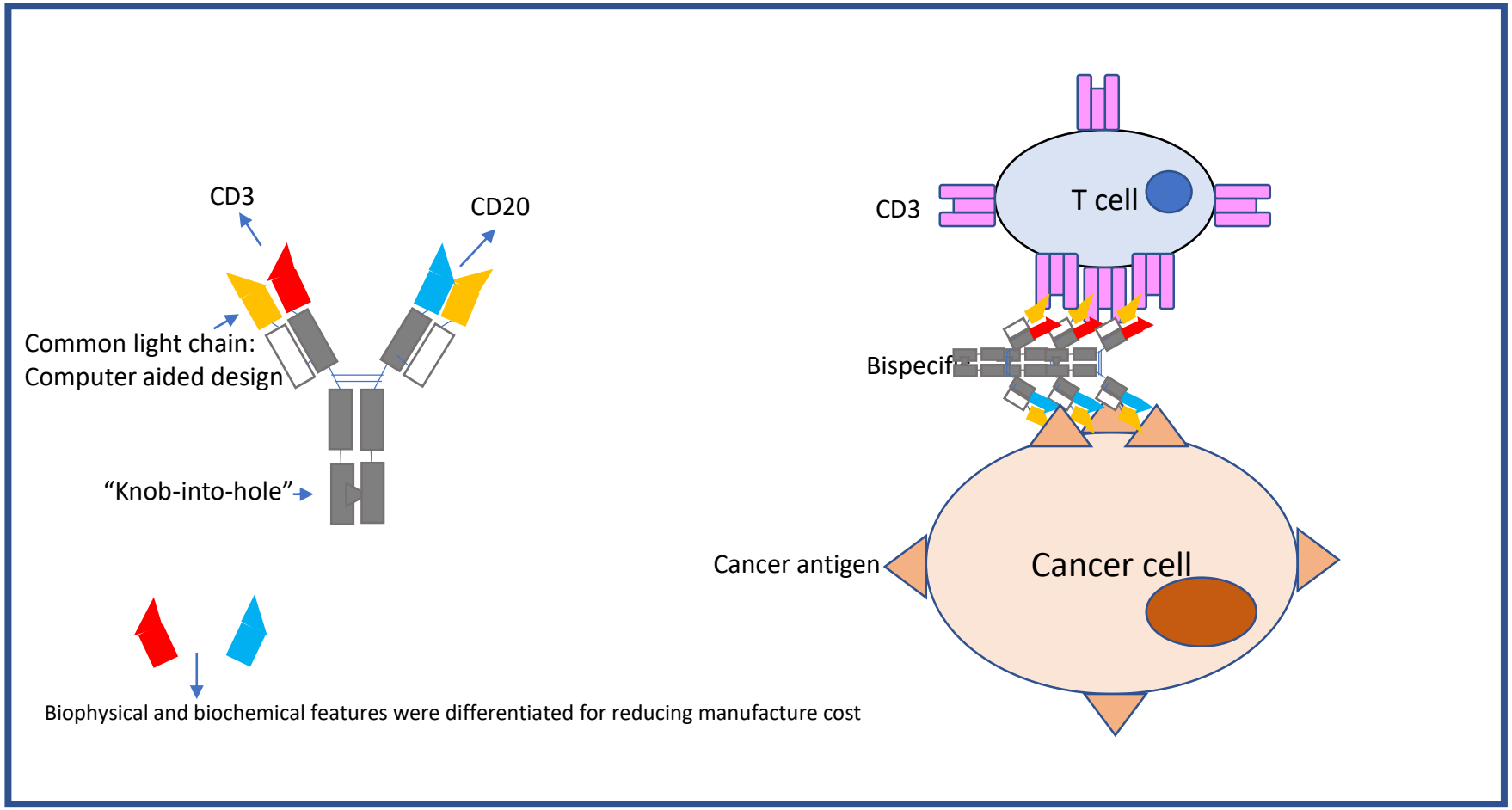
1) If VL-A and VL-B light chain homology >80%, design a VL-C based on VL-A via computer aided design: Maintain VH-A/VL-C’s affinity as close as that of VH-A/VL-A, allow VH-B/VL-C to have reduced affinity compared to VH-B/VL-B

2) If VL-A and VL-B light chain homology <80%, construct phage ScFV library:
VHs of the ScFV library are human germline VH library
VLs of the ScFV library are VL-A and its mutants derived from error prone PCR at less than 20% of mutation frequency

VH Design:

Use computer aided design to differentiate the biochemical and biophysical features of VH-A and VH-B in order to isolate ‘AB’ better from ‘AA’ and ‘BB’

Case Study: CD20/CD3 Bispecific



Difference to other CD20/CD3 bispecific: ADCC/CDC effector function is maintained

Current CD20+ Cancer Immuno-Therapy

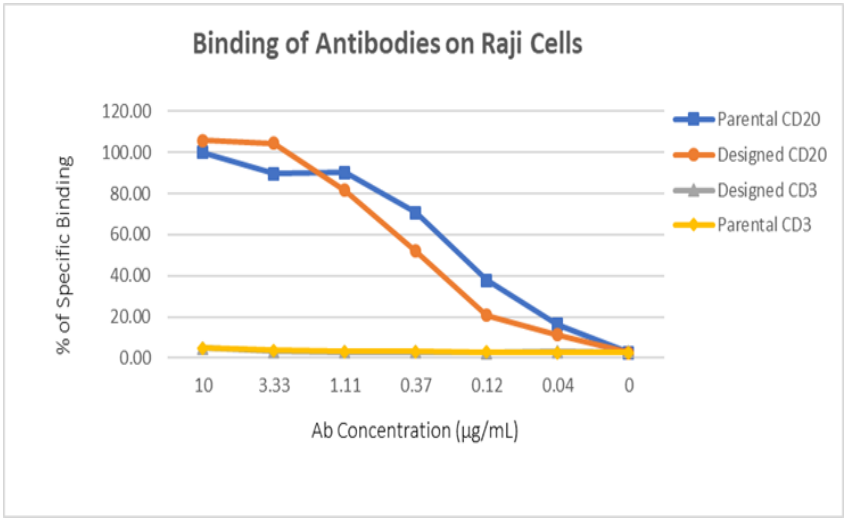
Mechanism of action: ADCC, CDC, Apoptosis
Representatives: Rituxan and its biosimilar, biobetter
Limitation: Other than antigen escape, different genetic makeup of patients' effector cells could cause different response to these therapies

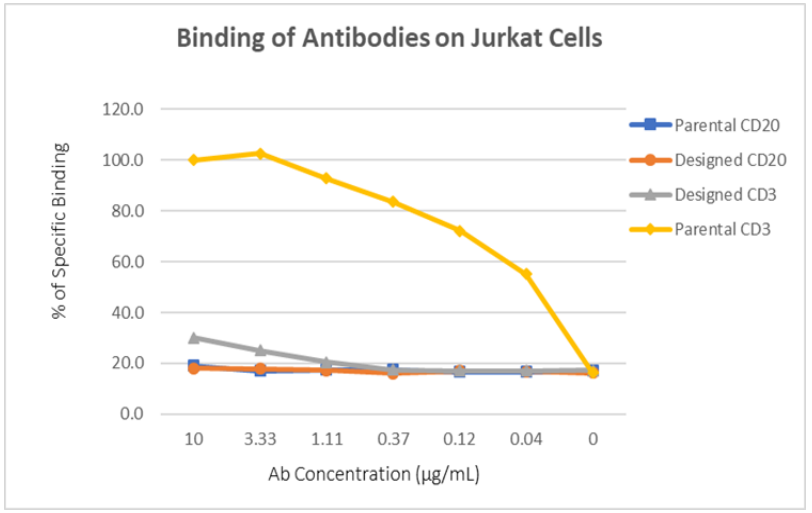
Mechanism of action: CAR-T, TCR-T, T cell engagement
Representatives: CD20/CD3 bispecific (–ADCC/CDC)
Limitation: Other than antigen escape, cancer cells with T cell immune suppressors such as PDL1 overexpressed may have worse response to T cell activation based therapies.

Novel "imbalanced" CD20/CD3 based cancer therapy

By making a CD20/CD3 bispecific antibody with CD3 binding affinity significantly reduced, we hope to "safely" maintain the effector function and reduce cytokine storm. Therefore, the novel "imbalanced" CD20/CD3 bispecific would have all mechanisms as described above.

Binding Ability of Designed Abs Compared to That of Parental Abs





After computational design, CD20 homodimer (hole-hole) IgG contains designed VH sequence and common VL sequence showed similar binding capacity for CD20 compared to that of parental CD20 IgG while CD3 homodimer (knob-knob) IgG contains designed VH sequence and common VL sequence showed significant reduced binding capacity for CD3 compared to that of parental CD3 IgG

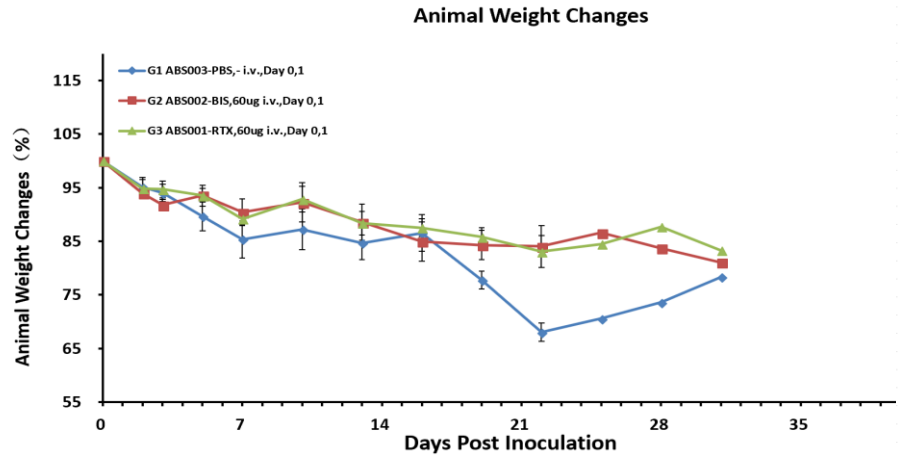
CD20/CD3 "Imbalanced" BsAb has Good Efficacy and Safety *in vitro*

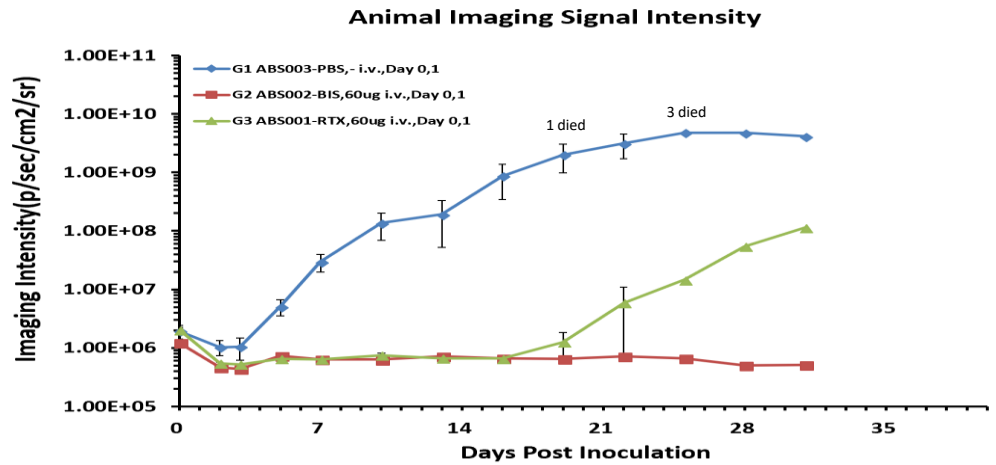
	Efficacy Assay				Safety Assay		
	ADCC on Raji cell	CDC on Raji cell	T cell killing on Raji cell	ADCC on Jurkat	CDC on Jurkat	T cell killing on Jurkat	T cell depletion from PBMC
Isotype Control	-	-	-	-	-	-	-
Rituxan	++	++	-	-	-	-	-
CD20 homodimer	++	++	-	-	-	-	-
CD20/CD3 BsAb	+/++	+	+++	-	-	-/+	-

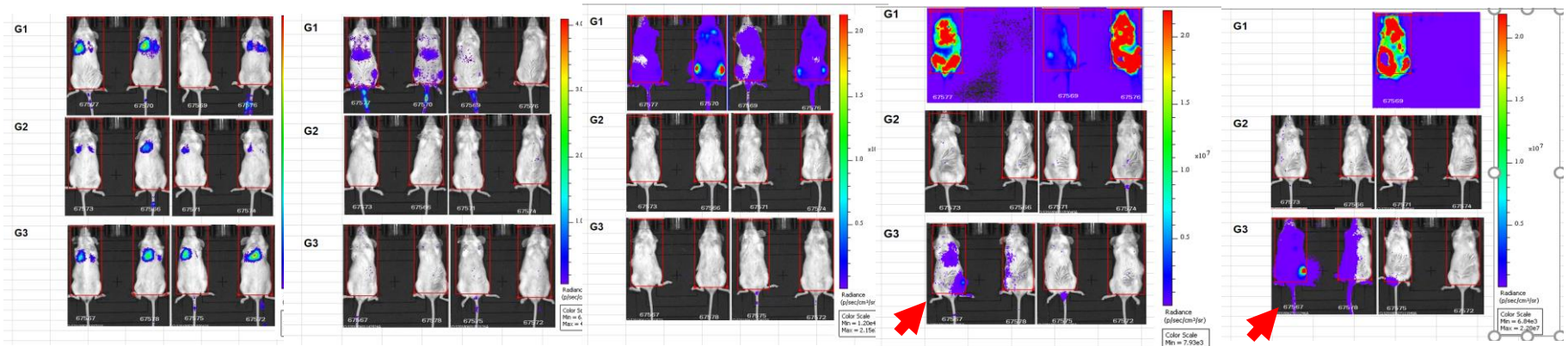
Raji cell: CD20+/CD3- NHL tumor cell

Jurkat cell: CD20-/CD3+ T cell tumor cell

CD20/CD3 "Imbalanced" BsAb has better Tumor Killing Potency than Rituxan *in vivo*

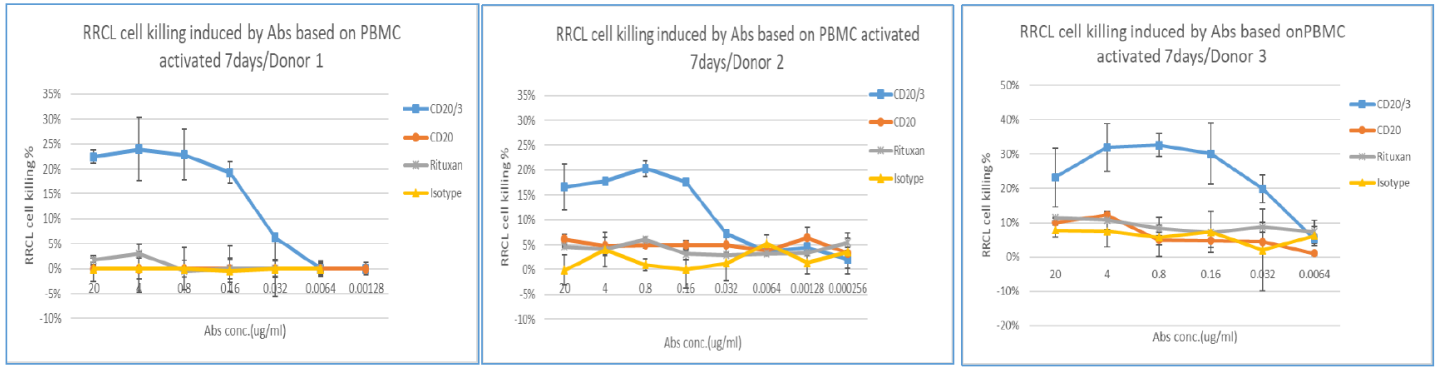






Day 0: IV injection with "Abs/PBS + Raji-Luc cell + human PBMC", first imaging: 15min post injection. G1: PBS, G2: BsAb, G3: Rituximab

Rituxan Resistant Raji Cell (RRCL) can be Efficiently Killed by Imbalanced CD20/CD3 BsMab Abs and PBMC with T cell Pre-activated for 7 days

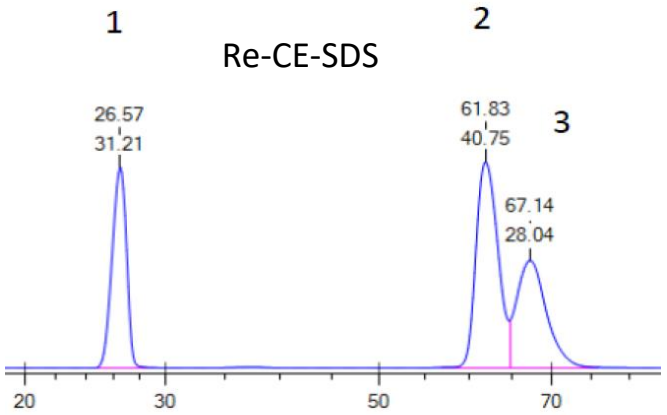


Lot number:3005555 Lot number:3005469 Lot number:3005683

Furthermore, We studied the most important question: Whether Rituxan resistant cancer can be treated by Imbalanced CD20/CD3 BsMab.

CD20/CD3 "Imbalanced" BsAb has good Developability

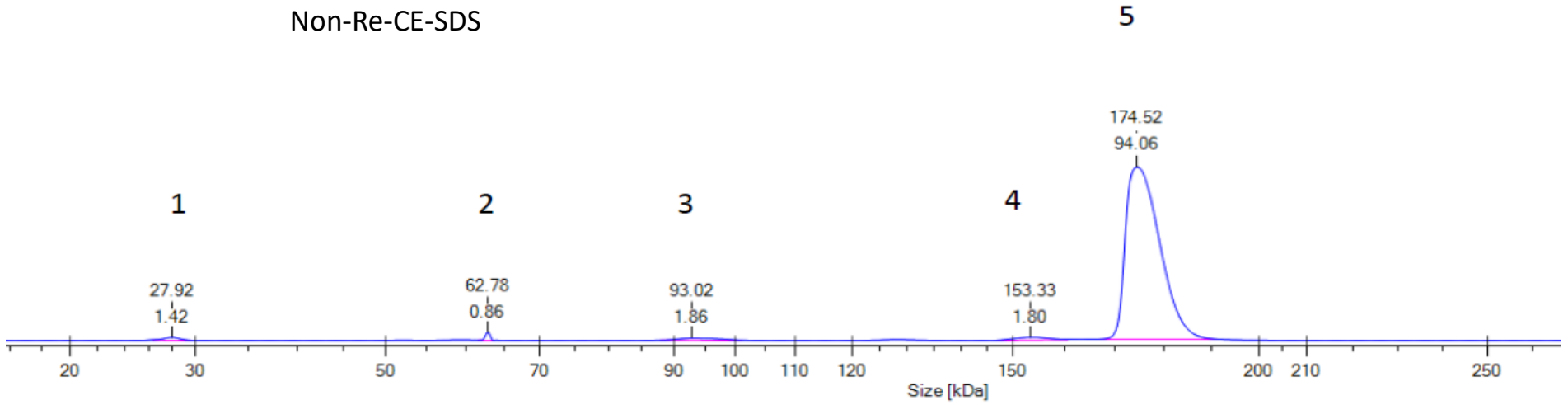
Re-CE-SDS



	DSF				SLS	
Temperature (°C)	Tm1	Tm2	Tm3	Tm4	Tagg 266	Tagg 473
	66.0	80.1	86.7		70.9	71.2

DLS	Peak #	Mode Diameter (nm)	Mass (%)	PDI
20 °C	Pk1	10.15	100	0.177
	Pk2			
	Pk3			

Non-Re-CE-SDS



Summary:

1) Computational designed Imbalanced CD20/CD3 BsMab has good efficacy, safety and developability *in vitro* and in animal model.

Item	m Ab	CAR-T	Bispecific Ab	Imbalanced Bispecific Ab
Efficiency of killing tumor	+	+++	++/+++	++
Cytokine storm	N/A	++	++	N/A
Mechanism	ADCC, CDC, Apoptosis	T cell	T cell	T cell engagement, ADCC, CDC
Dosage	High	N/A	Low	Low
Large-scale production	Yes	Personalized prodction, 3-4weeks, high cost	Yes	Yes

2) This imbalanced CD20/CD3 BsMab has potential to treat Rituxan resistant, difficult to treat and relapsed CD20+ cancer (unmet clinical needs, fast track for filing IND).

Development and Screening of Therapeutic Antibodies with Internalization Potential

Start from unknown target antigen

Disease cells incubate with human naïve ScFV or Fab phage library fat 4 degree for binding only, no internalization

↓

Wash away non-binder, put cells at 37 degree for internalization

↓

Use special buffer to further wash away cell surface binder and then lyse cells, use cell lysate to infect fresh E.coli.

↓

Repeat 3 rounds of panning, seq lead binders, convert to IgG for further characterization

↓

Co-IP and mass-spect to identify antigen

Start from known target antigen

Immunize mice with ECD of the target receptor and/or antigen overexpressed cell and/or target antigen gene

↓

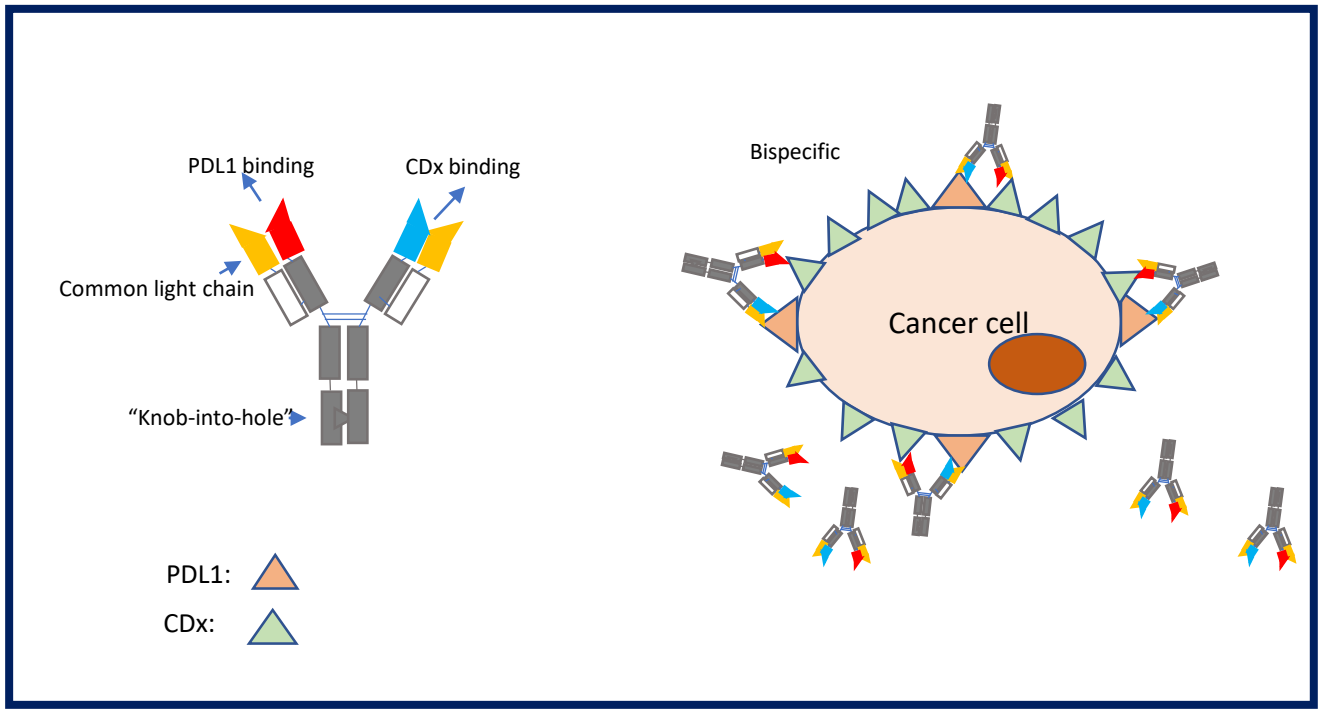
During hybridoma screening, add the following assay:
Incubate disease cells with hybridoma supernatant and a peptide-toxin. The peptide can bind to Mab's Fc domain. Select disease cell specific internalizer based on cytotoxicity.

↓

Further characterize leads from the screening assay

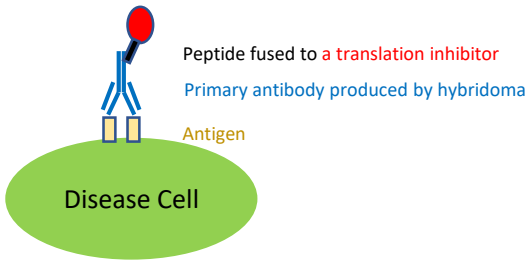
- Computer aided design for serial reduced internalization
- In house developed high-throughput screening assay

Case Study : PDL1/CDx Bispecific Antibody with Internalization Potential

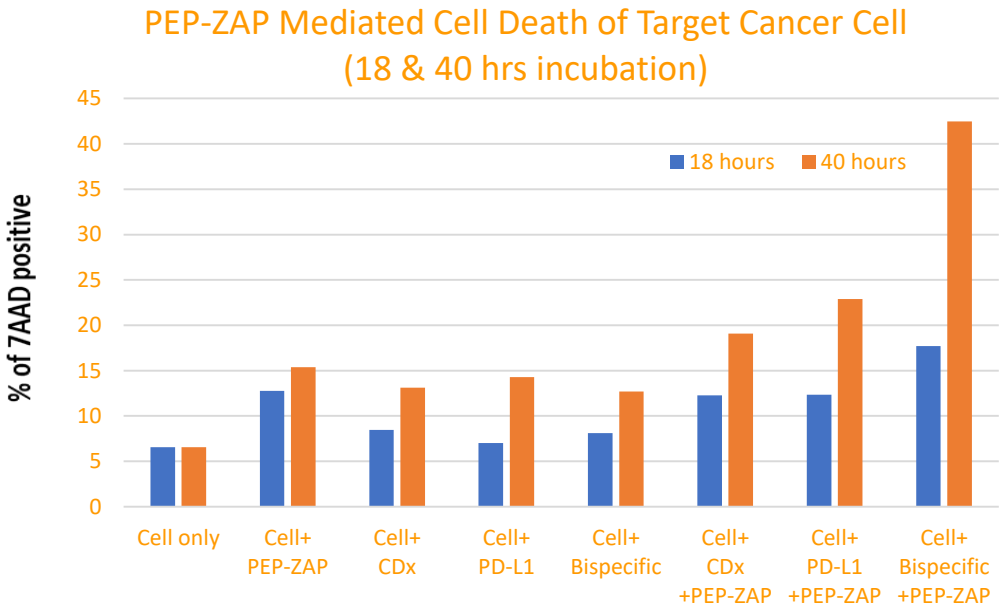


PDL1/CDx Bispecific Antibody Induces more Cell Death than PDL1 and CDx Homodimer in the Presence of Pep-ZAP

How PEP-ZAP works?



Pep-ZAP is a Fc binding peptide conjugated to a translation inhibitor protein, cell death is the readout of antibody internalization



Biological Significance:

This imbalanced bispecific may induce target cancer cell death at three different levels

1) Blocking PD1/PDL1 interaction

2) Induce PDL1 internalization

3) When conjugated to a drug, kill target cancer cells an Antibody drug conjugate

Thank you very much!