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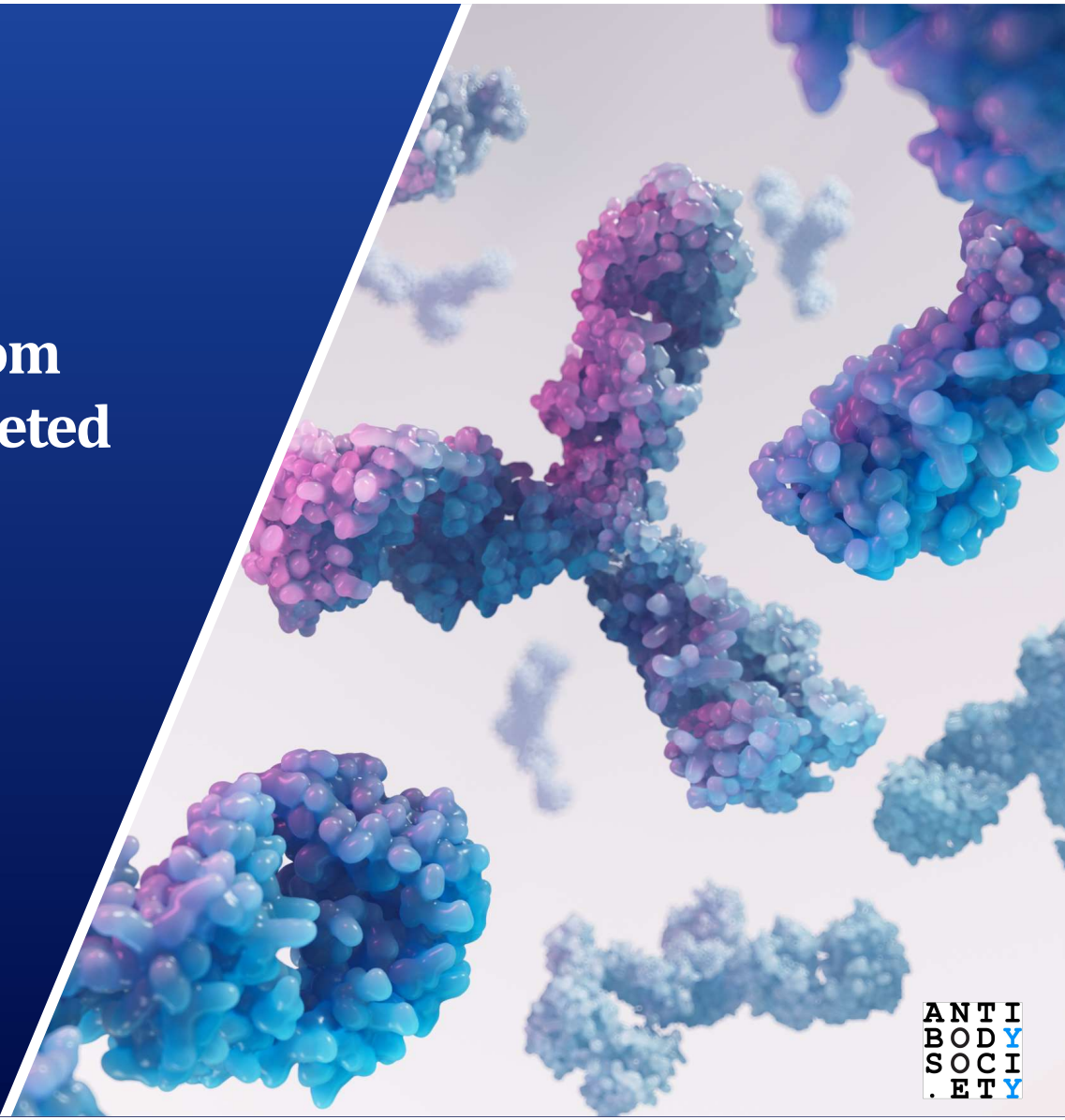
Avoid Thy Neighbor: Lessons from Successful LILRB1/LILRB2 Targeted Myeloid Suppression

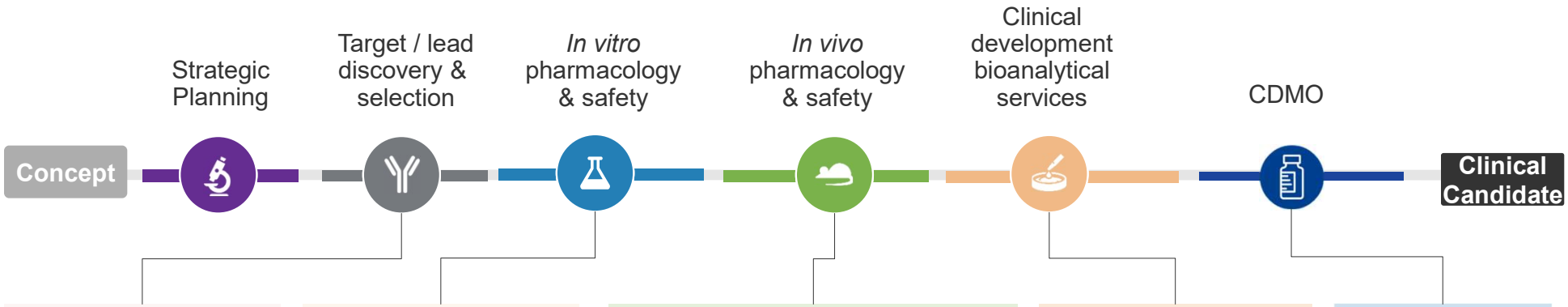
Kalyani Mondal
Charles River Laboratories

Antibody Society Webinar

October 17th, 2024



Integrated Drug Discovery & Development for Biologics



- Antibody Discovery (ScFv or VHH libraries)
- Optimization (Tumbler® Platform)

- Expression, purification & target binding
- Biological Activity (MoA) Functional cell assays
- *In vitro* Safety

- Broad range of models – CDX/PDX, autoimmune and infectious
- CYPRE 3D platform
- POC efficacy in disease models
- PK/PD study
- Tolerability studies in WT mice Exploratory Toxicity (non-GLP)

- GMP-compliant *in vitro* and *in vivo* assessments

- Lead to Manufacturability
- Lead to Tox
- Lead to Clinic

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Large Molecule Lead Discovery Approaches at Charles River

Library	Cosmic™ (Naïve ScFv Library)	SuperHuman™ 2.0 (Naïve ScFv Library)	Tungsten™ (VHH Library)	SLiC™ Single Light Chain
Downstream Applications	IgG, scFv, CAR-X, BiTE	IgG, scFv, CAR-X , BiTE	VHH, CAR-X , Diagnostic	Bispecific/Multispecific antibodies
Overview/Key Attributes	• Our largest library: **100 billion unique sequences** • Fully human CDRs • High diversity, manufacturability, non-immunogenicity	• Fully human scFv display library: **76 billion unique sequences** • >90 projects completed/ongoing	• Single domain libraries • **Humanized IGVH3-23 framework** library • >35 projects completed with 3 candidates in IND and beyond	• A SH2.0-based **single light chain library** • Enables rapid discovery & development of bi-specific antibodies with an **IGKV1-39 fully germline light chain**
Royalty-free	☑	☑	☑	☑
Clone Exclusivity	☑			
License for in-house use		☑	☑	☑

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Streamlining Your Path to the Clinic

An efficient approach for integrated biologic discovery and development

Hit Discovery | **Lead Characterization** | **Lead Specificity** | **Preclinical Development** | **Clinical Candidate**

140+

de novo antibody discovery programs completed/ongoing

1000+

Antibody characterizations in last 2 years

65+

Antibody IND submissions supported

6+

Antibodies in Clinical trials

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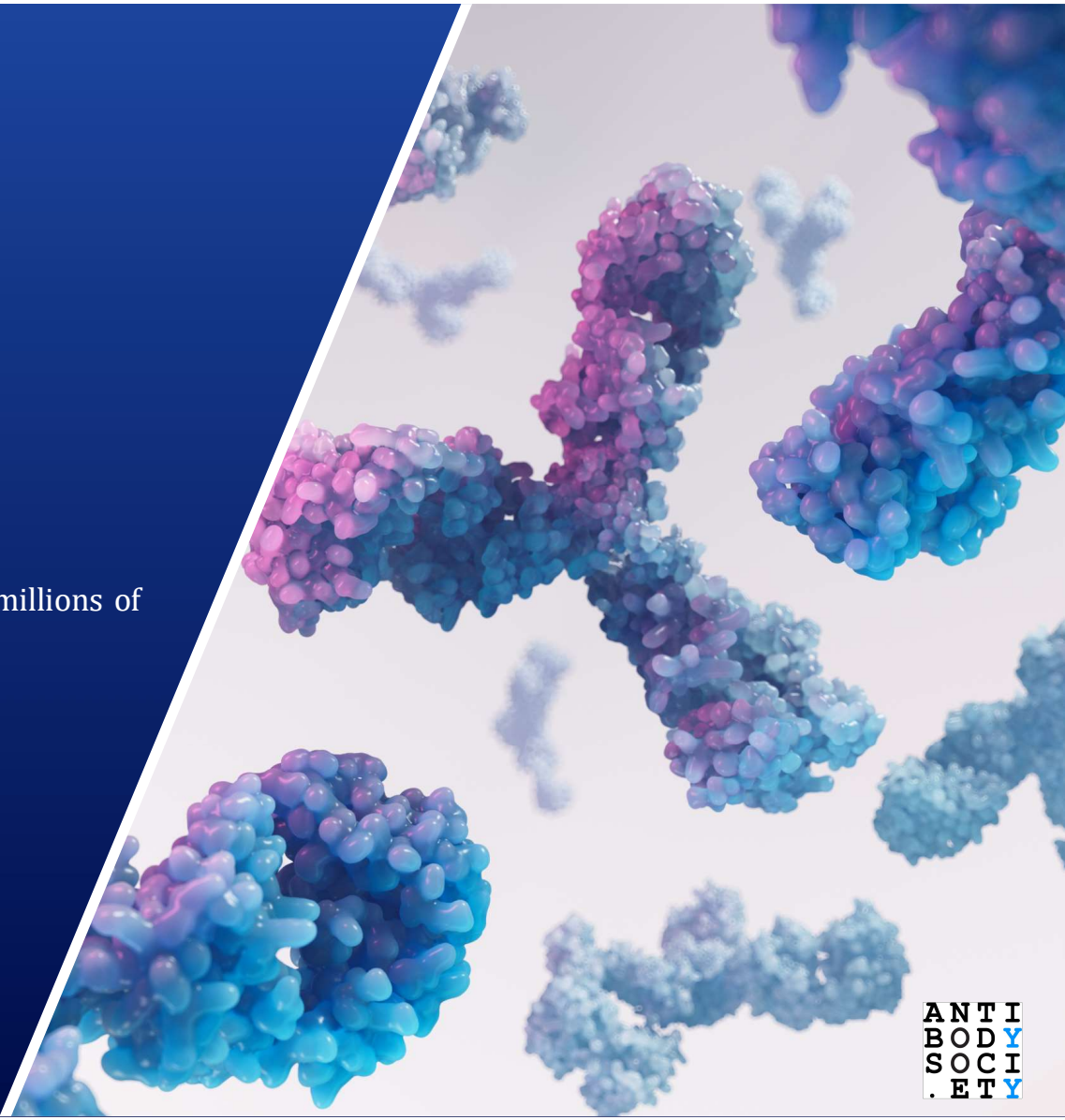
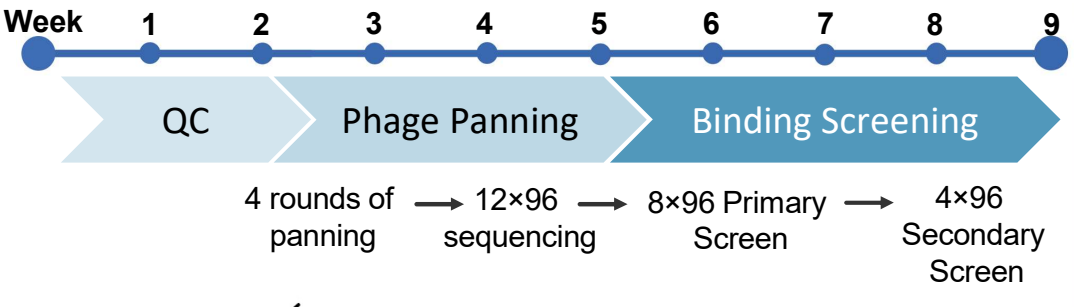
Yeast Display Technology to Generate High Affinity Hits

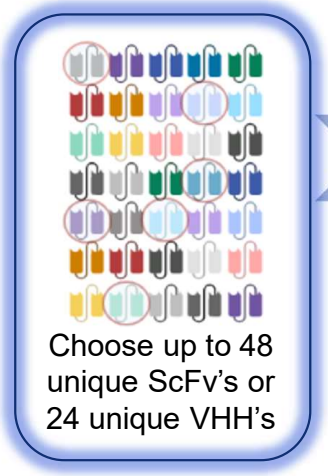
Best of Both Worlds for *In Vitro* Display

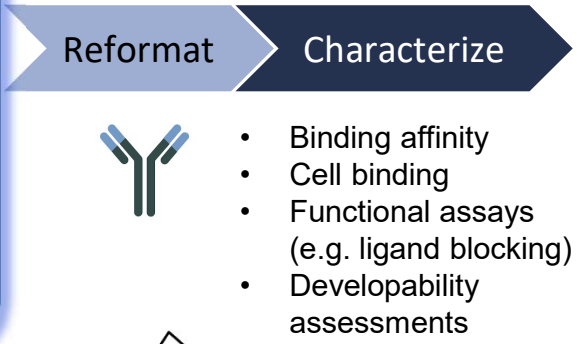
- Utilize existing industry-leading phage technology
- Leverages power of flow cytometry to screen through millions of hits and select the highest-affinity binders



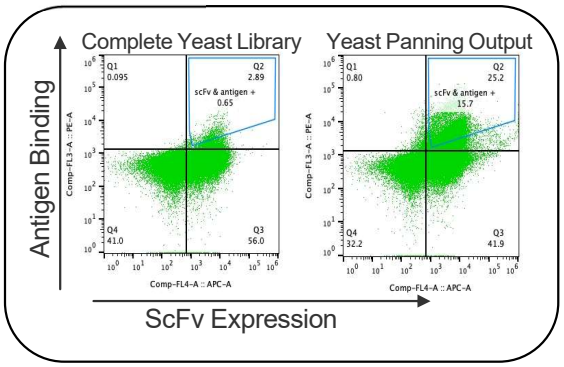
Yeast Display Complements and Enhances Our Phage Display Platforms

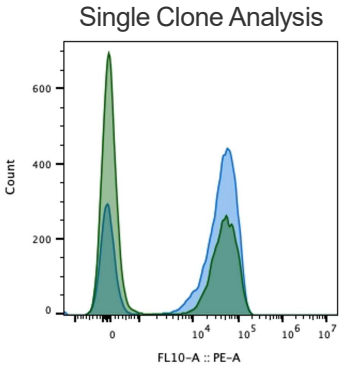






Post Round 2, in yeast





Sanger Sequencing

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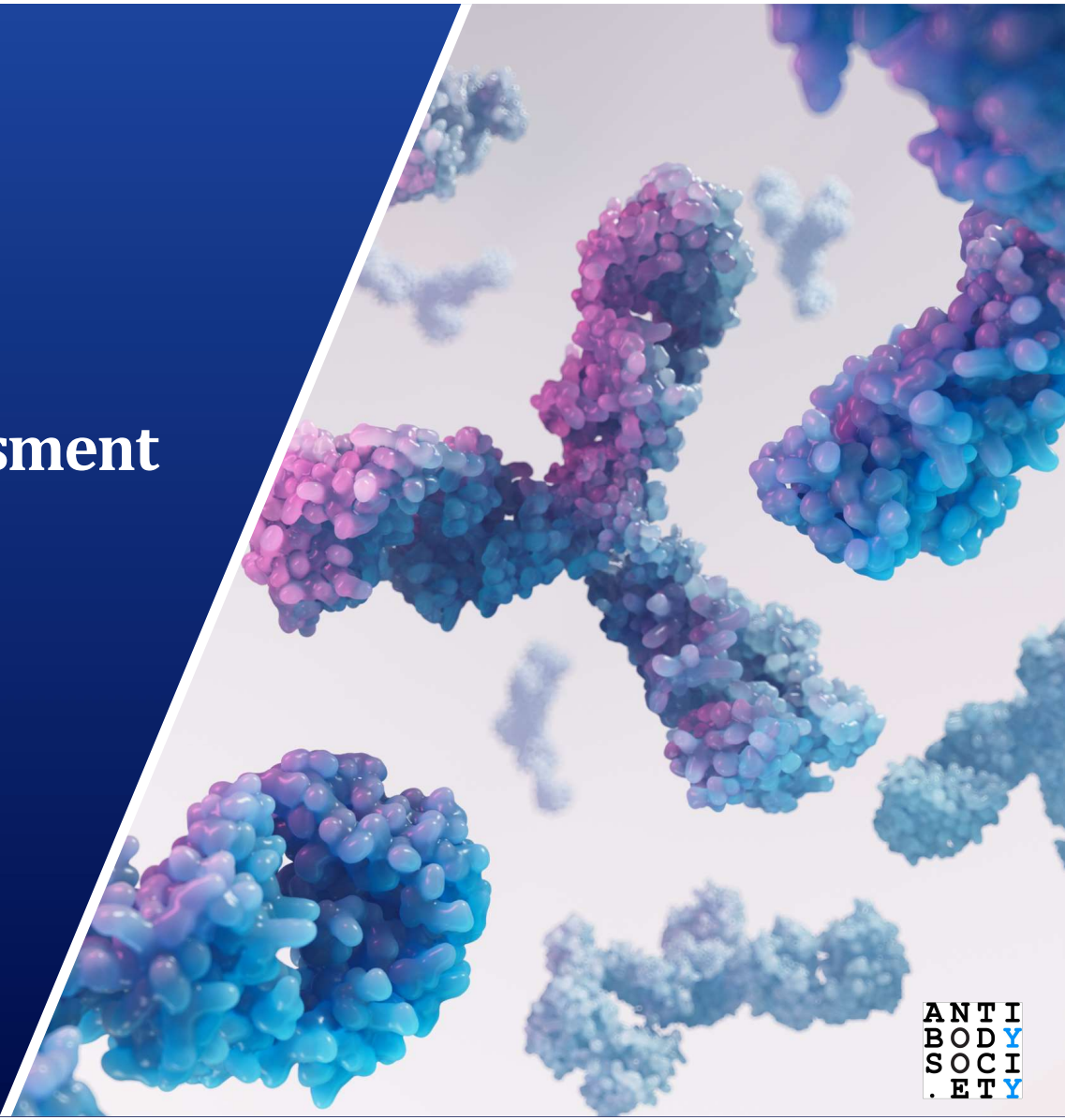
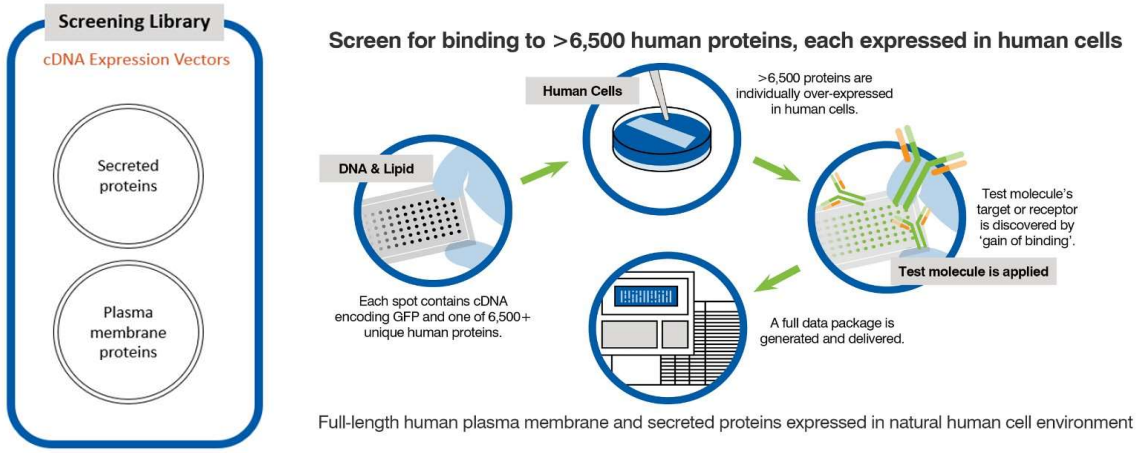
Specificity & Off-target Assessment using Retrogenix® Platform



Off-Target Assessment

Safety profiling to a final subset of clinical candidates using the Retrogenix® Cell Microarray Technology



Off-target/Specificity screening

- Lead candidate selection – filter out polyreactive candidates at earlier stage
- IND-enabling specificity data - support IND and BLA submissions to FDA, EMA etc.
- Aid selection of (healthy) tissues for further *in vitro* safety assays

Receptor identification/ligand de-orphanization

- Identify novel interactors for small and large molecules

Target deconvolution

- Elucidate MOAs from phenotypic screens

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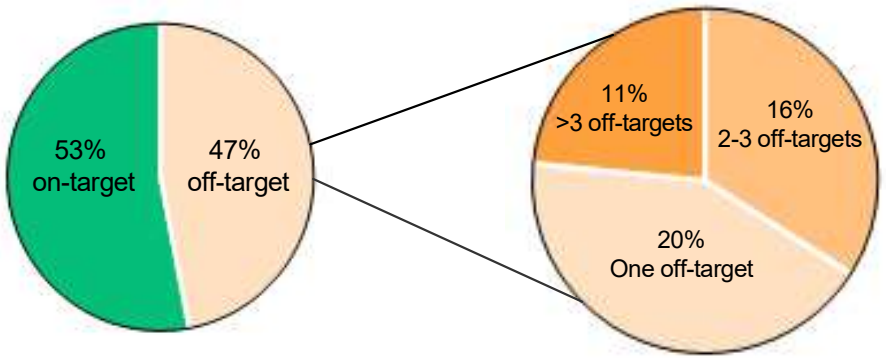
Approximately Half of mAbs Have Off-Target Liabilities

133 mAbs and related molecules screened for a large pharmaceutical company



- Assessing antibody specificity is a critical safety / de-risking step

Plenary Paper

CLINICAL TRIALS AND OBSERVATIONS

Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma

Analysis of HER2 and HER4 in Human Myocardium to Clarify the Cardiotoxicity of Trastuzumab (Herceptin™)

Ilka B. Fuchs, Solveig Landt, Helmut Bueler, Uwe Kuehl, Sarah Coupland, Anke Kleine-Tebbe, Werner Lichtenegger & Gerhard Schaller

Breast Cancer Research and Treatment **82**, 23–28 (2003) | Cite this article

Original Paper | Published: 07 May 2014

Do current therapeutic anti-Aβ antibodies for Alzheimer's disease engage the target?

Andrew D. Watt, Gabriela A. N. Crespi, Russell A. Down, David B. Ascher, Adam Gunn, Keyla A. Perez, Catriona A. McLean, Victor L. Villemagne, Michael W. Parker, Kevin J. Barnham & Luke A. Miles

Acta Neuropathologica **127**, 803–810 (2014) | Cite this article

Molecular basis for mid-region amyloid-β capture by leading Alzheimer's disease immunotherapies

Gabriela A. N. Crespi, Stefan J. Hermans, Michael W. Parker & Luke A. Miles

Antibody Therapeutics, 2018, Vol. 1, No. 1 13–17
doi:10.1093/abtlby/tby005
Advance Access Publication on 10 August 2018

Review

Trastuzumab-mediated cardiotoxicity: current understanding, challenges, and frontiers

Nishant Mohan, Jiangsong Jiang, Milos Dokmanovic and Wen Jin Wu*

Division of Biotechnology Review and Research 1, Office of Biotechnology Products, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD 20993, USA

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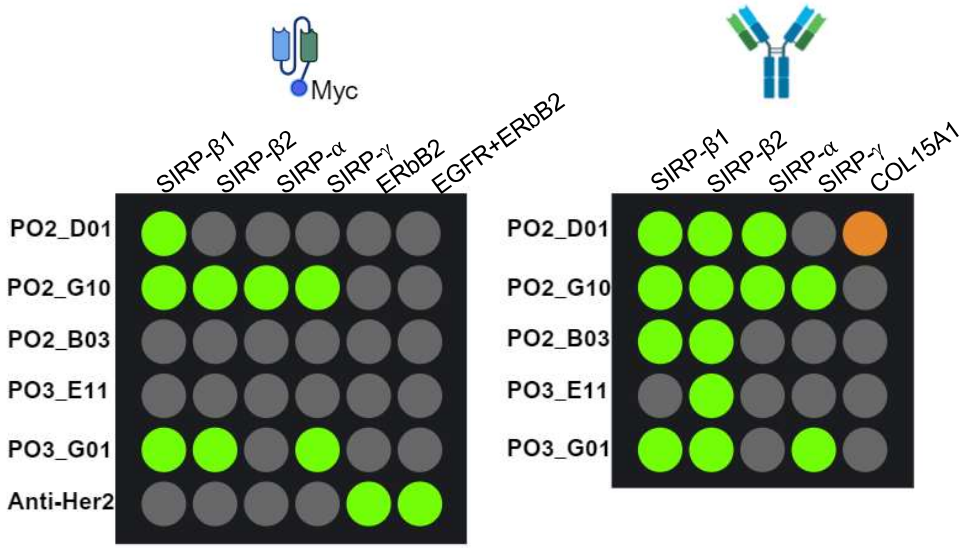
Early Off-Target Assessment Using Periplasmic Extract

Rapid Assessment of Up to 96 PPE Against ~300 Frequently Hit Off-targets



Figures@Biorender.com

- Anti-SIRPα ScFv clones showed binding to only SIRP family members, which is expected based on the panning strategy.
- A well behaved anti-Her2 was included as a negative control and showed binding to its specific target antigen.
- The same clones as mAbs showed higher sensitivity due to crosslinking with the expressed proteins.
- When reformatted to IgGs: no poly-reactivity against **~300 off-targets tested**, except for PO2_D01.

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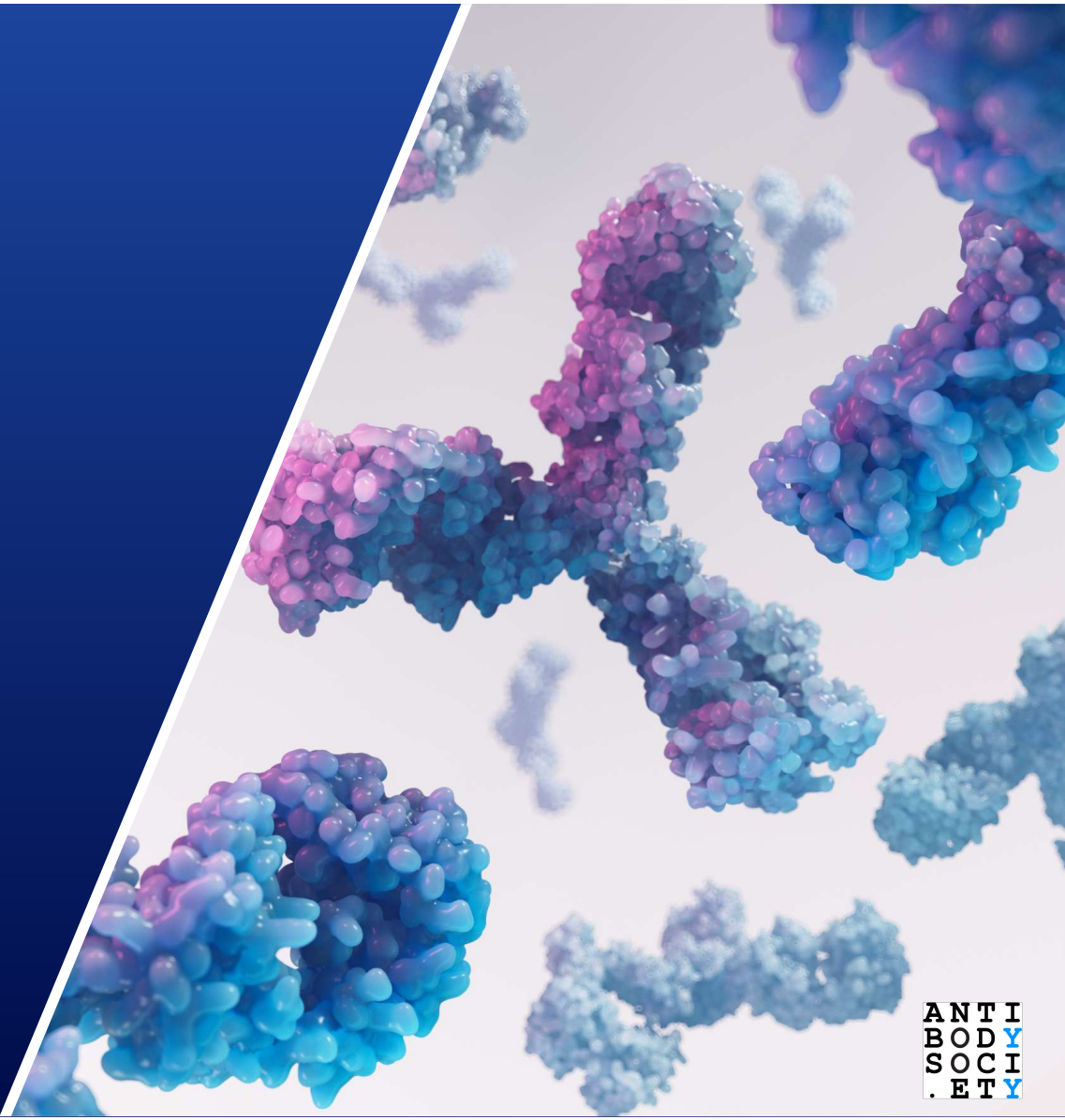
In Vitro Functionality



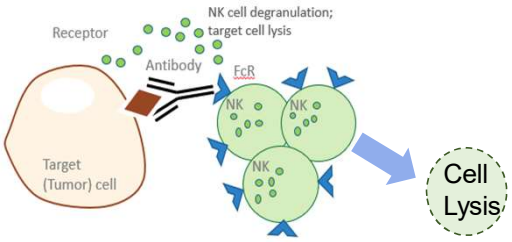
In Vitro Functionality

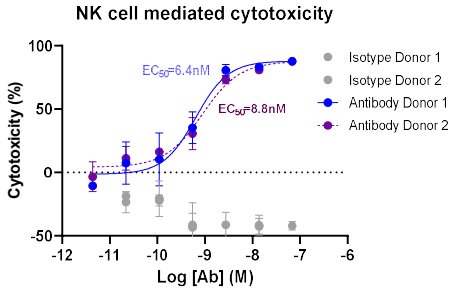
Mechanism of Action

Therapeutic effects of antibodies can be mediated by several mechanisms

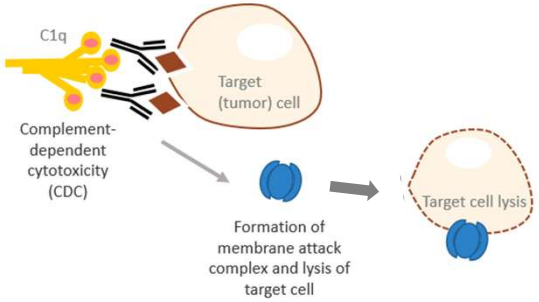
- Activation or inhibition of enzyme activity
- Activation or inhibition of signaling pathways
- Biochemical characterization of inhibition of ligand binding (IC_{50})
- Direct target cell killing (apoptosis)
- Antibody-dependent cellular cytotoxicity (ADCC)
- Antibody-dependent cellular phagocytosis (ADCP)
- Complement-dependent cytotoxicity (CDC)

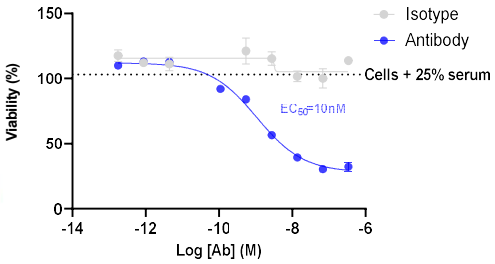
} Effector functions



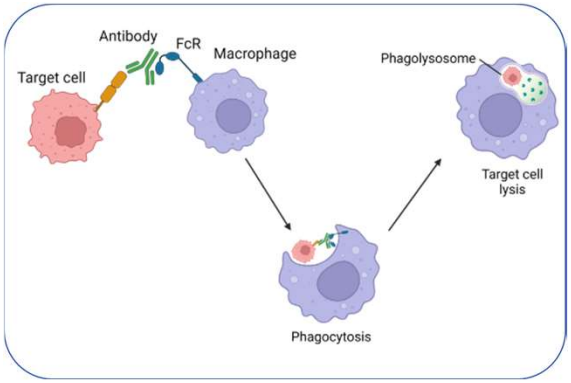


ADCC mediated by NK cells determined via flow cytometry (24h coculture A-431:NK cells)





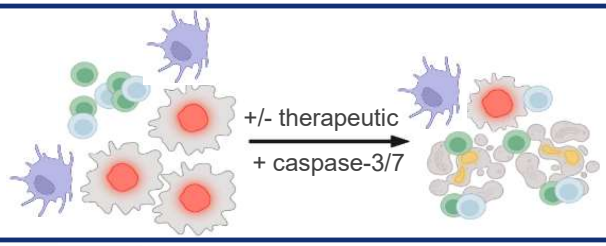
CDC monitored in Raji cells with target antibody using cell titre glo



2D & 3D Tumor Cell Killing Assay

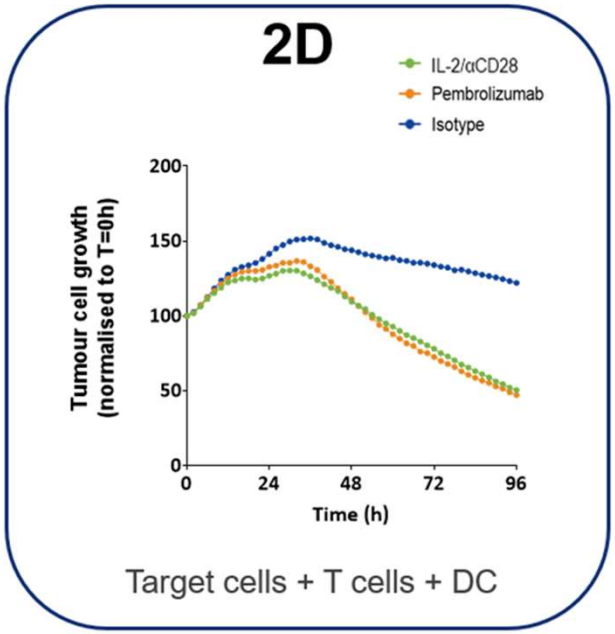
Building greater physiological relevance in tumor kill assays

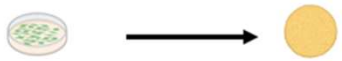
Target cells labelled with nucRFP and co-cultured T cells +/- mDC



Readouts:

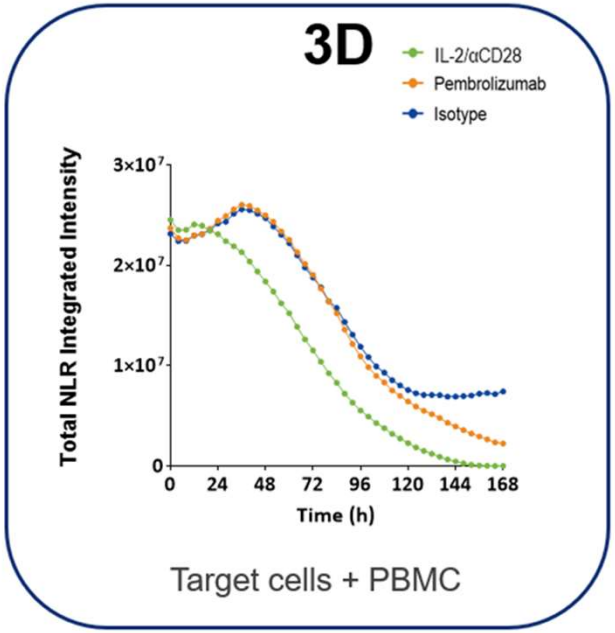
- Target number (IncuCyte)
- Target apoptosis (IncuCyte)
- Cytokine release (TR-FRET)
- Effector activation (flow cytometry)





Monitor immune mediated tumour cell killing in 2D or 3D over several days

Transition immune modulator efficacy from 2D to more physiologically relevant 3D models



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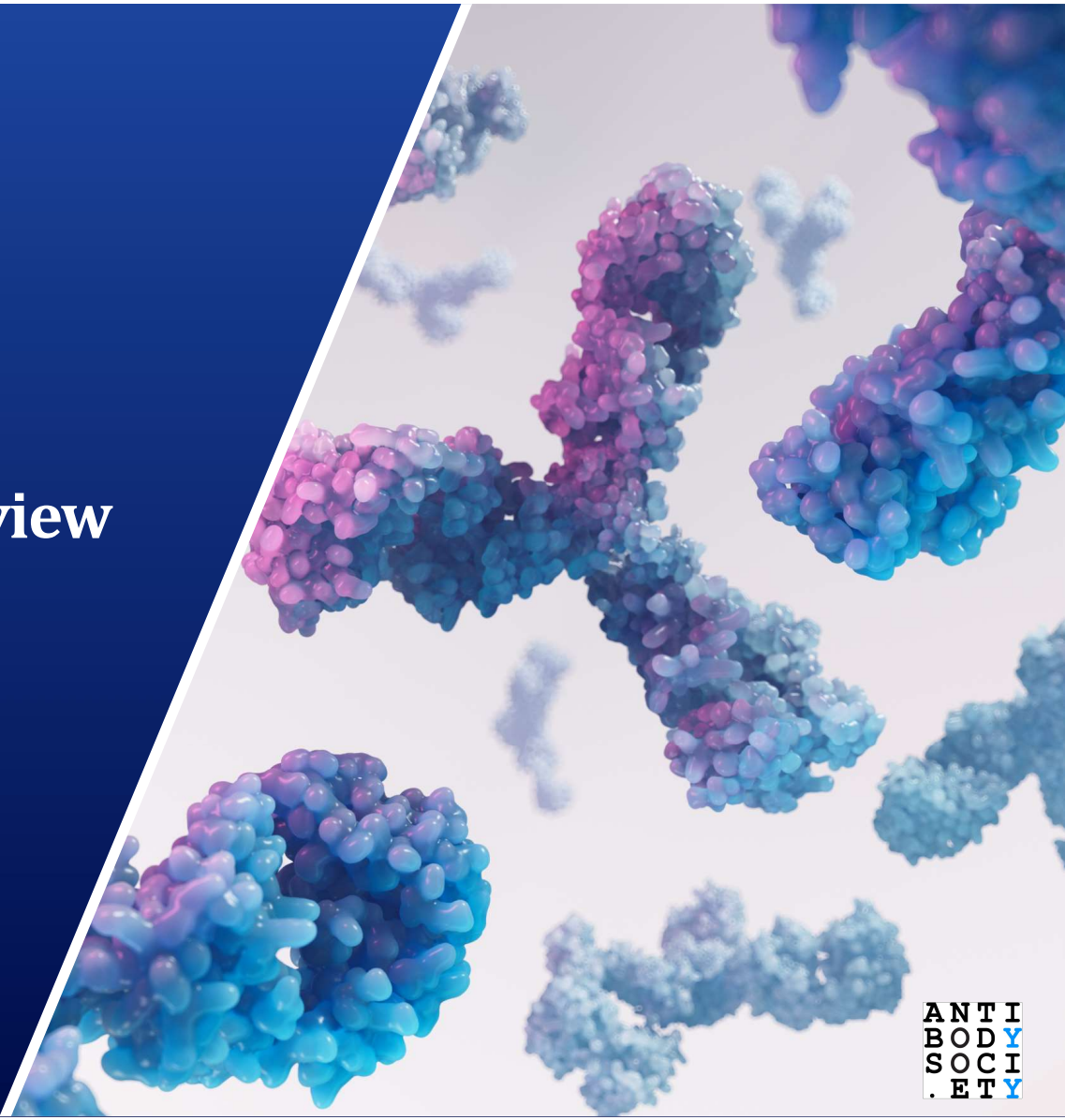
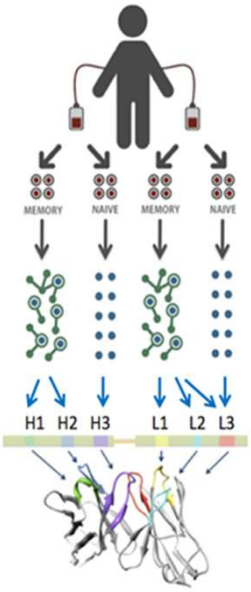
SuperHuman™ Library Overview



SuperHuman 2.0™: An NGS Informed Smart Phage Library



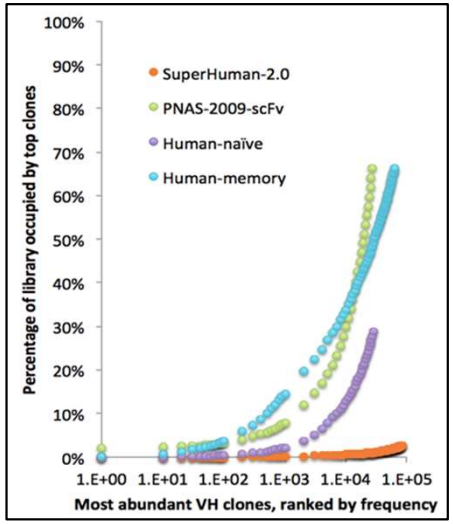
Drug worthy Frameworks

VK1-39	VH1-46
VK2-28	VH1-69
VK3-15	VH3-15
VK4-1	VH3-23

Optimizing the Repertoire

- First-generation natural naive libraries were highly redundant, resulting in relatively few hits and relatively weak binders.
- Synthetic libraries had greater sequence diversity but suffered from low "molecular fitness": synthetic clones that tended to not fold, to aggregate, and to be non-specific.
- **SH2.0™:** Through a combination of careful framework selection, using only human CDRs, thermal and expression selection pressures during construction, the library is engineered for enhanced thermostability, low immunogenicity, low aggregation.

High Sequence Diversity

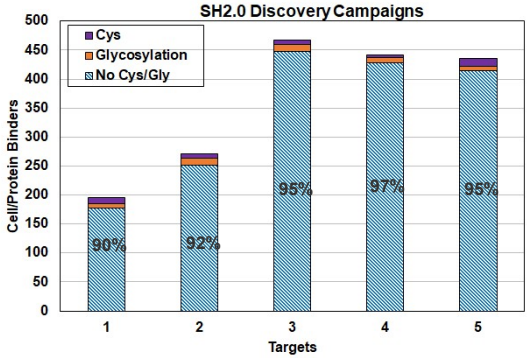


- No single clone is more than 0.03% of the total 76B member library

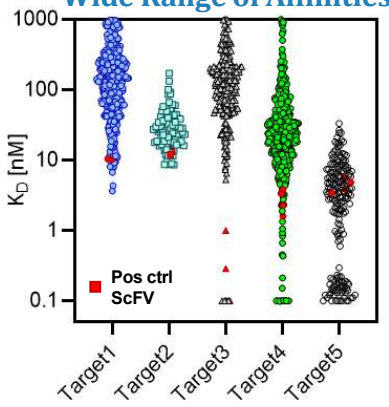
Glanville, G. et al. (2009) Proc. Natl. Acad. Sci. 106, 20216-20221
Zhai, W. et al (20111) J. Mol. Biol. 412, 55-71

SH2.0™ Outputs from Discovery Campaigns

Low liabilities;100s Unique Binders

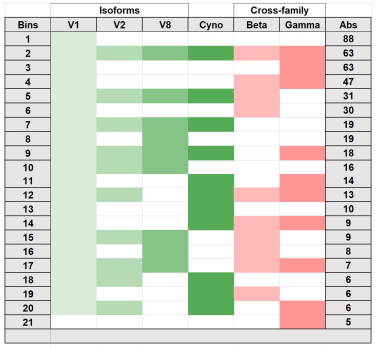


Wide Range of Affinities



Positive controls from immunizations

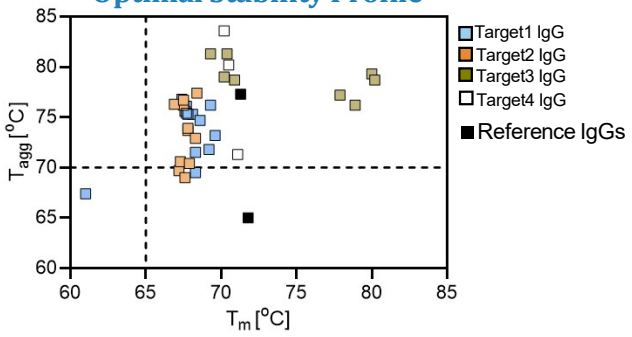
Broad Epitope Coverage



Bins	Isoforms V1	Isoforms V2	Isoforms V8	Isoforms Cyno	Cross-family Beta	Cross-family Gamma	Abs
1							88
2							63
3							63
4							47
5							31
6							30
7							19
8							19
9							18
10							16
11							14
12							13
13							10
14							9
15							9
16							8
17							7
18							6
19							6
20							6
21							5

- Over 178 binders grouped into 21 epitope bins

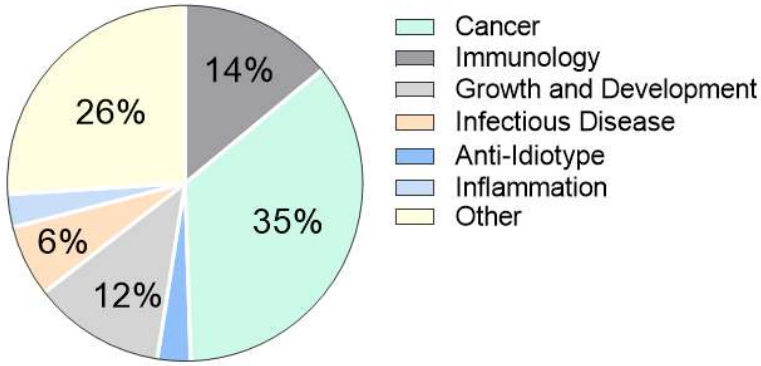
Optimal Stability Profile



Successful Application of SuperHuman 2.0™ Across Many Disease Indications

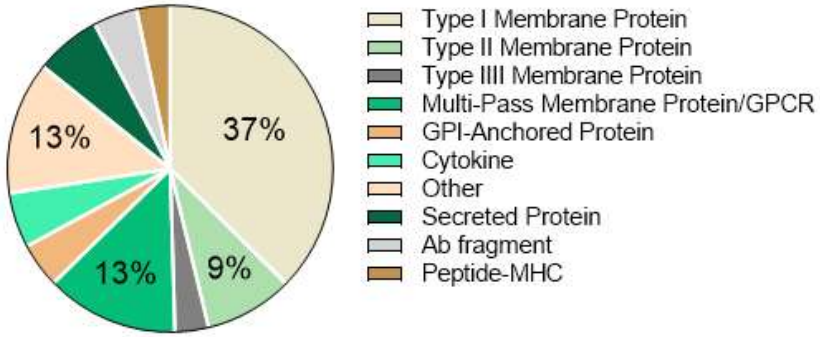
- SuperHuman (SH2.0™) released in 2017. It is the most utilized, hence also the preferred library amongst our clients.
- Over 90 projects completed or ongoing.

Application Across Different Disease Indications



- Other disease indications (26%) include pain, neurodegeneration, rare disorder.

Application Across Challenging Targets



- Other targets (13%) include small molecules, adhesion molecules, viral proteins/peptides, intracellular proteins

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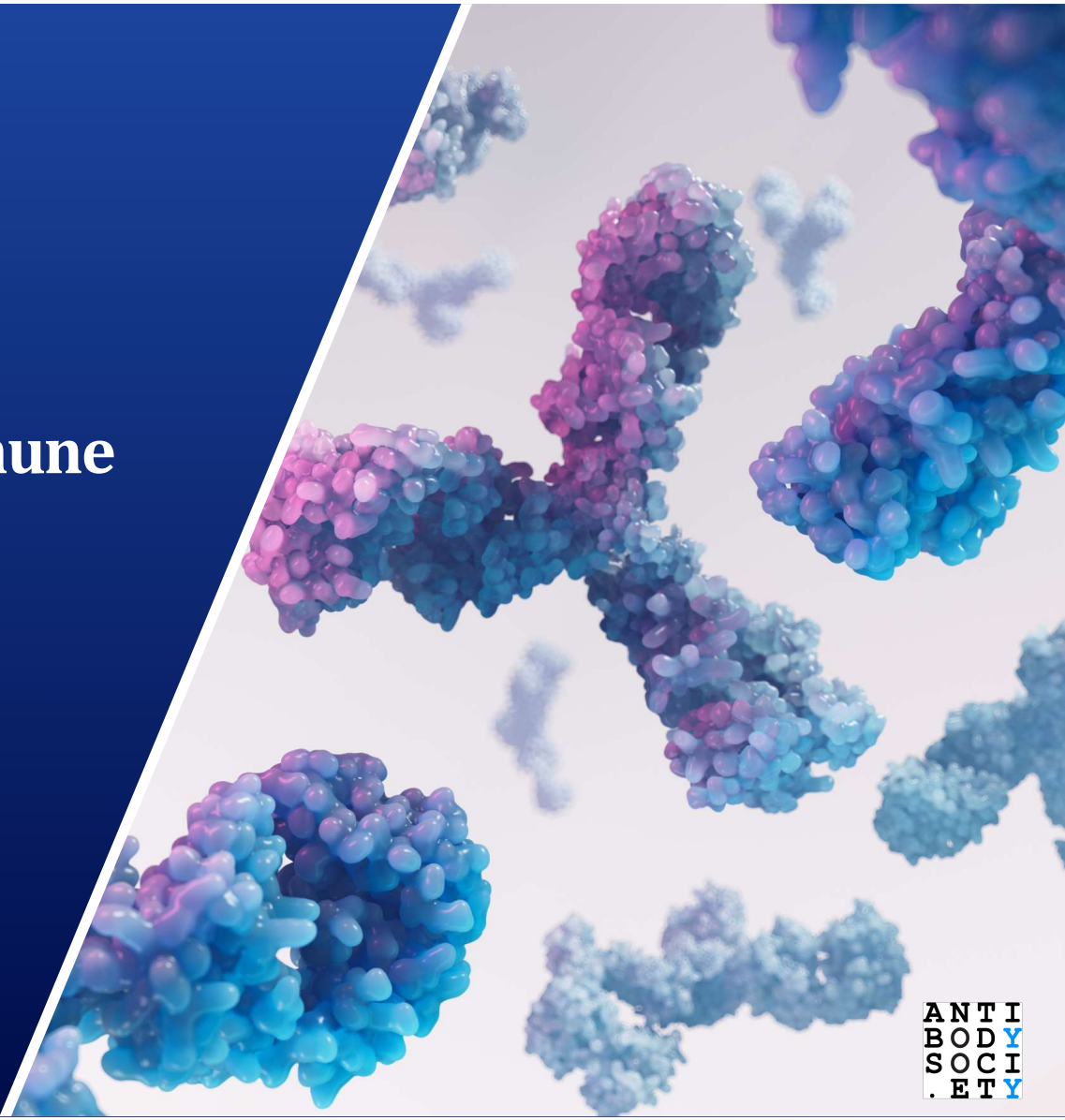
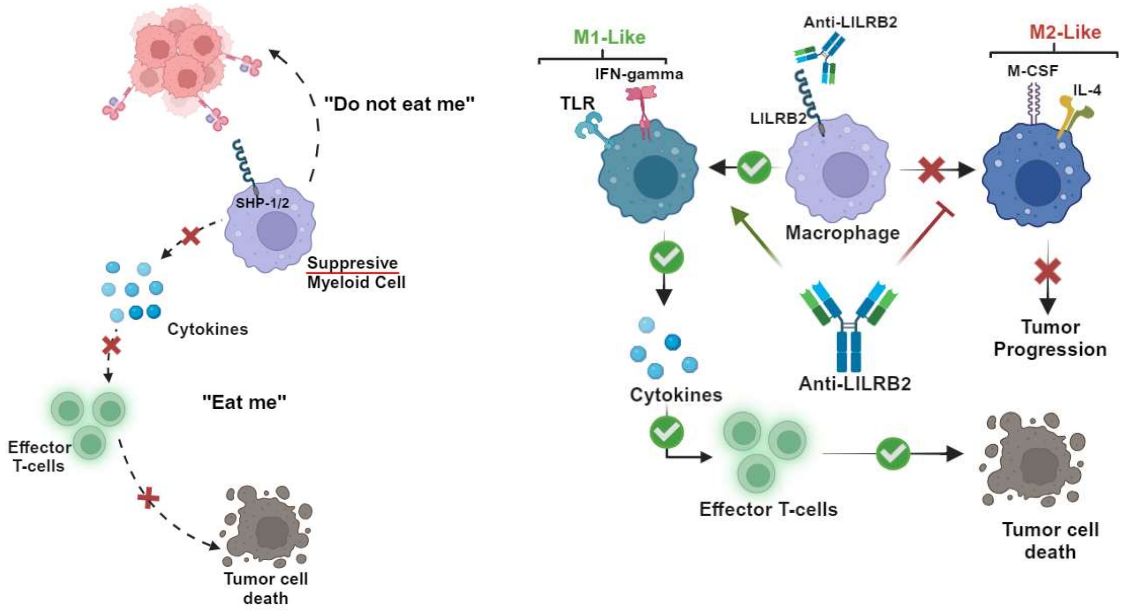
LILRB1/LILRB2 as Innate Immune Checkpoint Inhibitors



Overview

- Immune checkpoint blockade in T cells using antibodies has demonstrated clinical efficacy in different types of cancer.
- Beside T cells, innate immune cells (i.e., NK cells and macrophages) exert a pivotal role in the recognition and elimination of malignant cells in the tumor microenvironment.
- Inhibition of the innate immune system to disrupt “Don’t Eat Me” signals between tumor and macrophages is a rapidly growing area of drug development.
- LILRB1 and LILRB2 are two such immunomodulatory receptors
- LILRB1, LILRB2 (receptor) and HLA-G (ligand) are immune checkpoint factors that play a significant role in human immunosuppressive pathways
- 50% - 70% of malignant growth use HLA-G as a shield to disrupt the patient’s immune system’s from functionally normally.



Figures@Biorender.com

Chen et al. (2018) *J Clin Invest.*, 128(12):5647-5662
Yang, H et al. (2019) *Cancer Med. 8, 4245-4253*

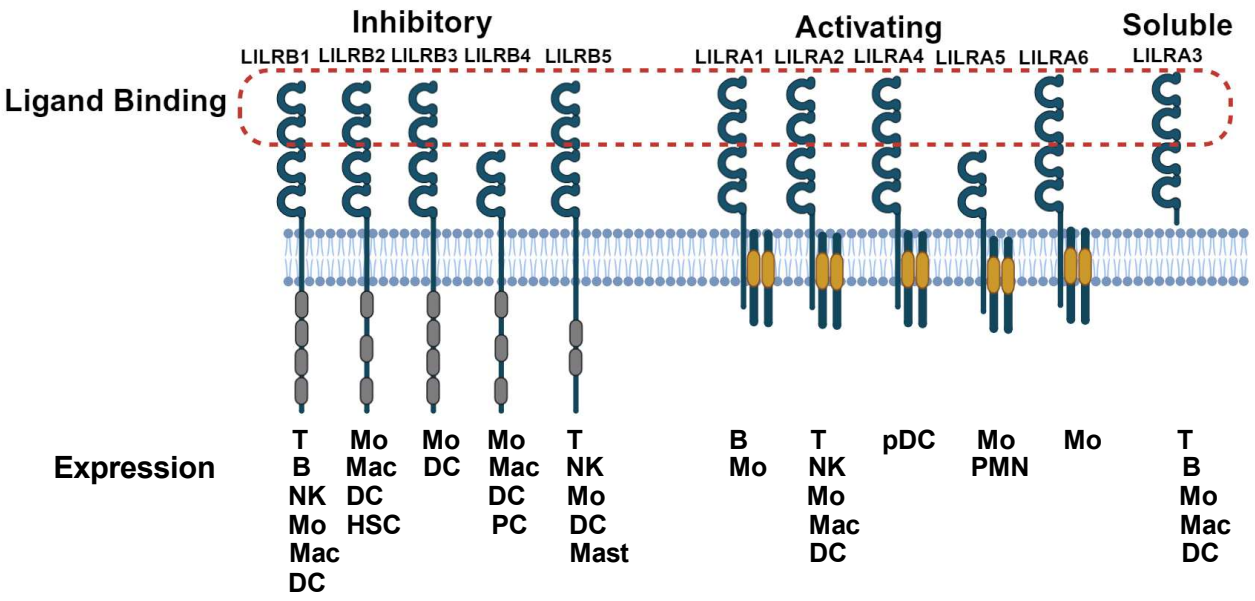
Clinical Antibodies Targeting LILRB1/LILRB2

Antibody	Target	Disease	Intervention	Phase	clinical trial.gov ID
BND-22/SAR444881 (Biond Biologics/Sanofi)	LILRB1	Advanced solid tumor	BND-22 alone BND-22+ Pembro BND-22+Cetuximab	I/II	NCT04717375
NGM707 (NGM Biopharmaceuticals)	LILRB1 LILRB2	Advanced solid tumor	NGM707 alone NGM707+Pembro	I/II	NCT04913337
AGEN1571 (Agenus)	LILRB1 LILRB2	Advanced solid tumor	AGEN1571 alone AGEN1571 + anti-PD-1 AGEN1571 + anti-CTLA-4	I	NCT05377528

Antibody	Target	Disease	Intervention	Phase	clinical trial.gov ID
MK-4830 (Merck)	LILRB2	Advanced solid tumor	MK4830 alone MK4830+Pembro	I	NCT03564691
JTX-8064 (Jounce therapeutics)	LILRB2	Advanced refractory solid tumor	JTX-8064 alone JTX-8064+Anti- PD1	I/II	NCT04669899
BMS-986406 (Bristol Myers-Squibb)	LILRB2	Advanced malignant tumors	BMS-986406 alone BMS986406+Nivo+Carboplatin	I	NCT05298592
CDX-585 (Celldex Therapeutics)	LILRB2/PD-1 bispecific	Advanced malignancies	CDX-585 alone	I	NCT05788484

Zeller, T. et al. (2023) *Frontiers Immunol*. 10.3389/fimmu.2023.1240275
Clinical Trials.gov

LIR Protein Family



Identity Matrix (%)

	B1	B2	B3	A1	A2	A3	A6
B1	100	84	72	77	74	84	64
B2	84	100	71	78	73	80	64
B3	72	71	100	65	64	66	90
A1	77	78	65	100	81	85	68
A2	74	73	64	81	100	82	68
A3	84	80	66	85	82	100	66
A6	64	64	90	68	68	66	100

Figures@Biorender.com

- LIR are grouped into two subfamilies:
  - **Subfamily A** consists of LILRA1, LILRA2, LILRA4-6 (cell surface receptors) that activate via their ITAM domain and the soluble member LILRA3.
  - **Subfamily B** consists of LILRB1-5, which inhibit via their ITIM domain.
- **Targeting LILRB1 & LILRB2 poses the challenge of avoiding ten highly homologous LILR family members that are also expressed on myeloid cells.**

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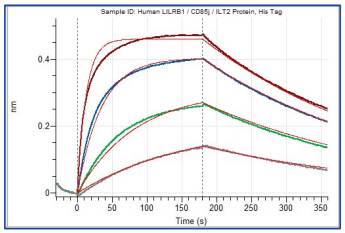
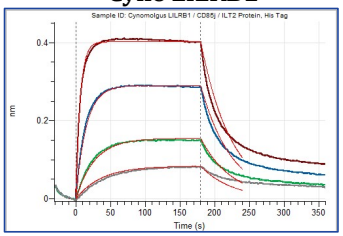
Clinical Anti-LILRB1 Antibody (15G8) Cross-Family Binding Profile

Hu LILRB1



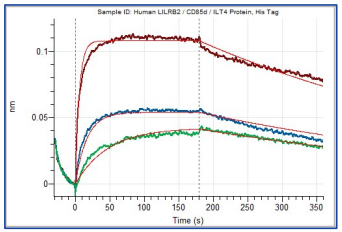
Hu LILRB1; K_D, 14.6nM

Cyno LILRB1



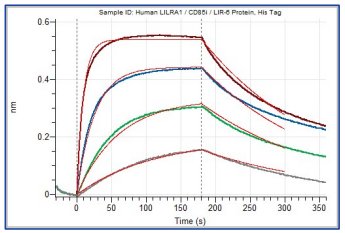
Cyno LILRB1; K_D, 24nM

Hu LILRB2



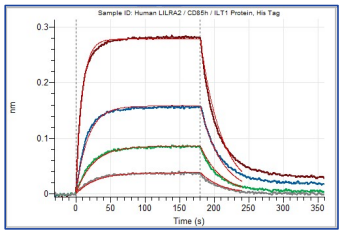
Hu LILRB2; K_D, 3.7nM

Hu LILRA1



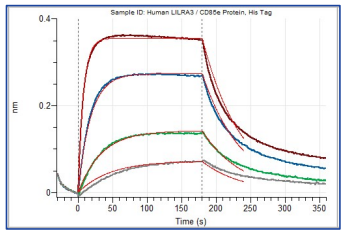
Hu LILRA1; K_D, 16.7nM

Hu LILRA2



Hu LILRA2; K_D, 126nM

Hu LILRA3



Hu LILRA3; K_D, 46.7nM

- First generation anti-LILRB1 antibody shows high affinity binding to human LILRB1 and cyno cross-reactivity, but also bind to other LILR family members with a range of affinities.

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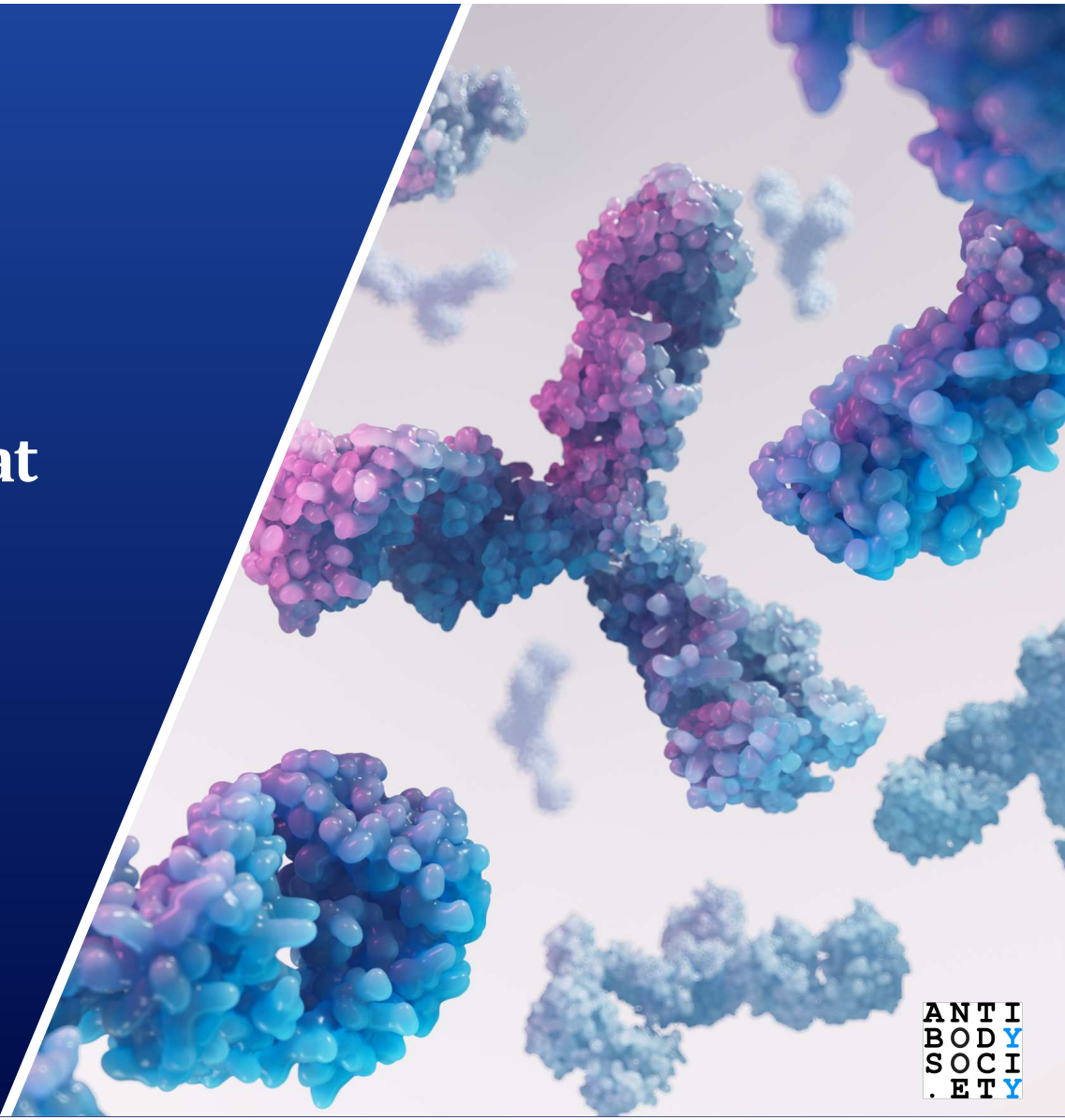
charles river

LILRB1 Antibody Discovery at Charles River



Goals for LILRB1 Discovery Campaign

Project Goals:

- Target: Leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1; Uniprot ID: Q8NHL6)
- Human-cyno cross reactivity preferred
- Off-target selectivity: LILRB2, LILRB3, LILRB4, LILRB5, LILRA1, LILRA2, LILRA3, LILRA4, LILRA5, LILRA6
- Desired Function: Specific binding to LILRB1 with blocking of HLA-A and HLA-G binding
- Final Format: IgG4
- For use in therapeutic applications

LILRB1 Workflow

- SDS-PAGE
- HPLC-SEC
- Tag accessibility
- Known receptor [HLA-G] binding

- Sequencing
- Rearray of unique clones

AlphaLISA HT screen for

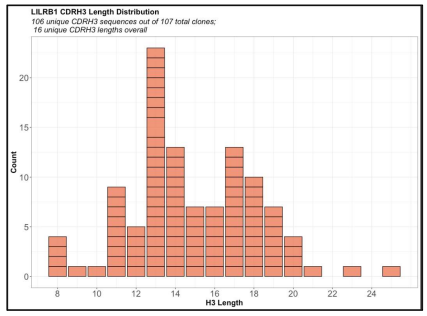
- binding to human/cyno LILRB1
- LILRBs, LILRAs off-target screen

- Binding kinetics to human/cyno LILRB1
- Ligand blocking (HLA-G)
- FACS confirmation of huLILRB1 binding

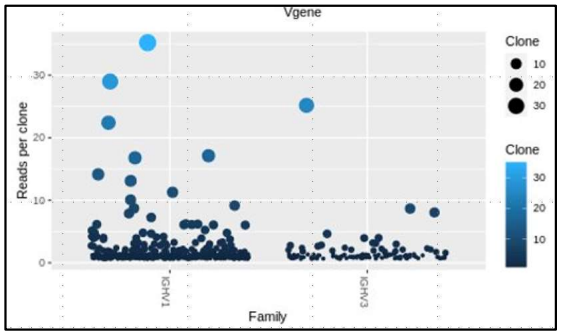
- HuIgG4

Panning Schema

Library	Panning Arm	Goal	R1	Competition	R2	Competition	R2 Off-target Deselection	R3	Competition	R3 Off-target Deselection	R4	Competition	R4 Off-target Deselection
SH 2.0	A	To find scFv binders to Human LILRB1 ECD that do not cross to LILRB2	HuLILRB1 ECD	None	HuLILRB1 ECD	HuLILRB1 ECD-His	HuLILRB2	HuLILRB1 ECD	HuLILRB1 ECD His	HuLILRB2	HuLILRB1 ECD	HuLILRB1 ECD-His	HuLILRB2
SH 2.0	B	To find human/cyno cross-binders with deselections to all LILR family members	HuLILRB1 ECD	None	CyLILRB1 ECD	None	LILRA1-6, LILRB2-5 proteins	CyLILRB1 ECD	None	LILRA1-6, LILRB2-5 proteins	HuLILRB1 ECD	HuLILRB1 ECD-His	LILRA1-6, LILRB2-5 proteins
SH 2.0	C	To find ligand blockers that cross with cyno with deselections to LILRB2, LILRA1, LILRA3	HuLILRB1 D1-D2 Domain	None	CyLILRB1 ECD	None	LILRB2, LILRA1, LILRA3	CyLILRB1 ECD	None	LILRB2, LILRA1, LILRA3	HuLILRB1 D1-D2 Domain	None	LILRB2, LILRA1, LILRA3



- Wide range of CDRH3 lengths observed



- Combination of enriched and singleton clones after 4 rounds of panning

LILRB1 ScFv Screening Workflow

Primary Screening (AlphaLISA)

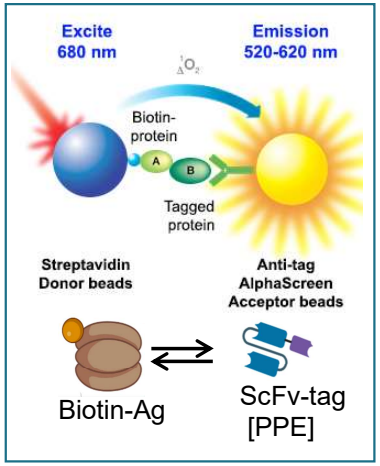
AlphaLISA-Yes/No Binding

1. Human LILRB1 ECD
2. Human LILRB1 D1-D2 Domain
3. Cyno LILRB1 ECD
4. Human LILRB2 (off-target)

Primary Screening (Off-Target Assessment)

AlphaLISA-Yes/No Binding

1. Human LILRB3
2. Human LILRB4
3. Human LILRB5
4. Human LILRA1
5. Human LILRA2
6. Human LILRA3
7. Human LILRA4
8. Human LILRA5
9. Human LILRA6



All clones that are Hu & CyLILRB1(+), low/no cross-reactivity to other LILR's

Secondary Screening

Binding Kinetics & Affinity

- Human LILRB1 ECD
- Cyno LILRB1 ECD

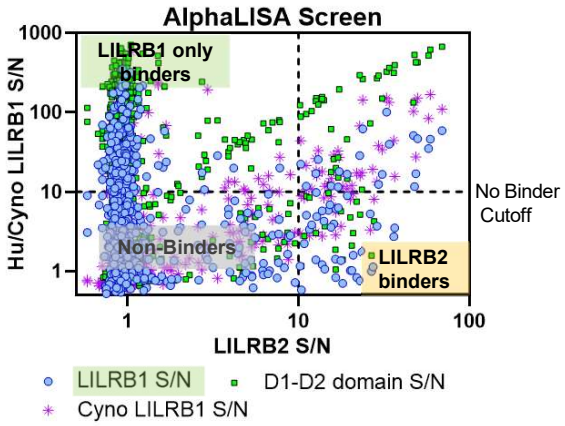
Competition

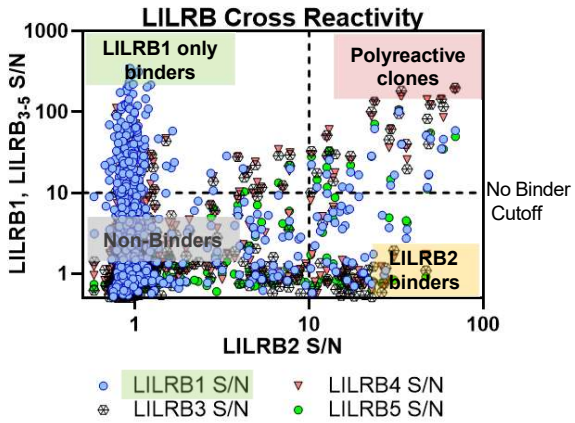
- Assess HLA-G ligand blocking

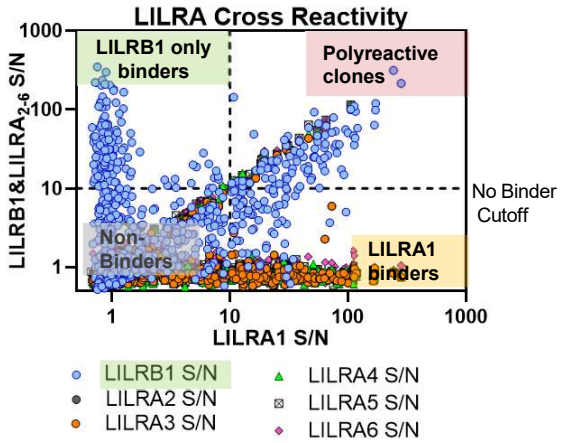
FACS

- Cell Binding to Human LILRB1 Overexpressing Cell Line

HT LILRA & LILRB Family Cross-Reactivity







15G8 ScFv (positive control) S/N>50
Negative control ScFvs S/N<2

- 153 unique clones that showed binding to D1-D2 domain and FL LILRB1 ECD.
- 18 unique clones that showed binding to D1-D2, human &cyno LILRB1 ECD.

- 194 of 672 screened unique clones showed no cross-reactivity to $LILRB_{2-5}$ family members.

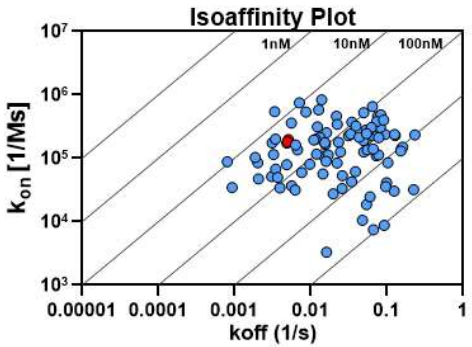
15G8 ScFv (positive control) S/N>50 for $LILRA_{1-3}$
Negative control ScFvs S/N<2

- 101 unique clones showed no cross-reactivity to LILRA & LILRB family members.

Secondary Screening Characterizations

Affinity/Ligand Blocking/FACS Binding of ScFvs from Periplasmic Extract

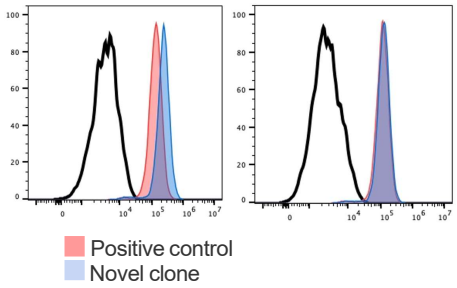
Isoaffinity Plot of all Cell Binders



15G8 ScFV, K_D: 25-27nM

- Several clones show higher affinity than the positive control, a few K_Ds ~nM range

FACS Screen with PPE



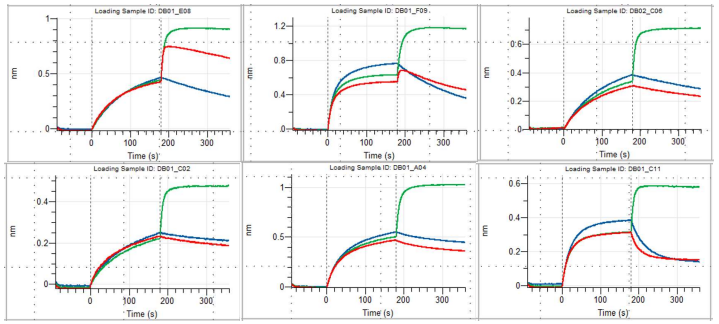
- ~30 clones showed MFI fold> positive control ScFV

Octet Based HLA-G Blocking Assay

Step1: Capture of ScFv via tag

Step2: Binding to huLILRB1-His

Step3: Dipping in HLA-G tetramer/ Anti-His



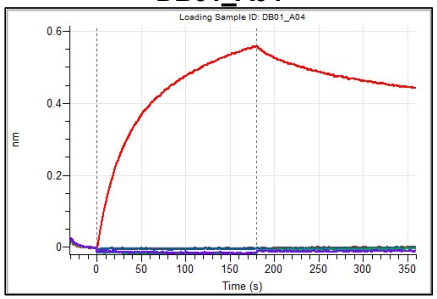
HLA-G tetramer blocking

Anti-His non-blocking

Buffer

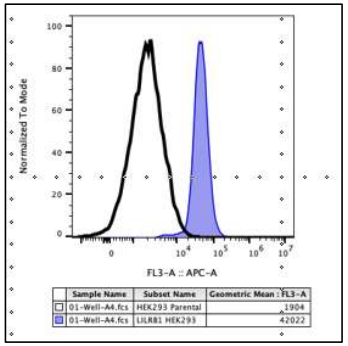
Example of Clones with No Cross-Family Cross-reactivity

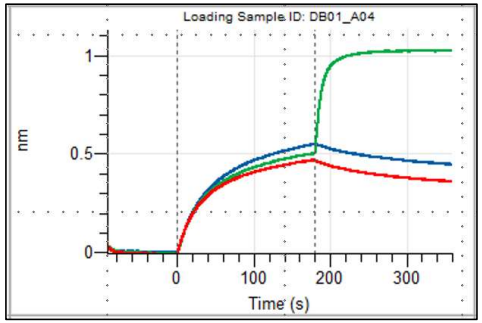
DB01_A04



Hu LILRB1; K_D, 17nM

15G8 ScFV, K_D: 25-27nM

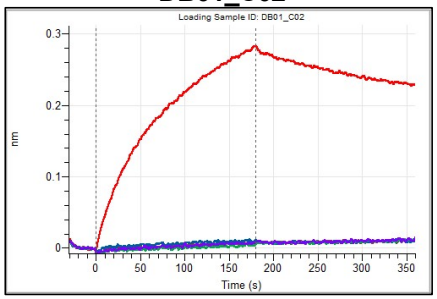




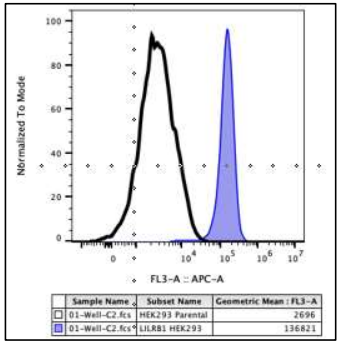
HLA-G tetramer blocking

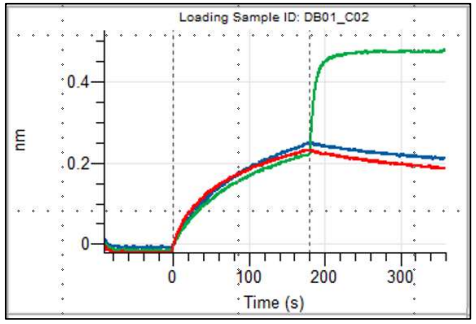
Anti-His non-blocking

DB01_C02



Hu LILRB1; K_D, 24.5nM

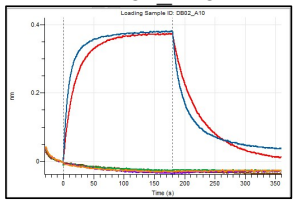




Example of Clones with Cyno Cross-reactivity

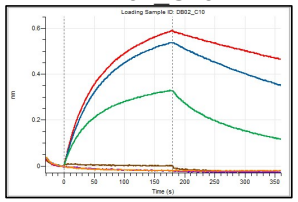
Introduction of Binding Interaction to 1/10 Cross-Family Members

DB02_A10



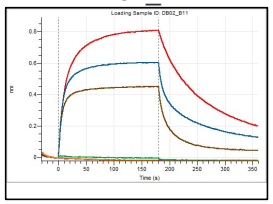
Hu LILRB1; K_D, 268nM
Cyno LILRB1; K_D, 179nM

DB02_C10



Hu LILRB1; K_D, 23nM
Cyno LILRB1; K_D, 48nM
Hu LILRA3; K_D, 116nM

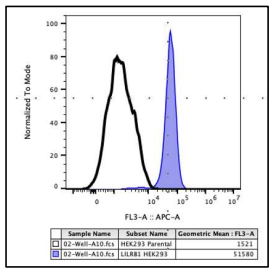
DB02_B11



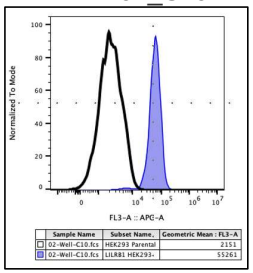
Hu LILRB1; K_D, 65nM
Cyno LILRB1; K_D, 74nM
Hu LILRA1; K_D, 120nM

- Hu LILRB1-His
- Cyno LILRB1-His
- Hu LILRB2-His
- Hu LILRA1-His
- Hu LILRA2-His
- Hu LILRA3-His

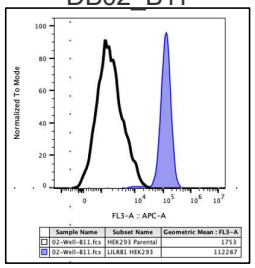
DB02_A10



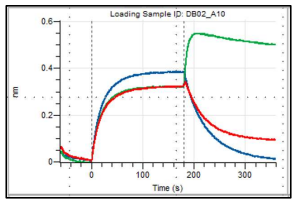
DB02_C10



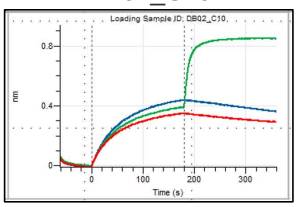
DB02_B11



DB02_A10



DB02_C10



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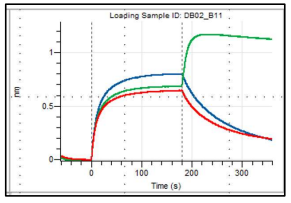

HLA-G tetramer blocking
Anti-His non-blocking
Buffer

Probable Rationale for Cyno & Cross-Family Binding

Alignment of Ligand Binding Domains

```
cyno     PKPMLWAEPDRVITQGSPVTLRCQGNLEALGYHLYRERKSASWITSIRPELVRKGQFPIP  60
LILRB1   PKPTLWAEPGSVITQGSPVTLRCQGGQETQEYRLYREKKTALWITRIPQELVKKGQFPIP  60
LILRA1   PKPTLWAEPGSVITQGSPVTLWCQGILETQEYRLYREKKTAPWITRIPQEIVKKGQFPIP  60
LILRA3   PKPTLWAEPGSVITQGSPVTLRCQGSLETQEYHLYREKKTALWITRIPQELVKKGQFPIL  60
         *** *****. ********** ***  *:  *:****:*:* *** *  *:*:******

cyno     SITWEDAGRYRCQYYSHS-WWSEHSDPLELVVTGAYSKPTLSALPSPVVASGGNVTLQCD  119
LILRB1   SITWEHAGRYRCYYGSDTAGRSESSDPLELVVTGAYIKPTLSAQPSPVVNSGGNVILQCD  120
LILRA1   SITWEHTGRYRCFYGSHTAGWSEPSDPLELVVTGAYIKPTLSALPSPVVTSGGNVTLHCV  120
LILRA3   SITWEHAGRYCCIYGSHTAGLSESSDPLELVVTGAYSKPTLSALPSPVVTSGGNVTIQCD  120
         *****.:*** * * *.:   ** ************ ****** ***** ***** ::*

cyno     SRVAFDGFILCKEGEDEHSQCLNSQPRTRGSSRAVFSVGPVSPSRRWSYRCYGYDSSFPY  179
LILRB1   SQVAFDGFSLCKEGEDEHPQCLNSQPHARGSSRAIFSVGPVSPSRRWWYRCYAYDSNSPY  180
LILRA1   SQVAFGSFILCKEGEDEHPQCLNSQPRTHGWSRAIFSVGPVSPSRRWSYRCYAYDSNSPH  180
LILRA3   SQVAFDGFILCKEGEDEHPQCLNSHSHARGSSRAIFSVGPVSPSRRWSYRCYGYDSRAPY  180
         *:***..* ********* *****: :::* ***:************ ****.***  *:

cyno     VWSLPSDLLELLVS- 193
LILRB1   EWSLPSDLLELLVL- 194
LILRA1   VWSLPSDLLELLVL  194
LILRA3   VWSLPSDLLGLLVP- 194
         ******** ***
```

Identity Matrix (%)

	Cyno	B1	A1	A3
Cyno	100	79	79	81
B1	79	100	87	88
A1	79	79	100	85
A3	81	88	85	100

AA different between cyno & human B1

AA different between cyno/hu B1/huA1/huA3

- High sequence identity (~80%) between cyno and human LILRB1, LILRA1, LILRA3 makes it challenging to ensure cyno cross-reactivity while avoiding off-target binding.

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Probable Rationale for Avoidance of Cross-Family Binding

Alignment of Ligand Binding Domains

```
LILRB1    PKPTLWAEPGSVITQGSPVTLRCQGGQETQEYRLYREKKTALWITRIPQELVKKGQFPIP 60
LILRA1    PKPTLWAEPGSVITQGSPVTLWCQGILETQEYRLYREKKTAPWITRIPQEIVKKGQFPIP 60
LILRA3    PKPTLWAEPGSVITQGSPVTLRCQGSLETQEYHLYREKKTALWITRIPQELVKKGQFPIL 60
          ********************* ***  *****:******** ********:********

LILRB1    SITWEHAGRYRCYYGSDTAGRSESSDPLELVVTGAYIKPTLSAQPSPVVNSGGNVILQCD 120
LILRA1    SITWEHTGRYRCFYGSHTAGWSEPSDPLELVVTGAYIKPTLSALPSPVVTSGGNVTLHCV 120
LILRA3    SITWEHAGRYCCIYGSHTAGLSESSDPLELVVTGAYSKPTLSALPSPVVTSGGNVTIQCD 120
          ******:*** * ***.*** ** ************ ****** *****.***** ::*

LILRB1    SQVAFDGFSLCKEGEDEHPQCLNSQPHARGSSRAIFSVGPVSPSRRWWYRCYAYDSNSPY 180
LILRA1    SQVAFGSFILCKEGEDEHPQCLNSQPRTHGWSRAIFSVGPVSPSRRWSYRCYAYDSNSPH 180
LILRA3    SQVAFDGFILCKEGEDEHPQCLNSHSHARGSSRAIFSVGPVSPSRRWSYRCYGYDSRAPY 180
          *****..* ***************: ::::* *************** ****.***.:*:

LILRB1    EWSLPSDLLELLVL- 194
LILRA1    VWSLPSDLLELLVL- 194
LILRA3    VWSLPSDLLGLLVP- 194
          ***********
```

Identity Matrix (%)

	B1	A1	A3
B1	100	87	88
A1	87	100	85
A3	88	85	100

AA common between human B1 and at least one of the off-targets

AA different between hu B1/huA1/huA3

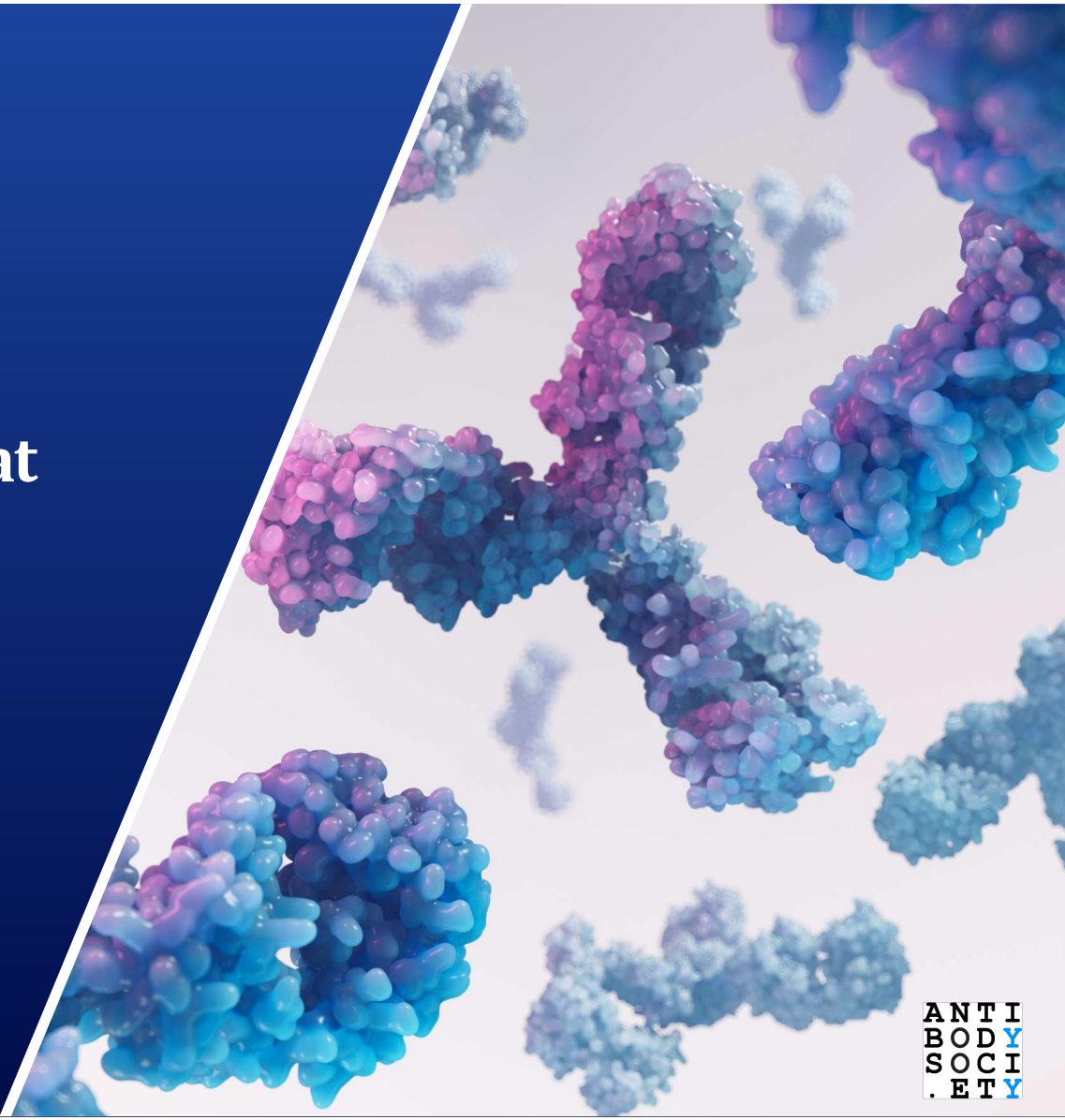
- Even though there exists high sequence identity (≥85%) between human LILRB1, LILRA1 & LILRA3, several AAs are different between the 3 proteins in the ligand binding D1-D2 domain to help avoid cross-family binding.

Summary of LILRB1 Discovery Campaign

- SH2.0™ was successfully applied in discovery of several anti-LILRB1 clones that showed improved off-target binding profile than a first-generation antibody that is currently in clinic.
- The data highlights the advantage of utilizing a large and diverse naïve ScFv library in combination with a well-designed panning strategy to identify multiple anti-LILRB1 antibodies that show low/no cross-reactivity to other LILR family members.
- Screening hundreds of antibody hits for multiple off-target binding can be laborious and time consuming.
- Here, we discussed the different screening methodologies applied to overcome these challenges.
- This includes utilizing automated HT screens to assess >8700 interactions within a day and complementing this with more rigorous cell-based functional characterizations.

charles river

LILRB2 Antibody Discovery at Charles River



Goals for LILRB2 Discovery Campaign

Project Goals:

- Target: Leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2; Uniprot ID: Q8N423)
- Human only binders a priority; Human-cyno cross reactivity is nice-to-have
- Off-target selectivity: LILRB2, LILRB3, LILRB4, LILRB5, LILRA1, LILRA2, LILRA3, LILRA4, LILRA5, LILRA6
- Desired Function: Specific binding to LILRB2 with blocking of HLA-G binding
- Final Format: IgG4
- For use in therapeutic applications

LILRB2 Workflow

Ag QC → Phage Panning SH2.0™ → Primary Screening → Secondary Screening → Reformat → Biophysical Characterization

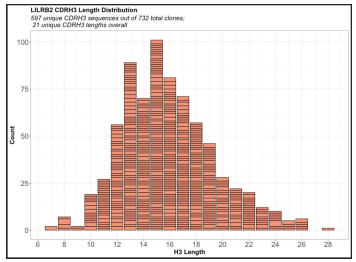
	R1-R4	
	Selection	Deselection
SH2.0	LILRB2	LILRB1

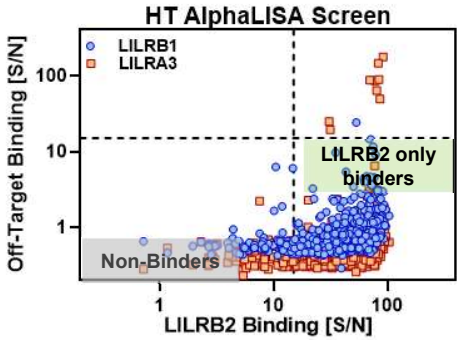
AlphaLISA HT screen for

- binding to human/cyno LILRB2
- LILRB1, LILRA3 off-target screen

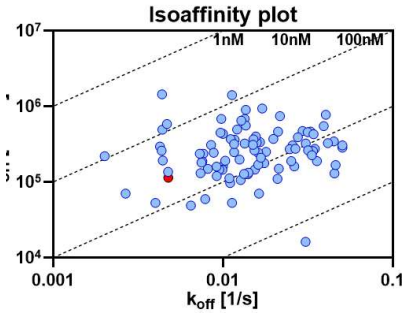
- Binding kinetics to human LILRB2
- Ligand blocking (HLA-G)
- FACS screen of 9 off-targets

- HuIgG4





- 634 LILRB2 only binders
- Low/no cyno cross-reactivity



1E1 ScFV, K_D: 42nM

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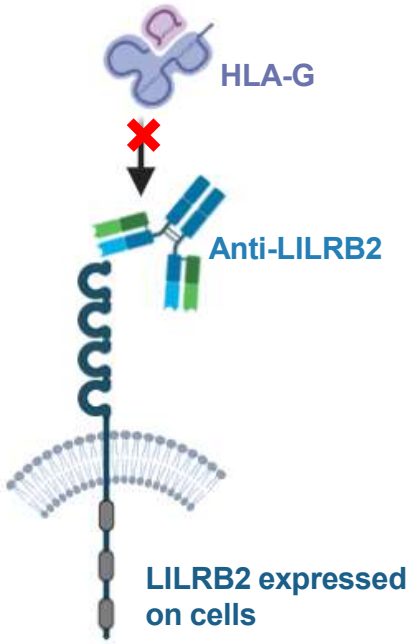
Blockade of LILRB2 and HLA-G Binding

Experimental Setup



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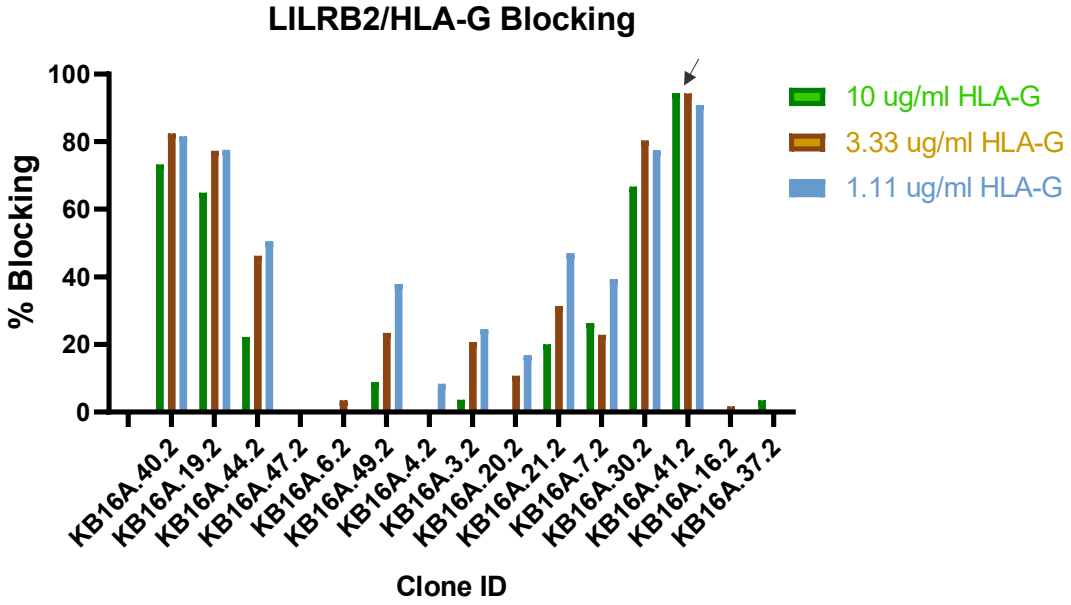
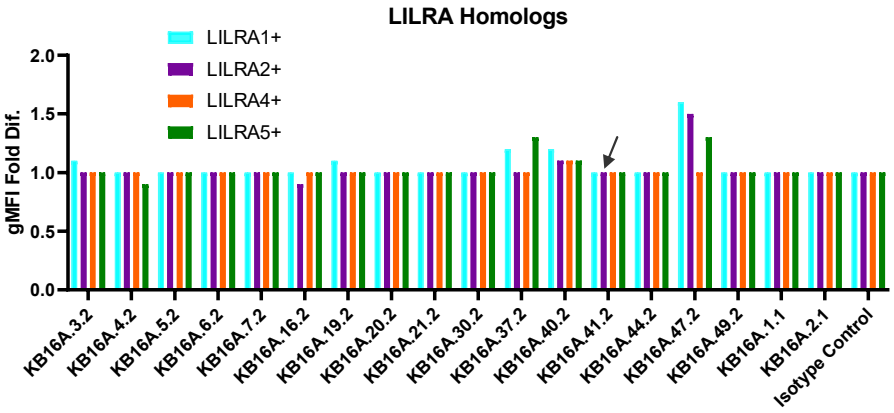
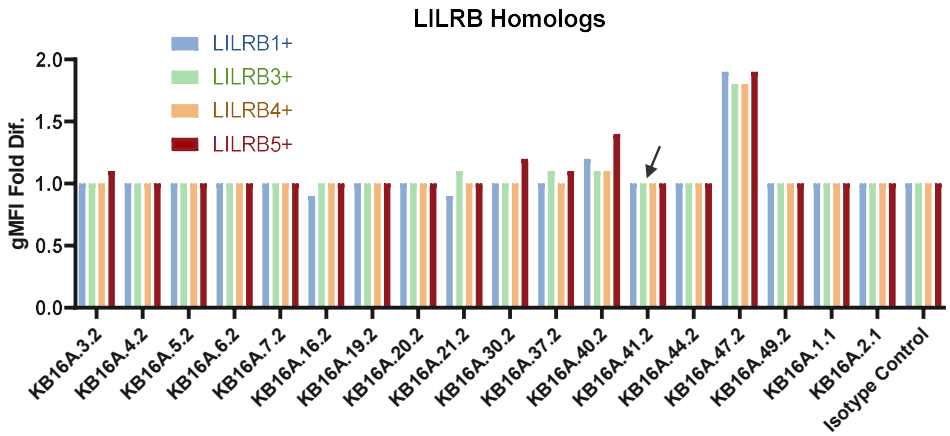


- 15 IgGs screened
- Data Courtesy, Joshua Royal, KBio
- KB16A.41.2 appear to have robust HLA-G blocking

Cell-Based Binding Specificity

Off-target **flow cytometric analysis** of mAb binding to LILR-expressing HEK293T cells

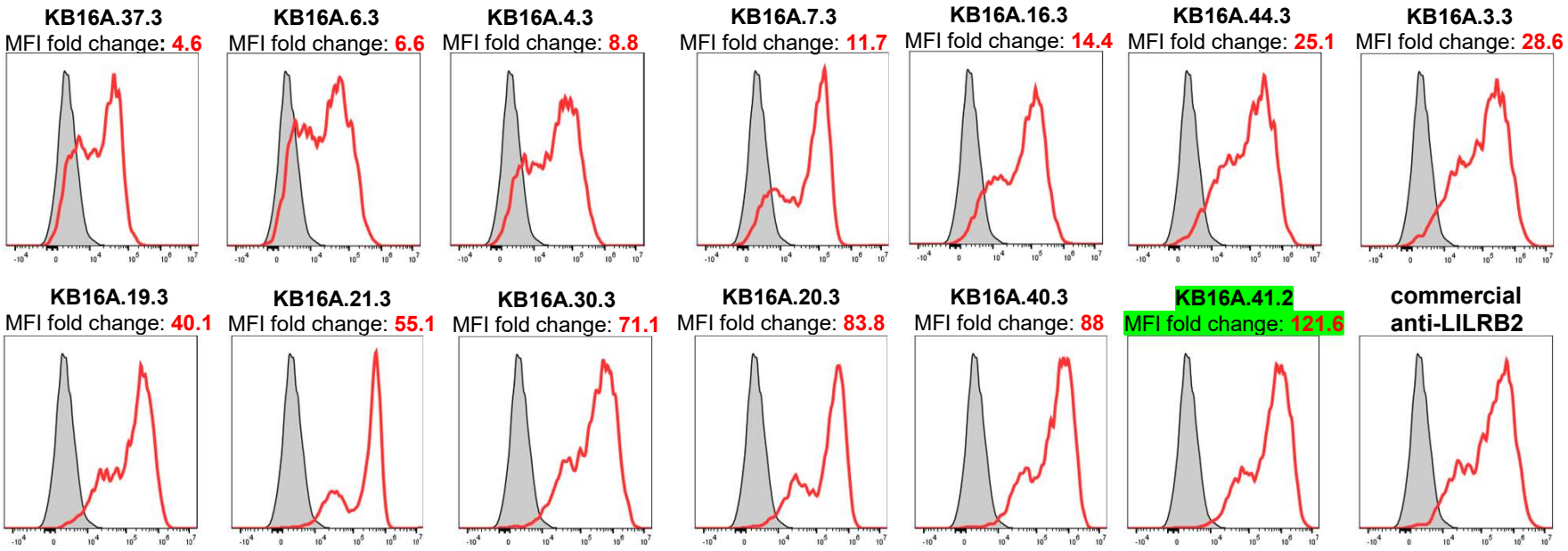




- Several mAbs showed low/no binding to LILRAs and LILRBs including KB16A.41.2

Cell Based Binding

Flow cytometric analysis of mAb binding to LILRB2-expressing HEK293T cells

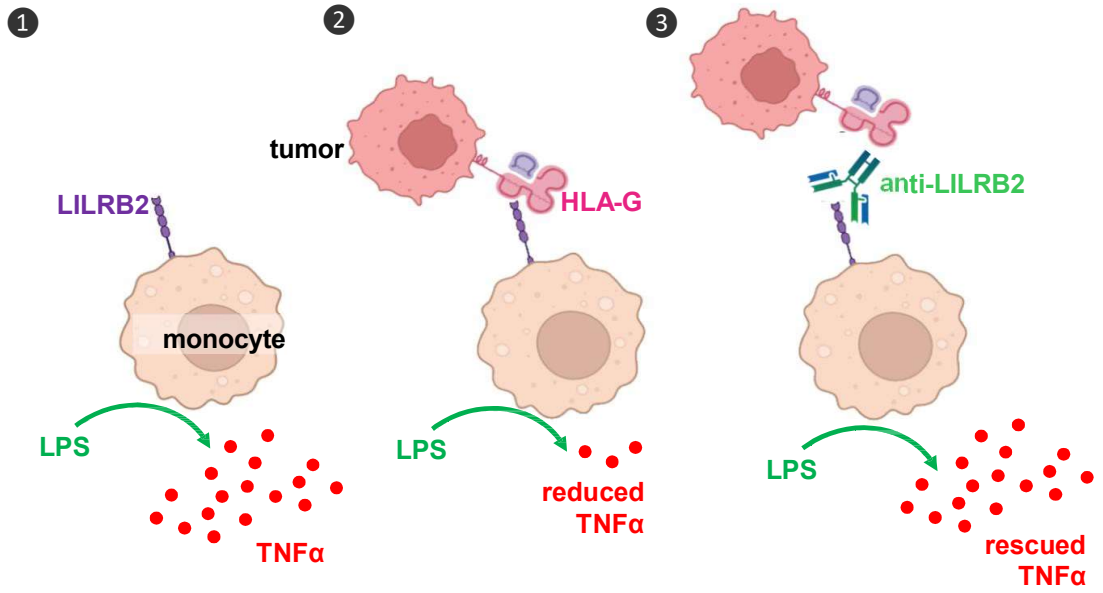


Human Cell-Based Functional Assay

1. LILRB2-expressing human monocyte produces inflammatory cytokine TNF in response to stimulus (LPS, bacterial cell wall component)

2. HLA-G-expressing tumour cells would engage the LILRB2 and suppress the monocytes, reducing TNF production

3. Blockade antibodies would bind to LILRB2 and prevent the suppressive activity



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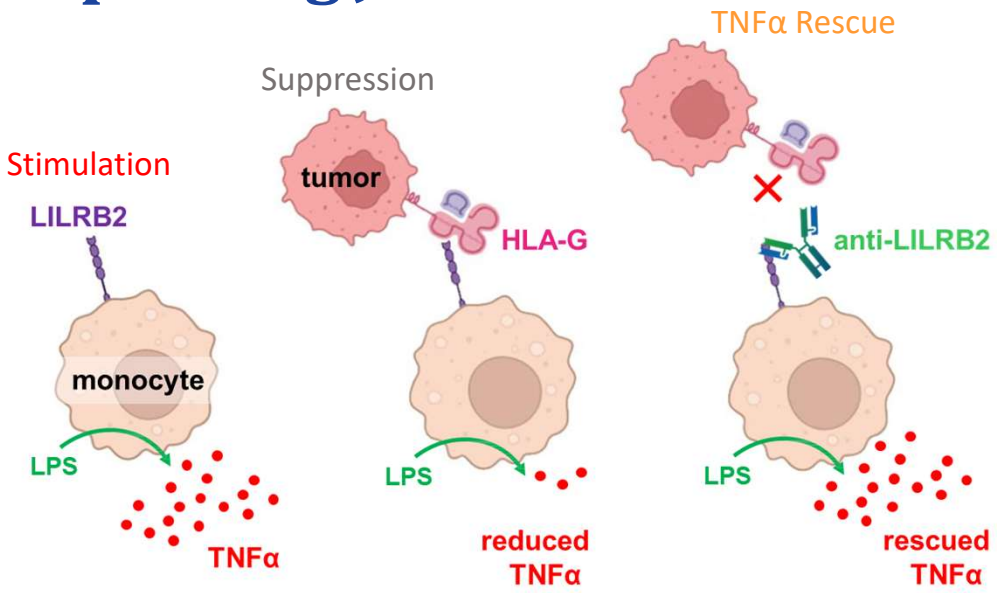
Co-culture Assay of Stimulated Monocytes with HLA-G - Expressing JEG-3 cells



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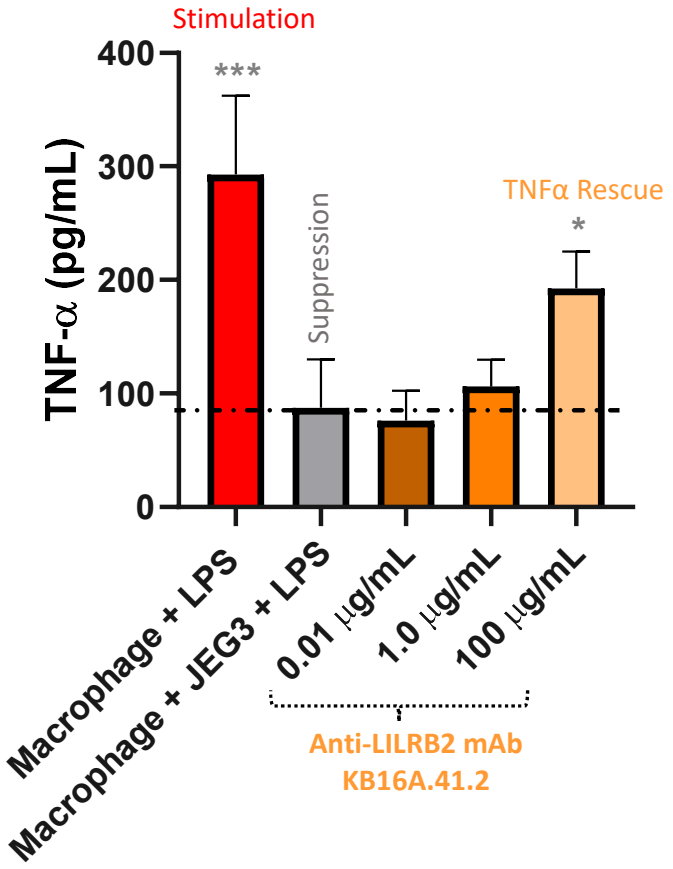
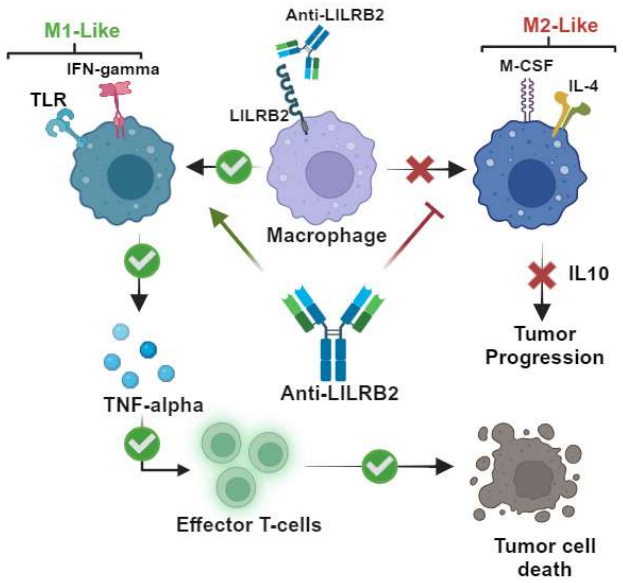


LILRB2 Antagonism Activates Macrophages



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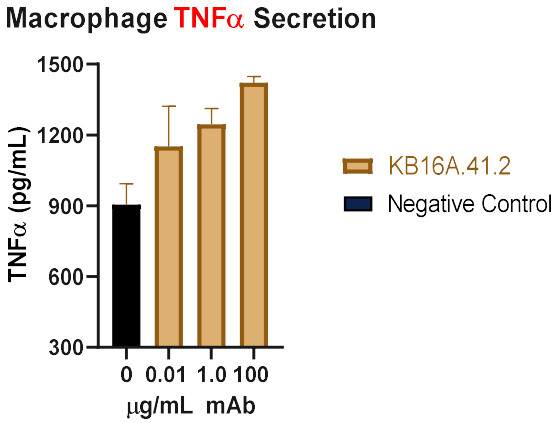
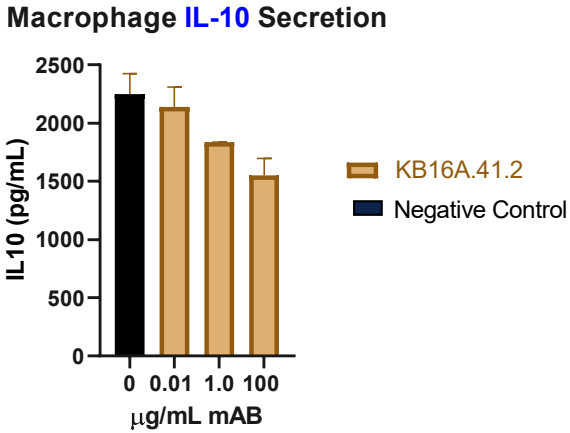
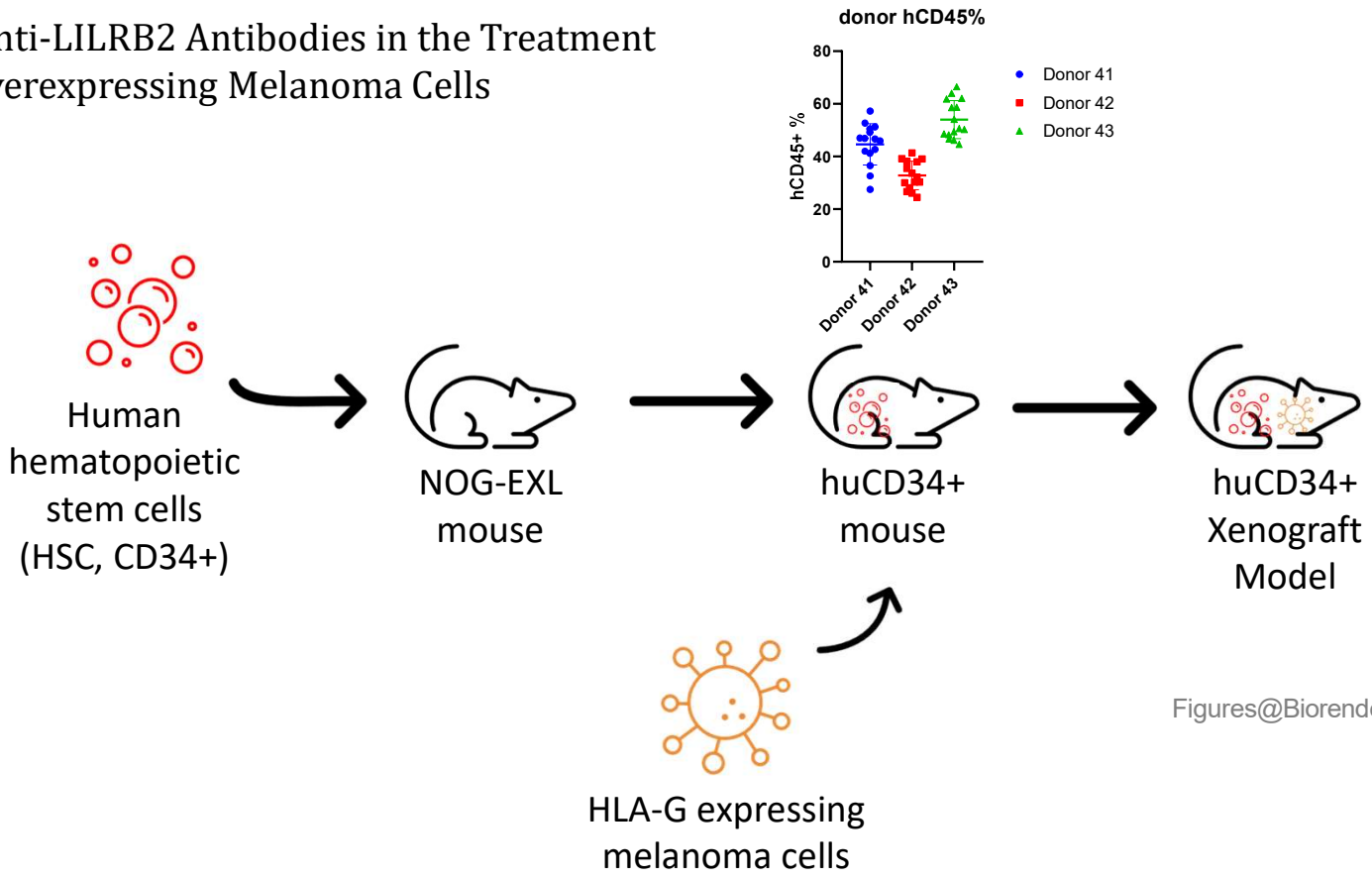




Chen et al., *J Clin Invest.* 2018;128(12):5647-5662.

Xenograft Model in HuHSC-NOG EXL Mice - Ongoing

In Vivo Efficacy Study of Anti-LILRB2 Antibodies in the Treatment of Subcutaneous HLA-G overexpressing Melanoma Cells



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Conclusions

- A rational and successful approach for specific targeting of LILRB1 and LILRB2 was discussed.
- Avoiding highly homologous LILR family members that are also expressed on myeloid cells can be challenging.
- The diversity of SH2.0™ naïve ScFv library in combination with a well-designed panning strategy helped identify multiple anti-LILRB1 and anti-LILRB2 antibodies with strong binding specificity.
- Anti-LILRB2 mAbs were shown to functionally antagonize LILRB2 on macrophages
  - + LILRB2 mAbs prevent HLA-G-induced immunosuppressive pathways
  - + LILRB2 mAbs polarized macrophages cells towards inflammatory phenotype
- Next Steps for anti-LILRB2 [KBio]: POC *In Vivo* Efficacy Studies
  - + POC *In vivo* efficacy study of anti-LILRB2 antibodies in the treatment of subcutaneous HLA-G overexpressing melanoma cells in HuHSC-NOG EXL xenograft model (on-going)
  - + Lead optimization and mAb (LILRB2, HLA-G, PD-L1 mAbs) combinational studies
- Next Steps for anti-LILRB1: Optimization/engineering to improve human/cyno LILRB1 affinity or reduce affinity to LILRA1/LILRA3.
- The lessons learned here are broadly applicable to next-generation immune checkpoint inhibitors that require recognizing multiple isoforms or necessitate avoidance of multiple close family members for effective immunotherapy.

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- Kyle Gruber
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Thank you!

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