

# Enable ADC & T Cell Engager Development with Highly Efficient in Vitro & in Vivo Characterization

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2023-11-22



**WuXi Biologics**  
Global Solution Provider

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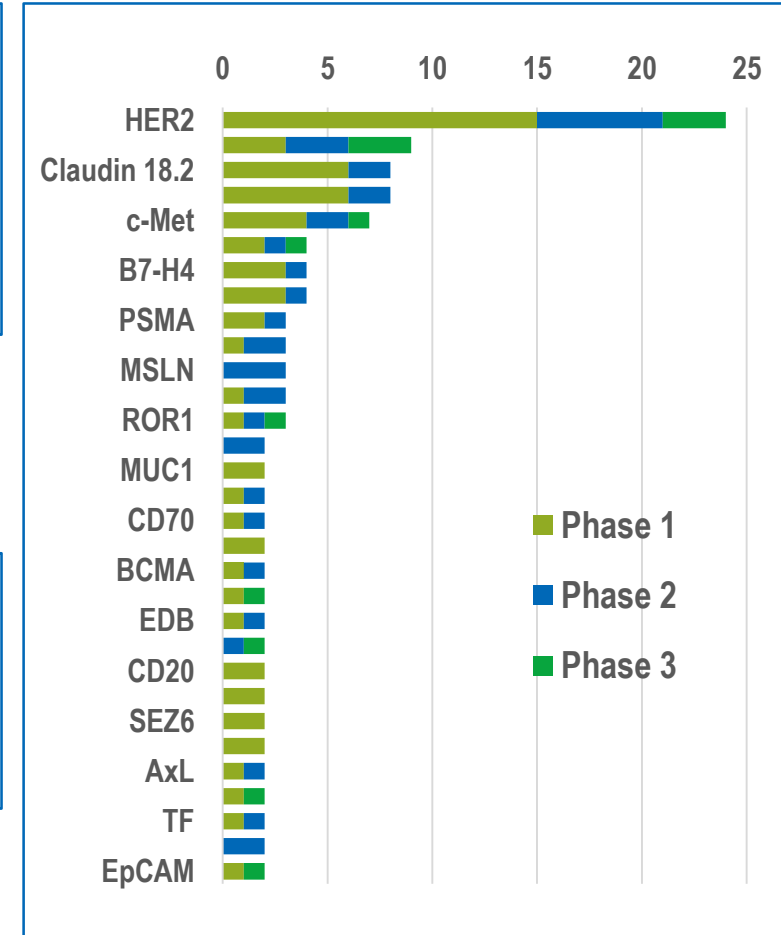
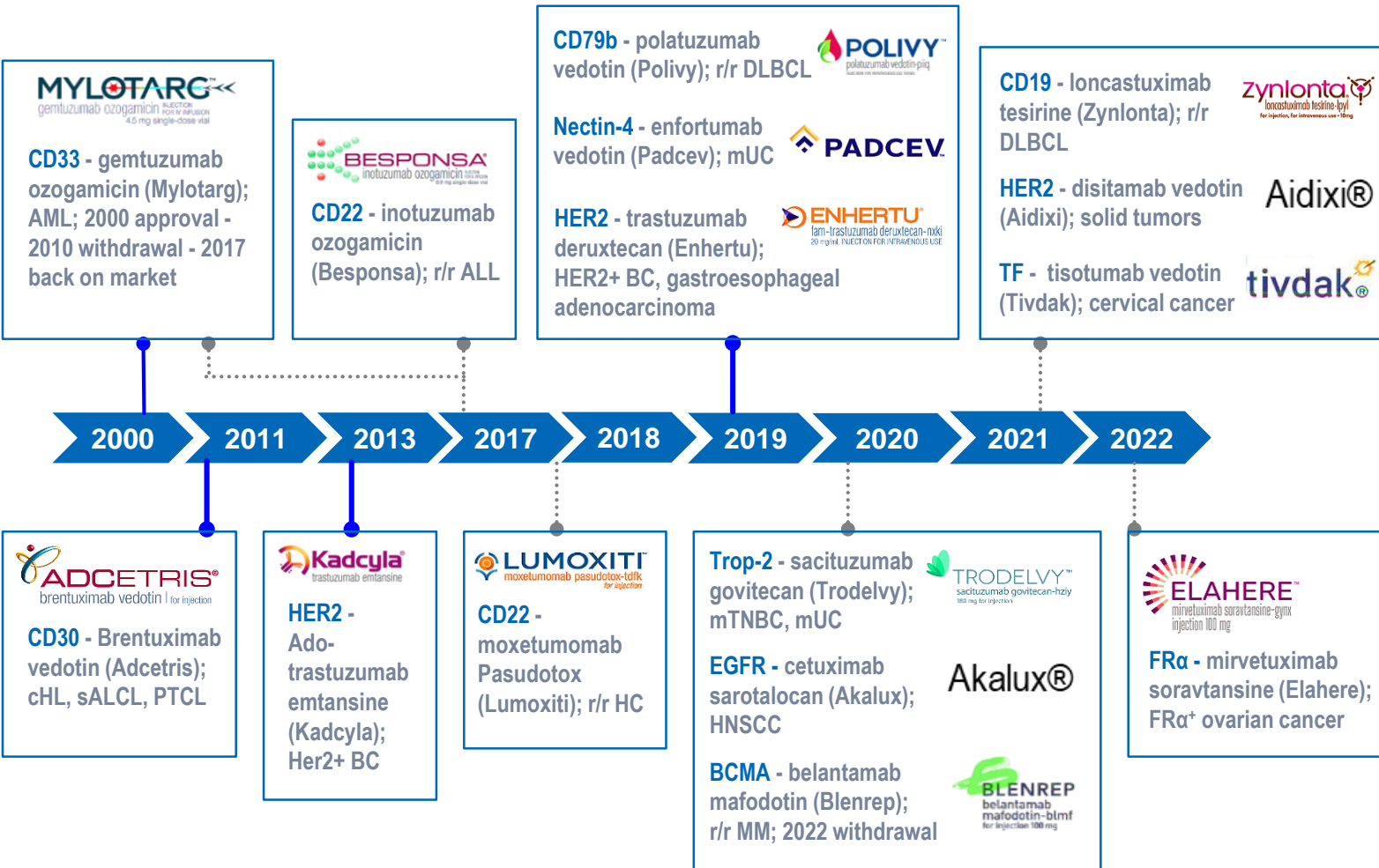
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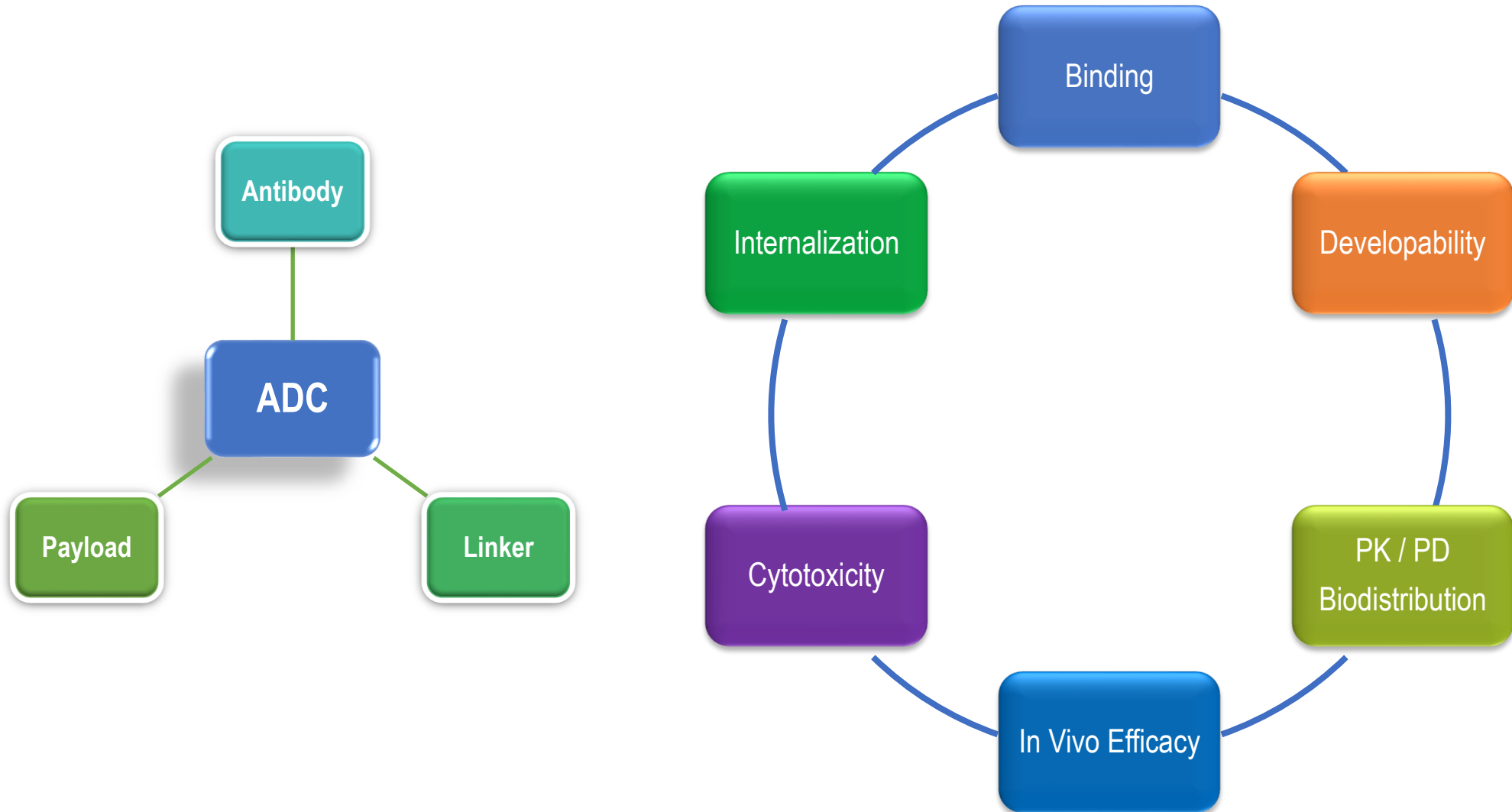
# ADCs Demonstrate Substantial Clinical Benefit

## Chronical of 15 ADC Approvals

## Top Targets by Number of ADCs in Clinical Trials



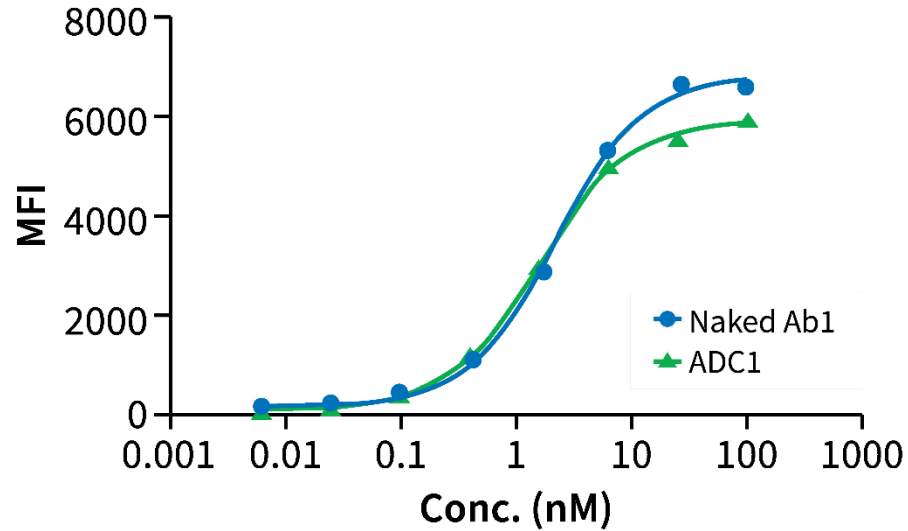
# ADCs Require Complex Pharmacological Evaluation



# Binding Before and After ADC Conjugation

## Binding affected by conjugation

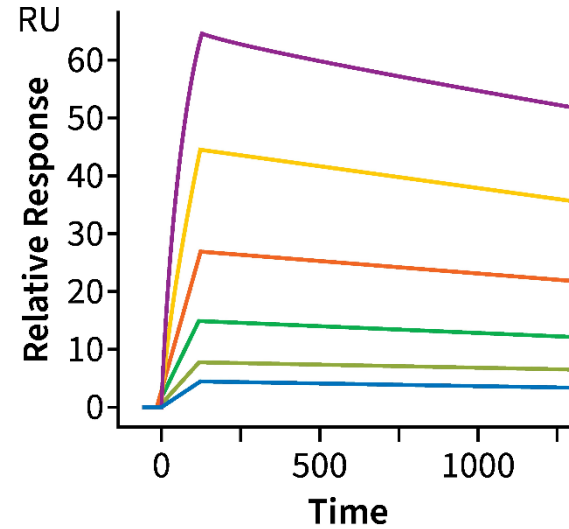
### Binding by FACS



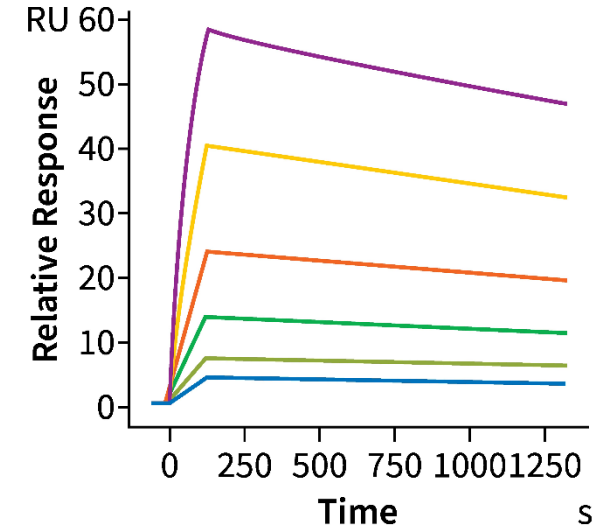
Molecule	EC <sub>50</sub> (nM)	Max MFI
Naked Antibody 1	2.1	6663
ADC1	1.6	5916

## Binding not affected by conjugation

### SPR Affinity - Naked Antibody



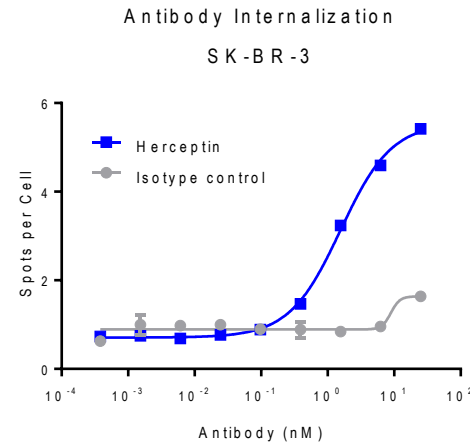
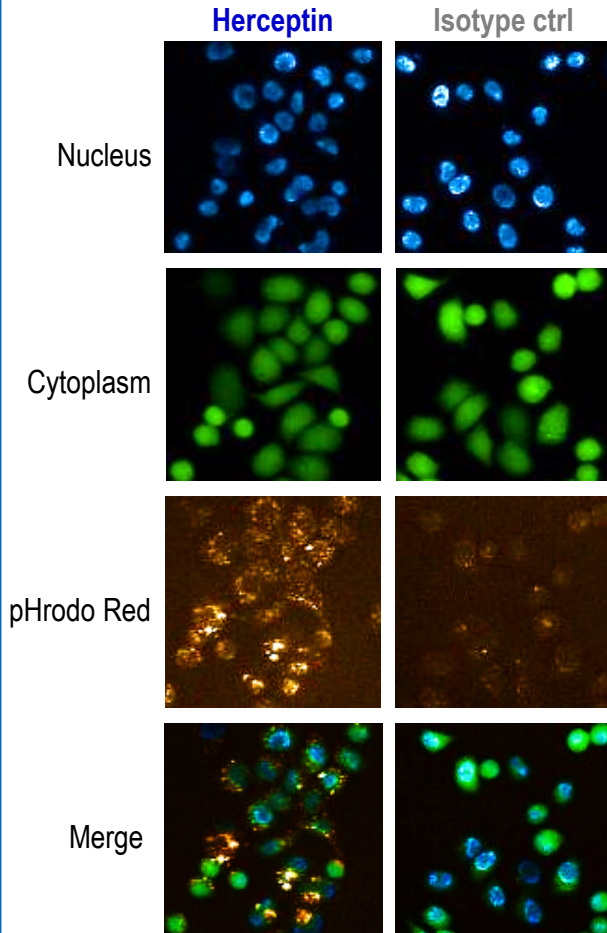
### SPR Affinity - ADC



Molecule	K <sub>a</sub> (1/ms)	K <sub>d</sub> (1/s)	K <sub>D</sub> (M)
Naked Antibody 2	1.25 E+5	1.73 E-4	1.39 E-9
ADC2	1.30 E+5	1.69 E-4	1.30 E-9

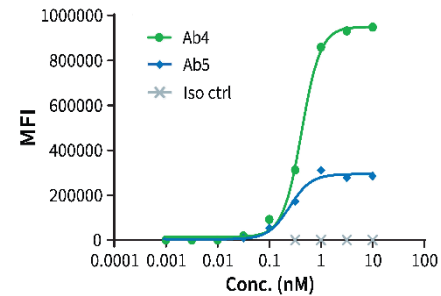
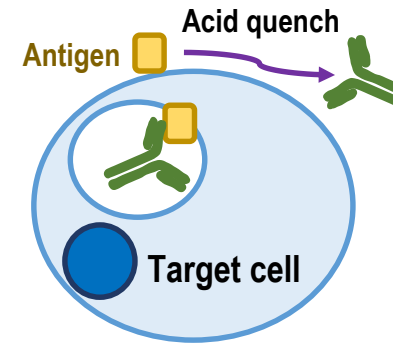
# Complementary Internalization Assays

## pHrodo Based



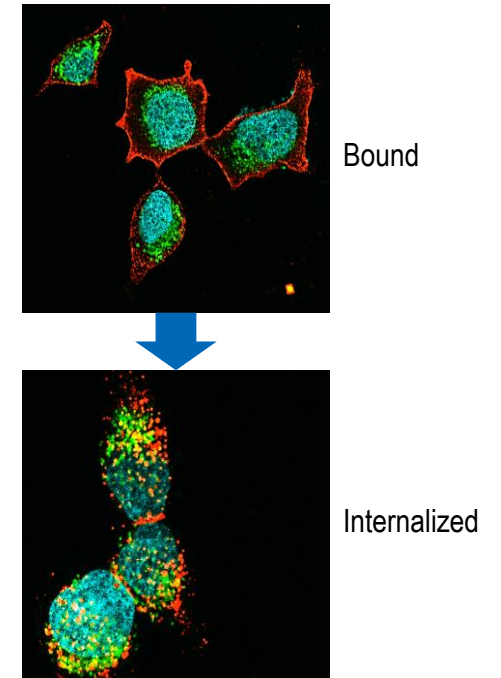
Molecule	EC <sub>50</sub> (nM)	Max Spot
Trastuzumab	~1.6	5.4
Iso CTRL	NA	1.7

## Acid Quenching



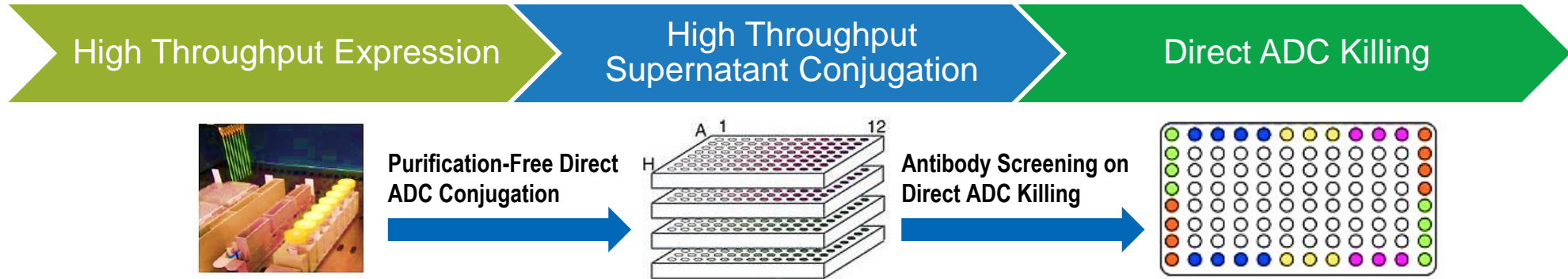
Molecule	EC <sub>50</sub> (nM)	Max MFI
Antibody 1	0.42	947659
Antibody 2	0.25	311610
Iso CTRL	NA	80

## Lysosome Tracking



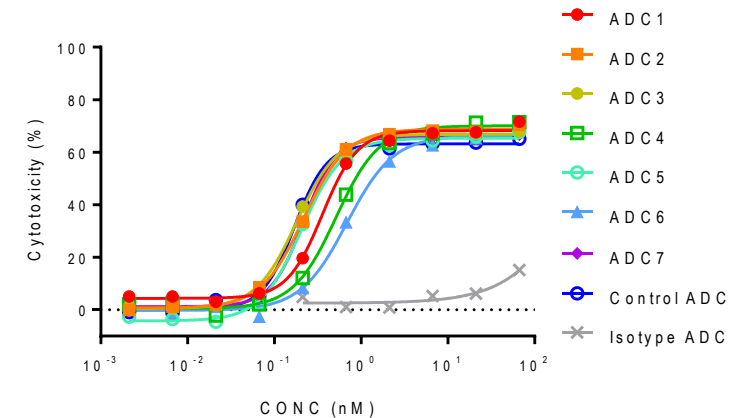
**Red:** Ab1 + anti-IgG PE  
**Green:** FITC lysosome marker  
**Blue:** DAPI  
**Yellow:** Ab1 co-localized with lysosomes

# Streamlined Supernatant ADC Conjugation Workflow



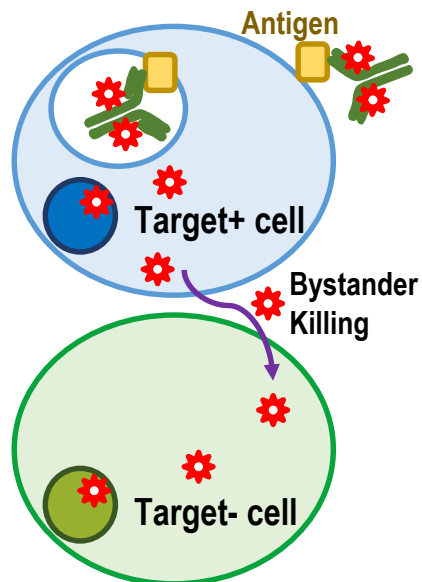
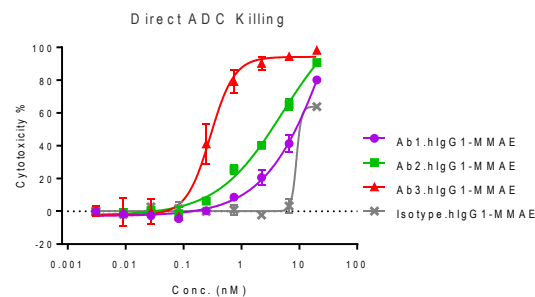
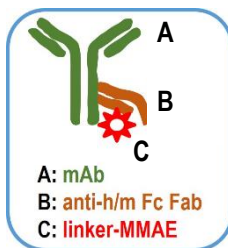
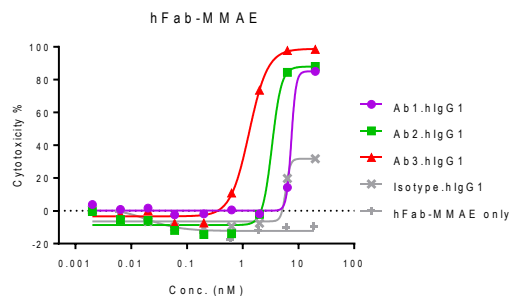
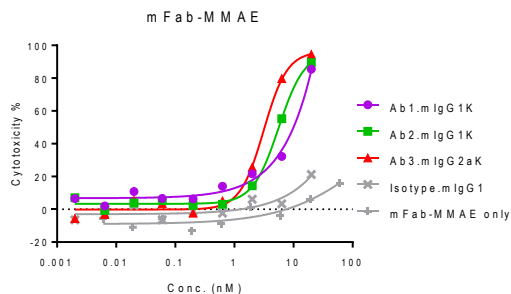
Economical	Robust
<ul style="list-style-type: none"> <li>• Mini-scale expression</li> <li>• Microgram scale conjugation</li> </ul>	<ul style="list-style-type: none"> <li>• Antibody concentration accommodative</li> <li>• DAR = 4 / 8 for vc-MMAE / GGFG-Dxd</li> </ul>
High Quality	Efficient
<ul style="list-style-type: none"> <li>• Low residual free drug</li> <li>• Low aggregation</li> </ul>	<ul style="list-style-type: none"> <li>• Up to hundreds of samples per batch</li> <li>• Direct ADC killing for antibody selection</li> </ul>

In Vitro Killing of Tumor Cells

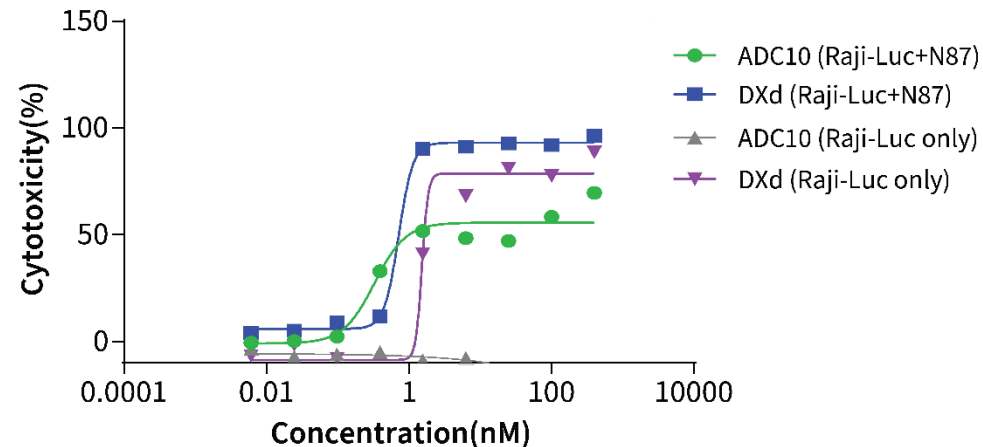


# Specialized Cytotoxicity Assays for ADC Selection

## Fab-MMAE Indirect Killing Assay



## Bystander Killing Assay

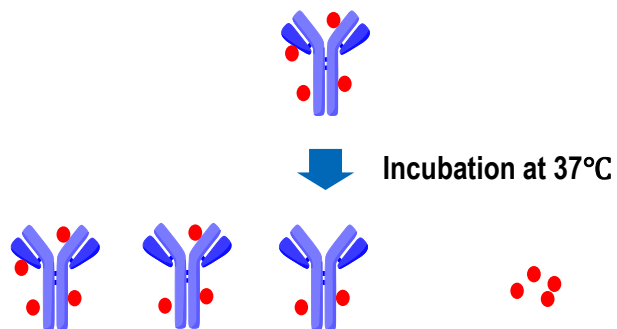


Cells	Compound	IC <sub>50</sub> (nM)	Max Killing (%)
NCI-N87 (HER2+) Raji-Luc (HER2-)	T-Dxd	0.33	70
NCI-N87 (HER2+) Raji-Luc (HER2-)	Dxd	0.72	96
Raji-Luc (HER2-)	T-Dxd	NA	-3
Raji-Luc (HER2-)	Dxd	~ 1.5	89



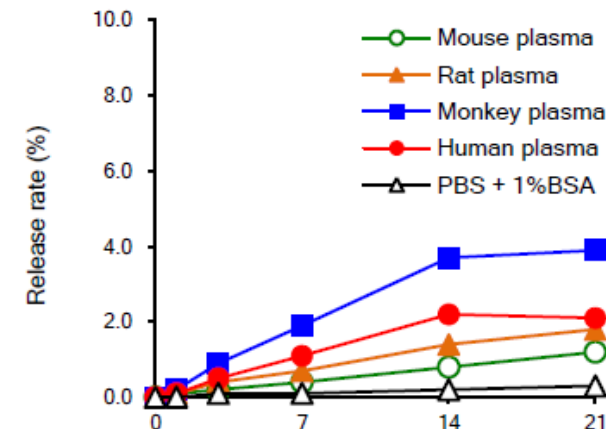
# Serum & Plasma Stability

## Mouse/Rat/Cyno/Human Serum/Plasma Stability

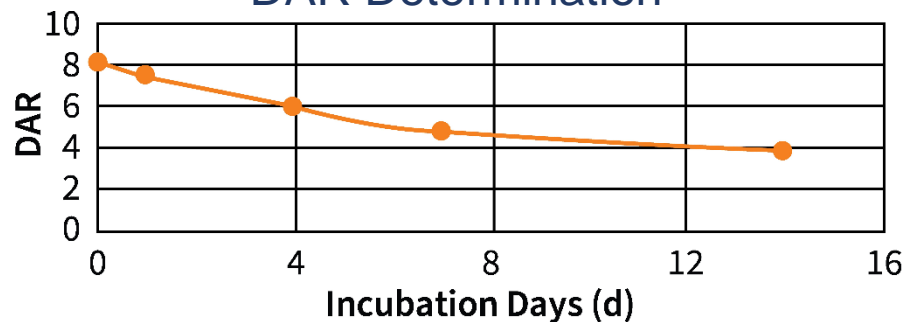


- Affinity measurement
- DAR value determination
- Free payload detection

## Free Drug Release



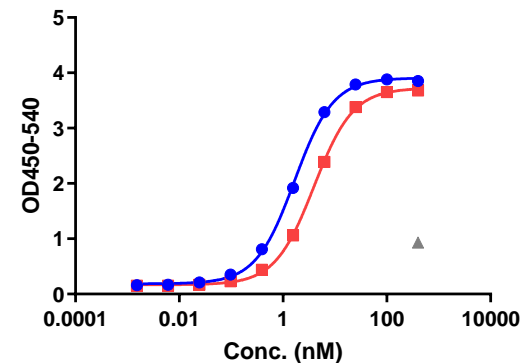
## DAR Determination



Time	Day 1	Day 1	Day 4	Day 7	Day 14
Avg. DAR	8.00	7.43	5.86	4.65	3.76

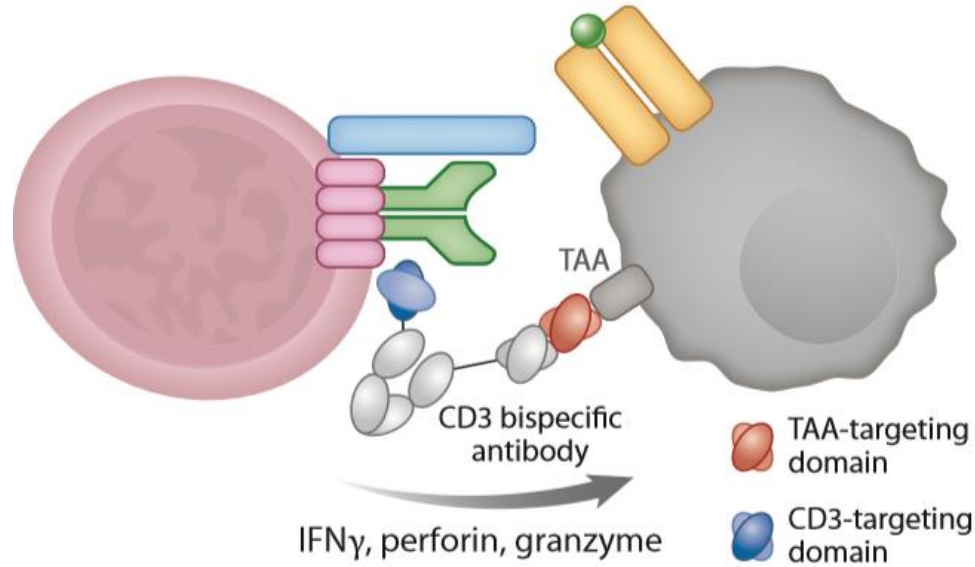
## Bio-Activity

### ELISA of Human Serum-treated Samples to Antigen



Time	EC <sub>50</sub> (nM)	Max OD
Day 0	1.6	3.88
Day 14	4.0	3.68

# T Cell Engagers Redirect T Cells to Kill Tumor Cells

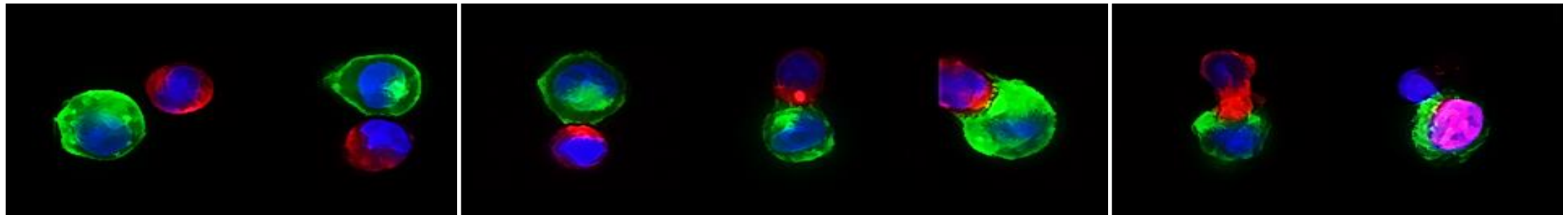


TCEs induce the formation of cytotoxic synapses to mediate TCR / MHC-I independent killing of tumor cells

Adhesion

Polarization

Degranulation



Granzyme B (T Cell) EGFR (Tumor Cell) DAPI (Nuclei)

Clynes RA & Desjarlais JR, Annual Review of Medicine 2018  
Bardwell, PD et.al., Protein Cell 2018

# TCEs Have Established Clinical Efficacy in Patients

## Proven Record in Liquid Cancers



MRD+ B-cell Precursor ALL  
R/R B-cell Precursor ALL

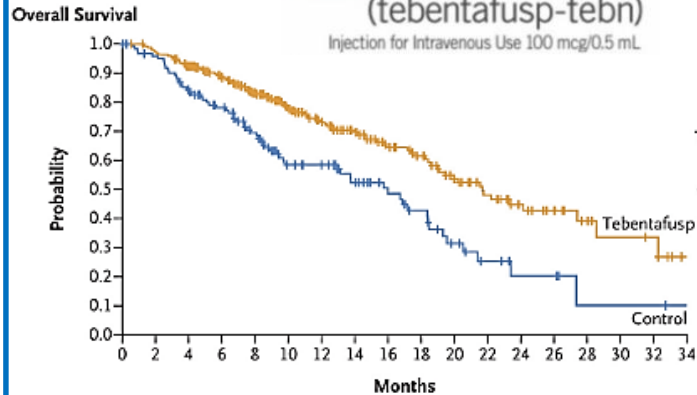


R/R follicular lymphoma

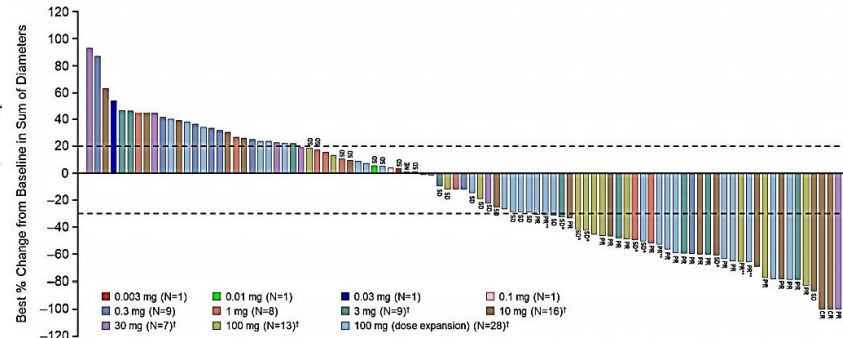


R/R multiple myeloma

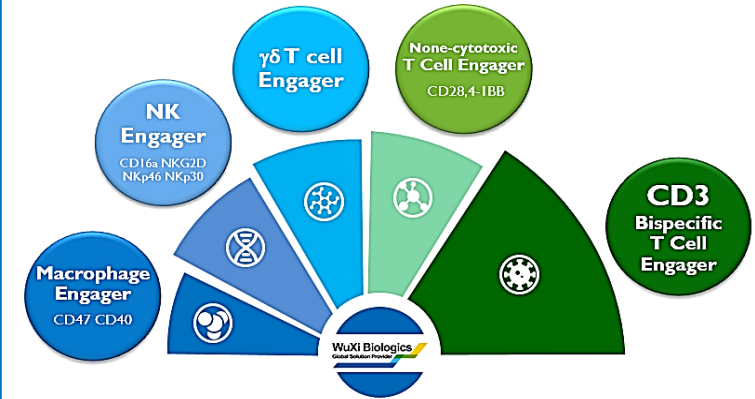
## Encouraging Efficacy in Solid Tumors



## AMG 757 (DLL3 x CD3) in SCLC

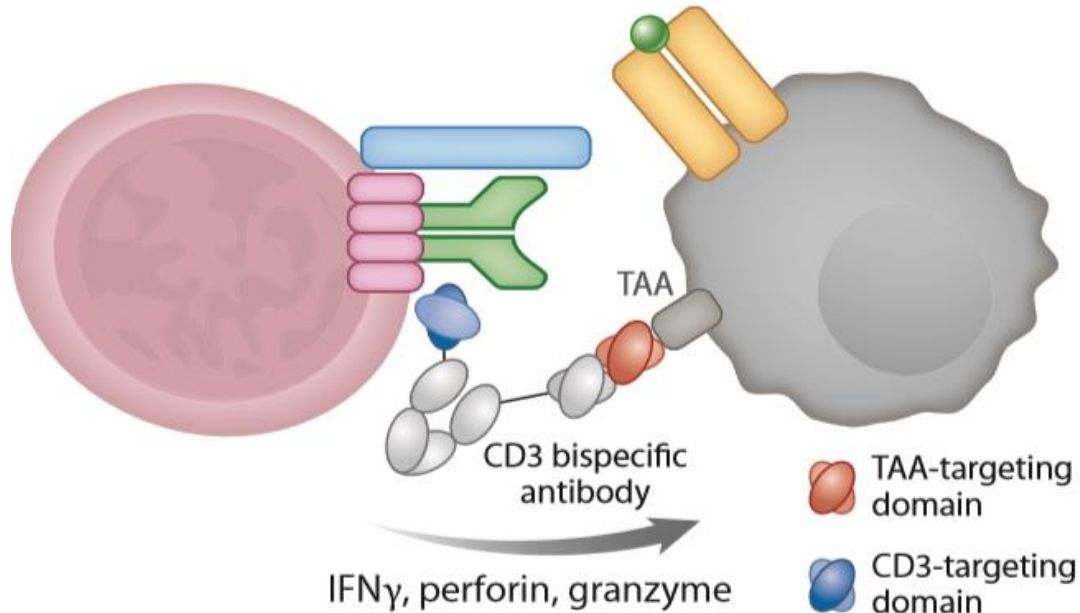


## New Immune Cell Engagers Being Studied



N Engl J Med. 2021. PMID: 34551229 Clinical Trial  
ONCOLOGY CLINICAL UPDATE, WCLC 2022, Amgen

# Determining Factors of TCE Efficacy & Safety

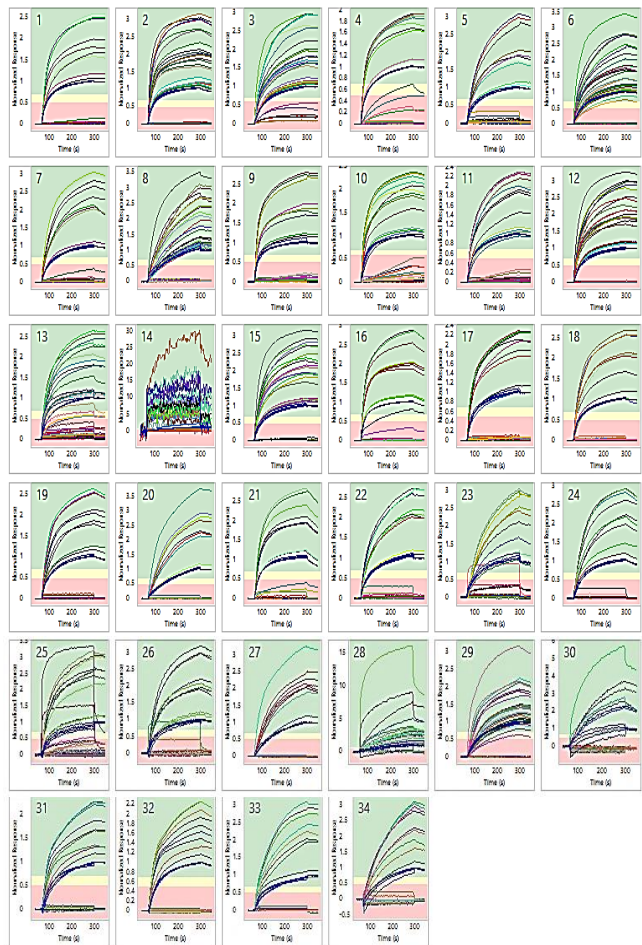


Clynes RA & Desjarlais JR, Annual Review of Medicine 2018

- 1 Tumor Associated Antigen (TAA) Selection
- 2 Format Optimization
- 3 T Cell Engagement through CD3
- 4 Additional Immune Modulation

# High-Throughput SPR Binning for Antibody Diversity

## Pairwise SPR Binning



## Improving Epitope Diversity in Antibody Discovery

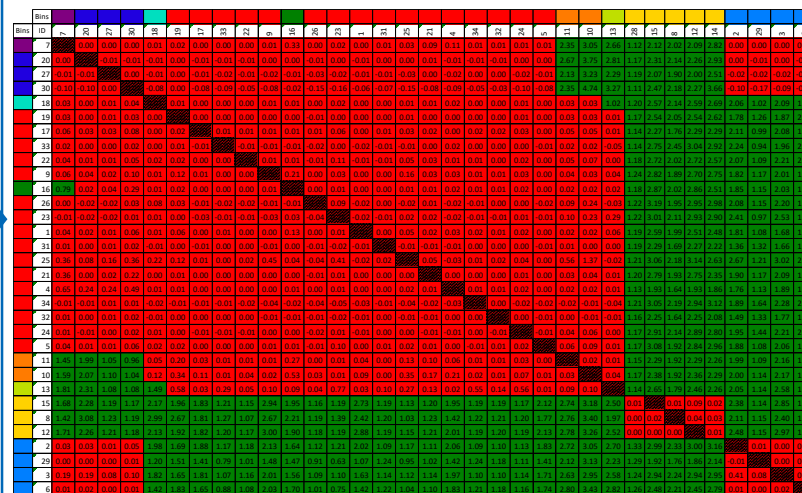
### Traditional competition assays $\times$

- Bi-directional competition difficult to scale up (up to 10 x 10)
- Time and sample consuming

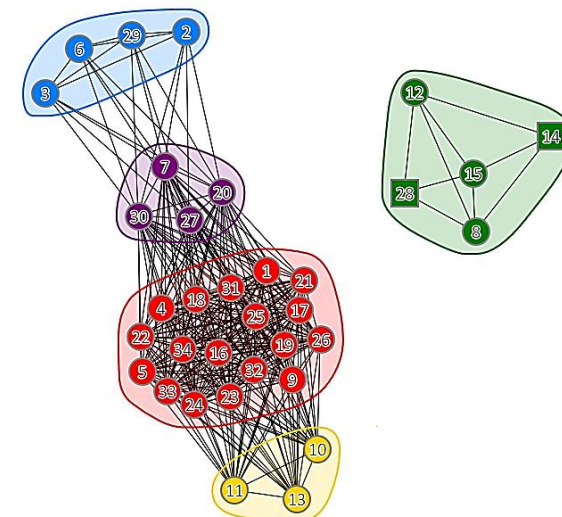
### SPR-based high-throughput epitope binning $\checkmark$

- Scales up linearly
- Fast with limited sample uptake
- > 30 x 30 pairwise binning in 1 day

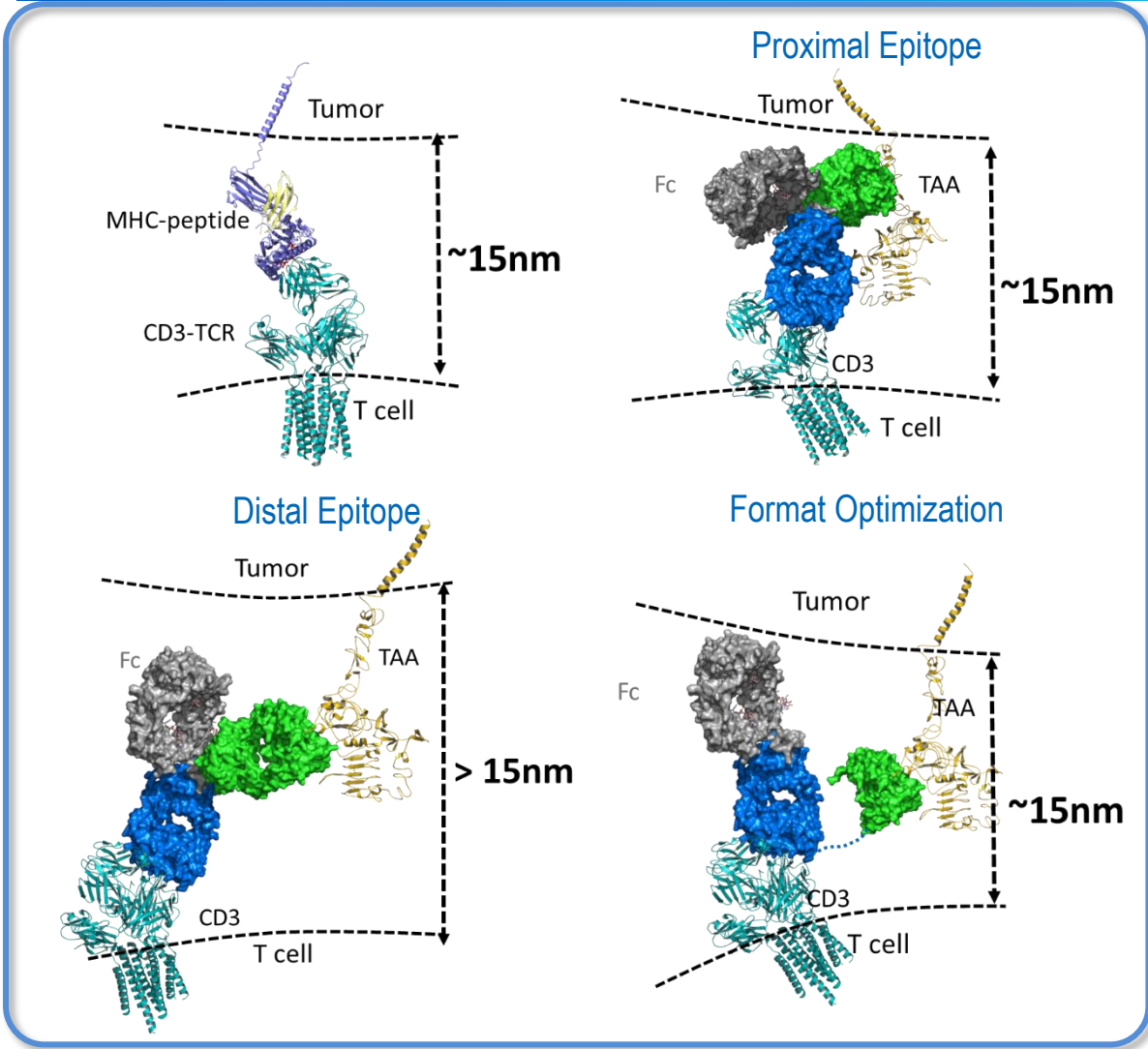
## Competition Matrix




## Network Blocking Plot




# WuXi Biologics' Multiple Platforms Enable Fit-for-Target Format Selection and Optimization



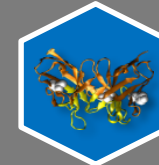
Multiple formats enable optimization on valency, distance and geometry for best T cell activation / safety profile




WuXiBody™



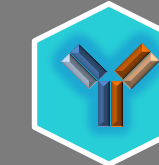
SDArBody™



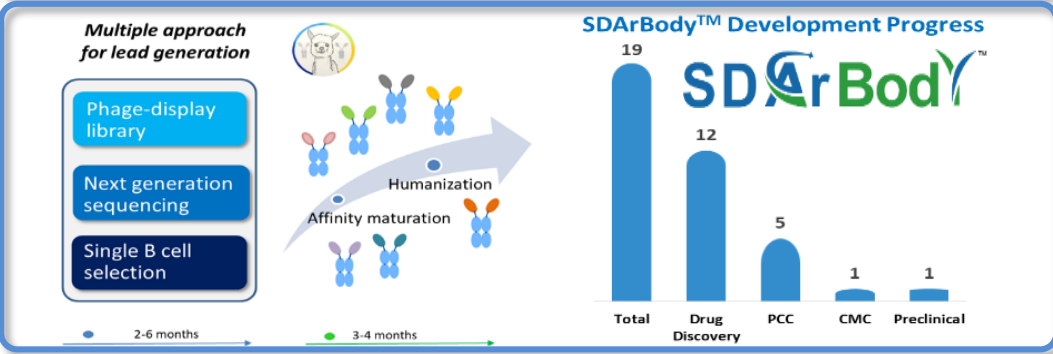
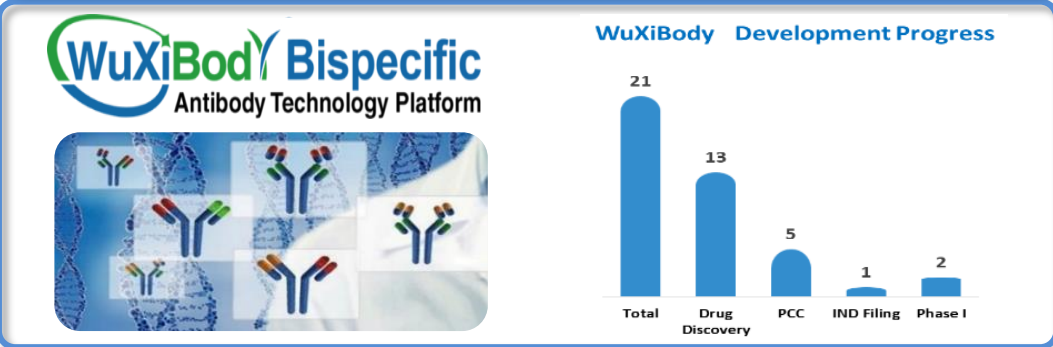
SkyBody



Common LC BsAb



Other BsAb



# Comprehensive TCE In Vitro Characterization

## Assays optimized at WuXi Biologics

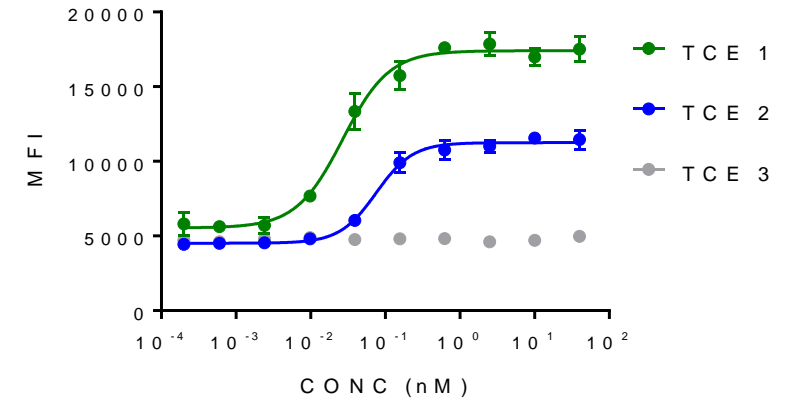
### Standard Assays

- Binding
- Cytotoxicity
- T cell activation
- Cytokine release

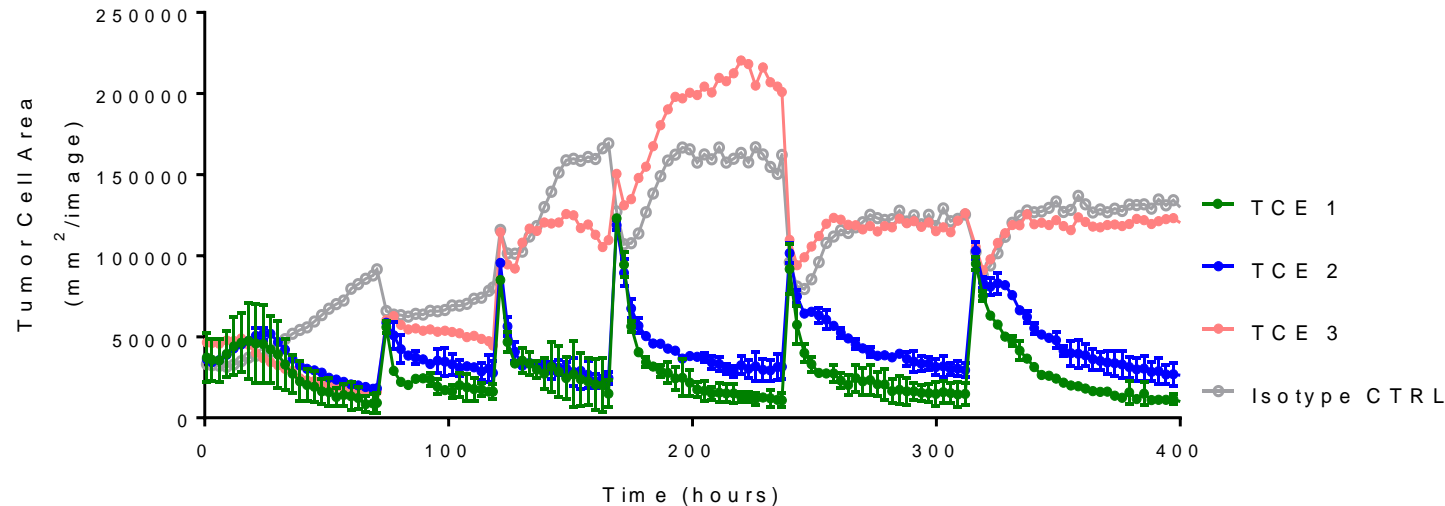
### Special Assays

- Engineered tumor cell-lines
- Engineered reporter cells
- AICD by Phospho Flow
- Repeated tumor cell killing

B c L - x l P h o s p h o F l o w



Repeated Tumor Cell Killing



# In Vitro Pharmacology Solution Provider

## Molecular Assays

- Binding assays
- Competition binding assays
- Epitope binning assays
- Dual-binding assays
- Dimerization assay
- Receptor occupancy assays

## Biochemical Assays

- Epitope Enzymatic assays
- Phosphorylation assays

## Metabolic Assays

- LDL uptake assay
- cAMP assay
- CRE-luc RGA assay
- Lipid content determination

## Cellular Assays

- Proliferation assays
- Internalization assays
- Apoptosis assays
- Cell cycle analysis
- Cell migration assay
- Agglutination assay
- Polarization and differentiation
- 3D cell culture

## ADC Assays

- Potency: internalization, cytotoxicity
- Serum/plasma stability: DAR, free drug

## Infectious Disease Platform

- Pseudovirus neutralization assay
- Antibody-dependent enhancement

## Immune Assays

- Reporter gene assays
- Mixed lymphocyte reaction
- T cell activation assay
- Ag-specific T cell proliferation
- Regulatory T cell expansion
- T cell killing assay
- T cell exhaustion
- NK cell proliferation / degranulation / cytotoxicity assays
- APC activation assay
- Treg suppression assay
- Cytokine release assays

## Fc Function Assays

- ADCC assay
- CDC assay
- Phagocytosis assay



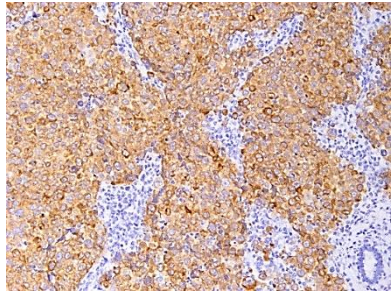
# Extensive CDX Model Collection at WuXi Biologics

NO.	Origin	Model	NO.	Origin	Model	NO.	Origin	Model	NO.	Origin	Model
1	Bladder	HT-1376	19	Colon	LoVo	37	Lung	NCI-H82	55	Liver	HepG2
2	Bladder	5637	20	Colon	T84	38	Lung	Calu-6	56	Liver	Huh7
3	Breast	MDA-MB-231	21	Colon	NCI-H716	39	Lung	NCI-H69	57	Connective tissue	HT1080
4	Breast	MCF-7	22	Leukemia	Daudi	40	Lung	NCI-H446	58	Neuroblast	SK-N-FI
5	Breast	JIMT-1	23	Leukemia	RPMI 8226	41	Lung	NCI-H520	59	Skin	A375
6	Breast	HCC1954	24	Leukemia	Ramos	42	Lung	NCI-H1650	60	Skin	A431
7	Breast	HCC70	25	Leukemia	WSU-DLCL2	43	Lung	NCI-H524	61	Skin	A2058
8	Breast	MDA-MB-468	26	Leukemia	CCRF-CEM	44	Lung	NCI-H211	62	Skin	SK-MEL-28
9	Breast	SK-Br-3	27	Leukemia	Raji	45	Lung	A549	63	Stomach	NCI-N87
10	Breast	MX-1	28	Leukemia	MOLM-13	46	Lung	NCI-H358	64	Stomach	NUGC4
11	Breast	BT474	29	Leukemia	Karpas 299	47	Ovary	OVCAR3	65	Stomach	SNU-16
12	Bone marrow	OPM-2	30	Leukemia	Nalm-6	48	Ovary	SK-OV-3	66	Stomach	MKN-45
13	Bone marrow	MOLP-8	31	Leukemia	THP-1	49	Ovary	PA-1	67	Pharynx	Fadu
14	Bone marrow	NCI-H929	32	Bone	Saos-2	50	Pancreas	BxPC3	68	Prostate	22Rv1
15	Colon	LS1034	33	Lung	NCI-H1975	51	Pancreas	HPAF-II	69	Prostate	Lncap
16	Colon	HCT116	34	Lung	NCI-H2110	52	Pancreas	AsPC-1	70	Prostate	PC-3
17	Colon	HT29	35	Lung	HCC827	53	Pancreas	HPAC	71	Prostate	C4-2
18	Colon	LS174T	36	Lung	SHP77	54	Pancreas	PANC-1	72	Brain	U-251MG

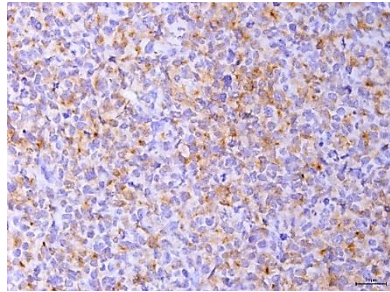
Capability to build engineered cell-lines and establish new models on demand

# Comprehensive IHC and IF Capabilities

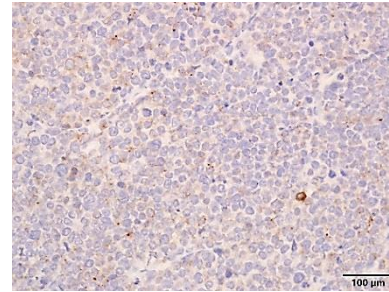
## Tumor Antigen IHC for Model Characterization & Selection



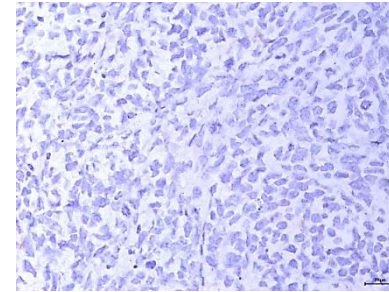
SHP-77



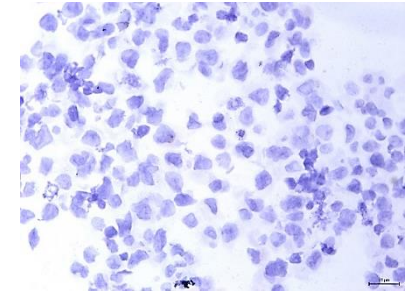
H69



H221

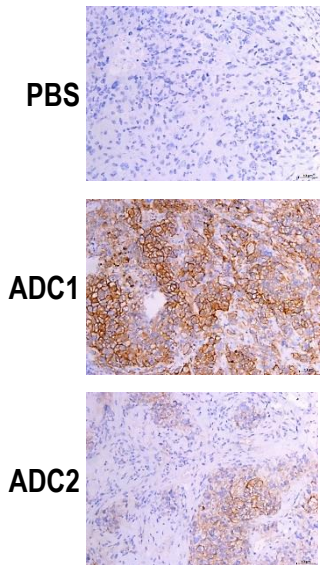


H446

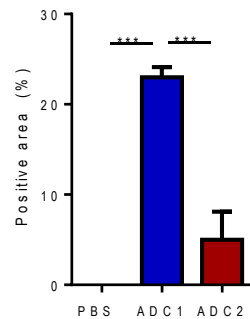


HEK293 Pellet

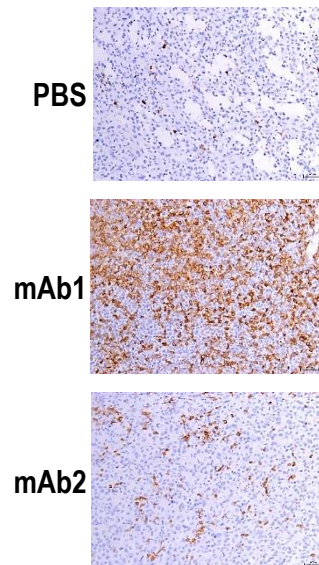
## Human Fc IHC for Exposure



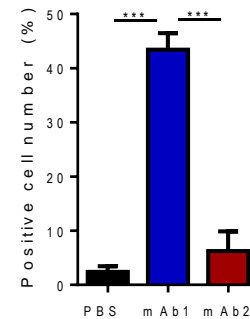
Relative quantification



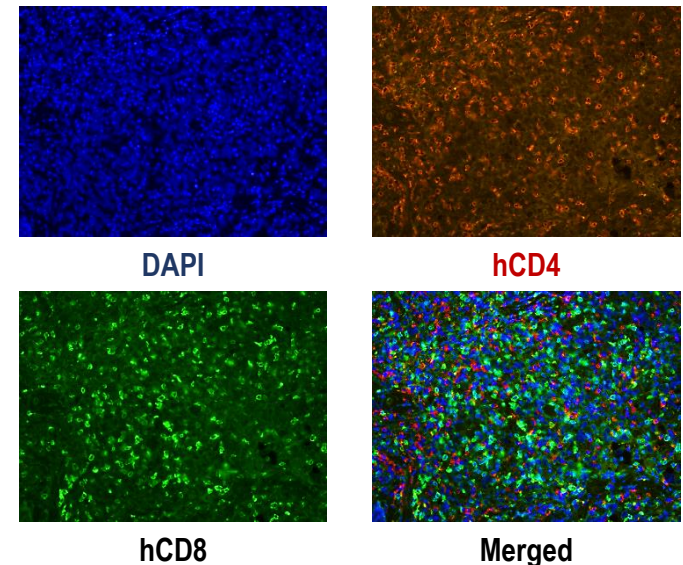
## Human CD4 IHC for Infiltration



Relative quantification



## Multiplex IF for Immuno-Profiling



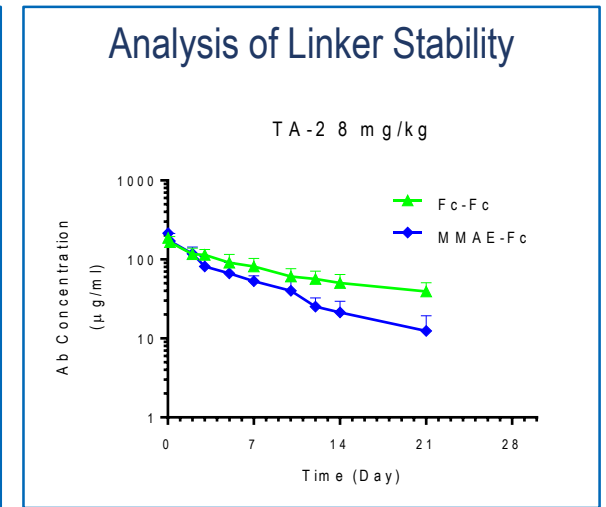
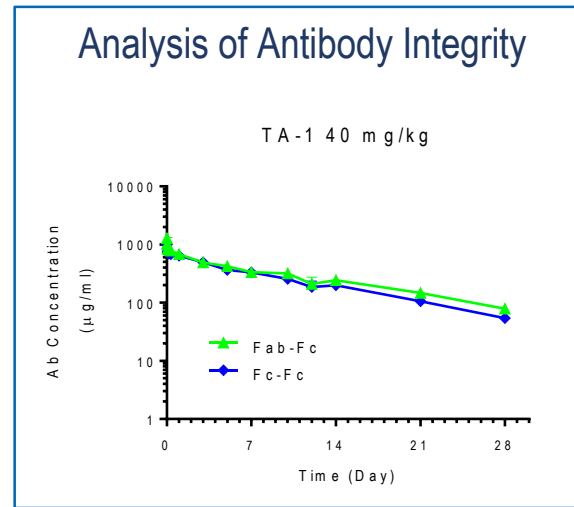
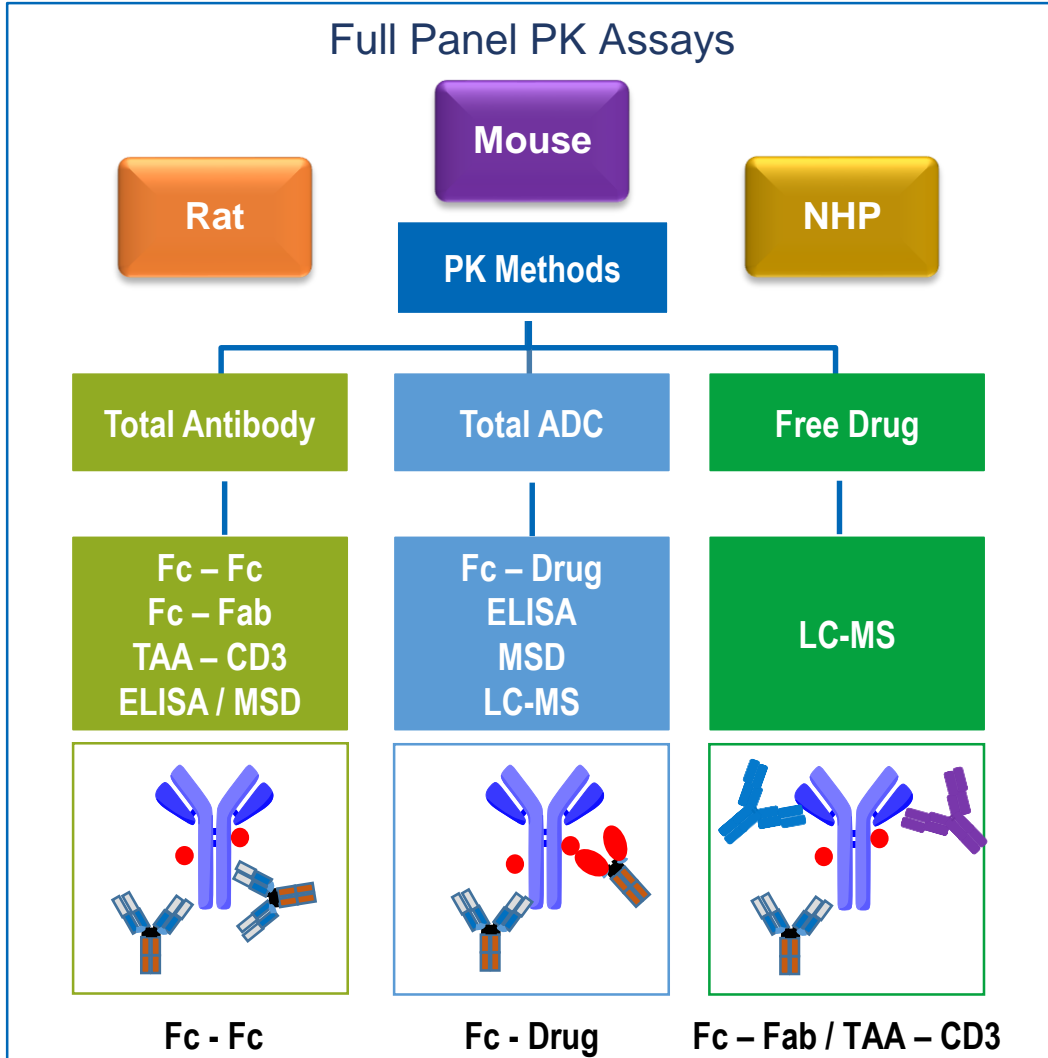
DAPI

hCD4

hCD8

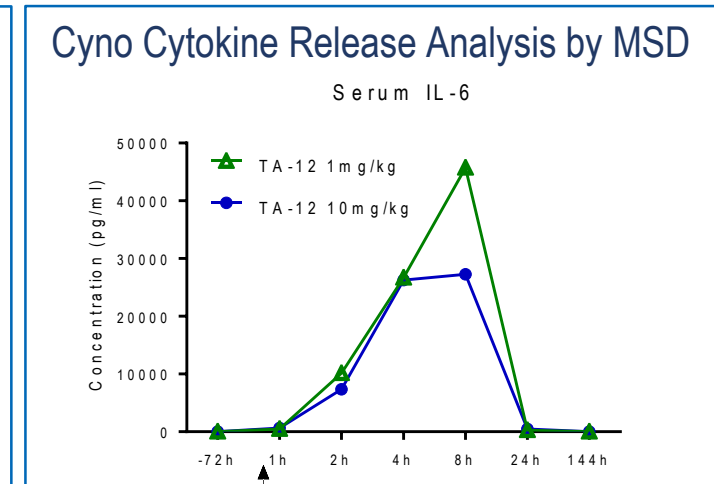
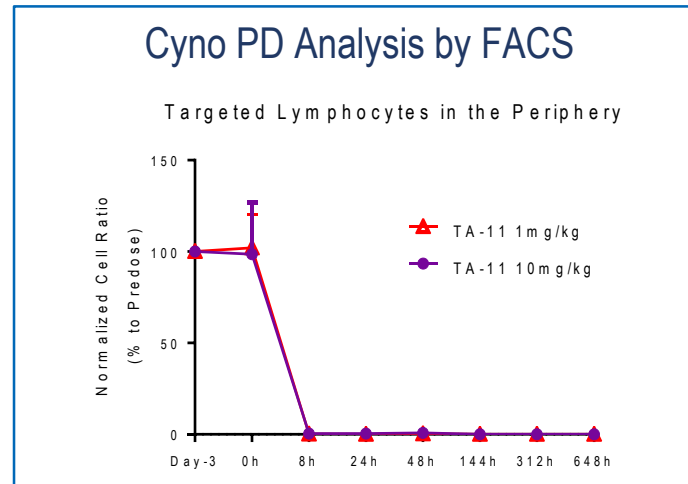
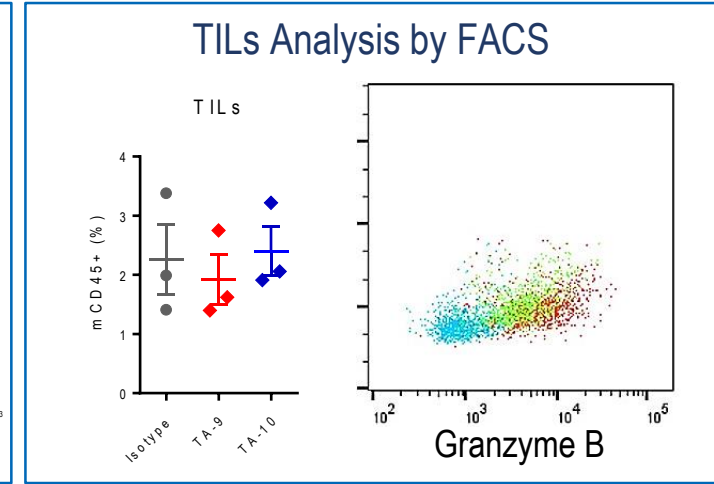
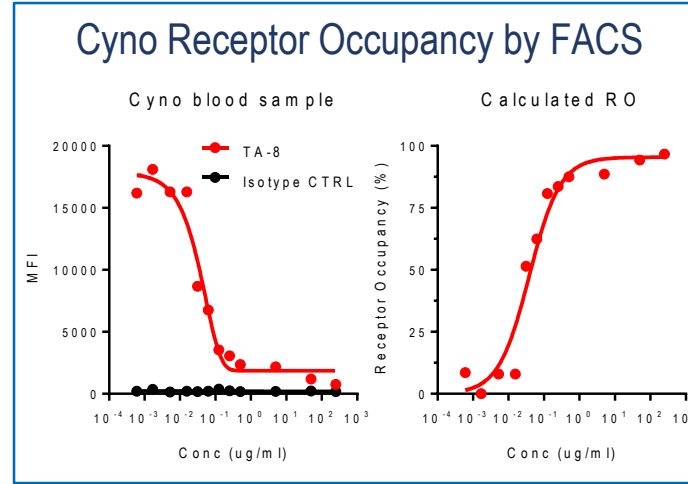
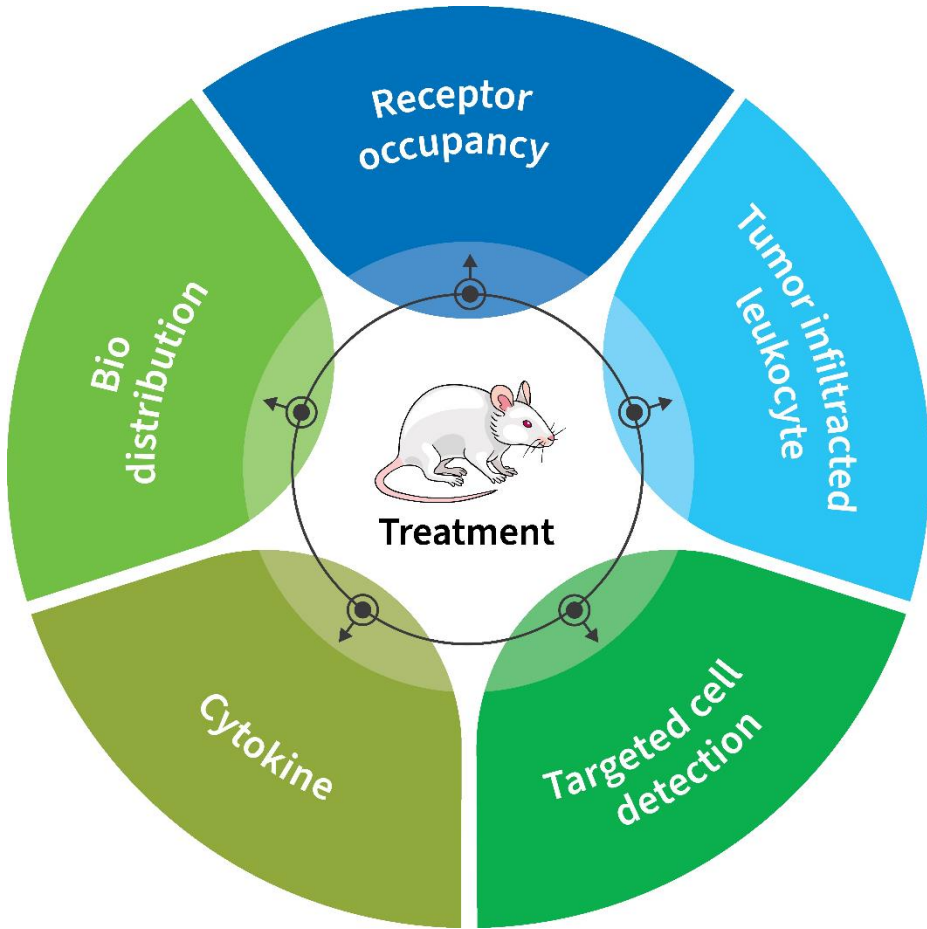
Merged

# Fit-For-Purpose PK Assays



NO.	Class	Payload	NO.	Class	Payload
1	Auristatin	MMAE	6	Benzodiazepine	PBD
2	Auristatin	MMAF	7	Maytansine	DM1
3	Camptothecin	Dxd	8	Maytansine	DM4
4	Camptothecin	Extecan	9	Duocarmycin	Duocarmycin
5	Camptothecin	SN-38	10	Doxorubicin	Doxorubicin

# Comprehensive PD Evaluation



Beckman Coulter CytoFLEX LX with 5 Lasers, 19 Channels, and Plate Reader

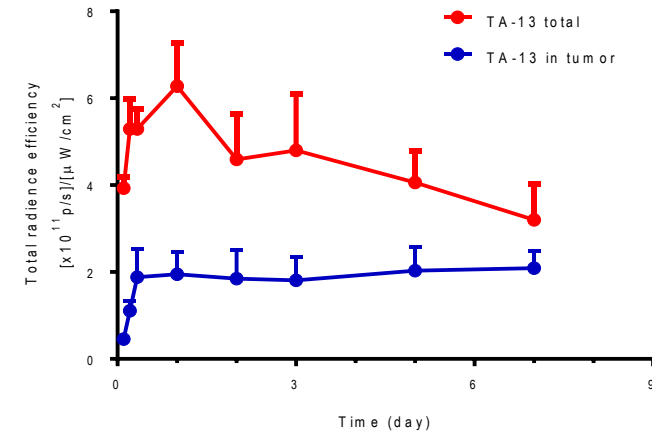
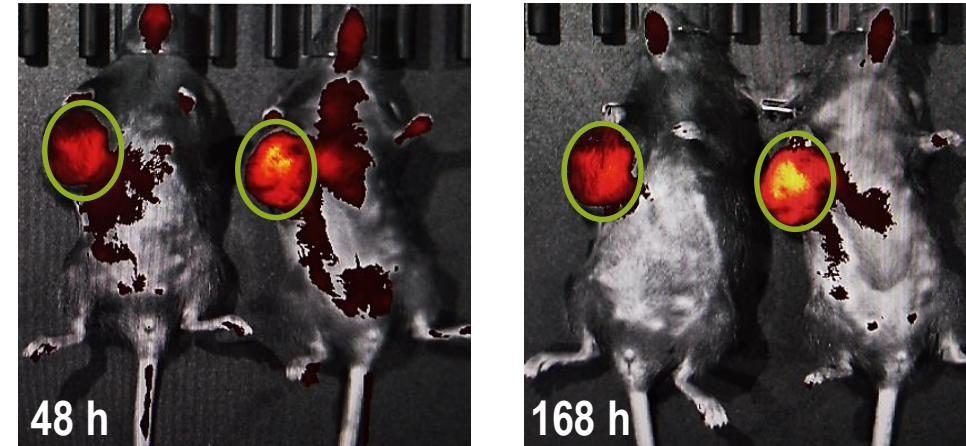
# Applications with IVIS Imaging System

## IVIS Spectrum In Vivo Imaging System



- High-sensitivity in vivo imaging of fluorescence and bioluminescence
- Up to 5 mice at the same time with a 23 cm field of view
- High resolution (up to 20 microns) with a 3.9 cm field of view
- Twenty eight high efficiency filters spanning 430-850 nm
- Supports spectral unmixing applications
- Ideal for multiple bioluminescent and fluorescent applications

## ADC Tissue Distribution by IVIS



# In Vivo Pharmacology Solution Provider

## Efficacy

- State-of-the-Art Experimental Animal Center
  - Variety of immunocompetent or immunodeficient mice
  - Selection of transgenic mice
- Extensive Collection of Models
  - Syngeneic models
  - CDX models
  - PBMC / ATT models
  - TAA humanized cell-lines
  - IVIS enabling cell lines
- Autoimmune Disease Models
- Metabolic Disease Models
- IVIS Spectrum Imaging Platform

## PK / PD

- Species
  - Rat, mouse, NHP, human, etc.
- FACS platform
- MSD platform
- ELISA platform
- PK methods for mAb, multi specific Ab, ADC detection
- ADA detection
- Cytokine measurement
- TILs analysis
- Receptor occupancy

## Histology

- Section
  - Paraffin, frozen, cell smear
- Staining
  - HE, IHC, IF, EHC, TCR
- Specific Staining
  - Masson, Luxol Fast blue, etc
- Scanner
  - Leica Automated Scanner
- Analysis
  - Whole slide computerized analysis



# WuXi Biologics Vision

“Every drug can be made and every disease can be treated” by building an open-access platform with the most comprehensive capabilities and technologies in the global biologics industry.

Learn More



Contact Us

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Website: [wuxibiologics.com/discovery](http://wuxibiologics.com/discovery)

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