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Screening Novel Format Antibodies to Design Bispecific ADCs that Address Target Heterogeneity

PEGS Boston 2024
Engineering Bispecific Antibodies
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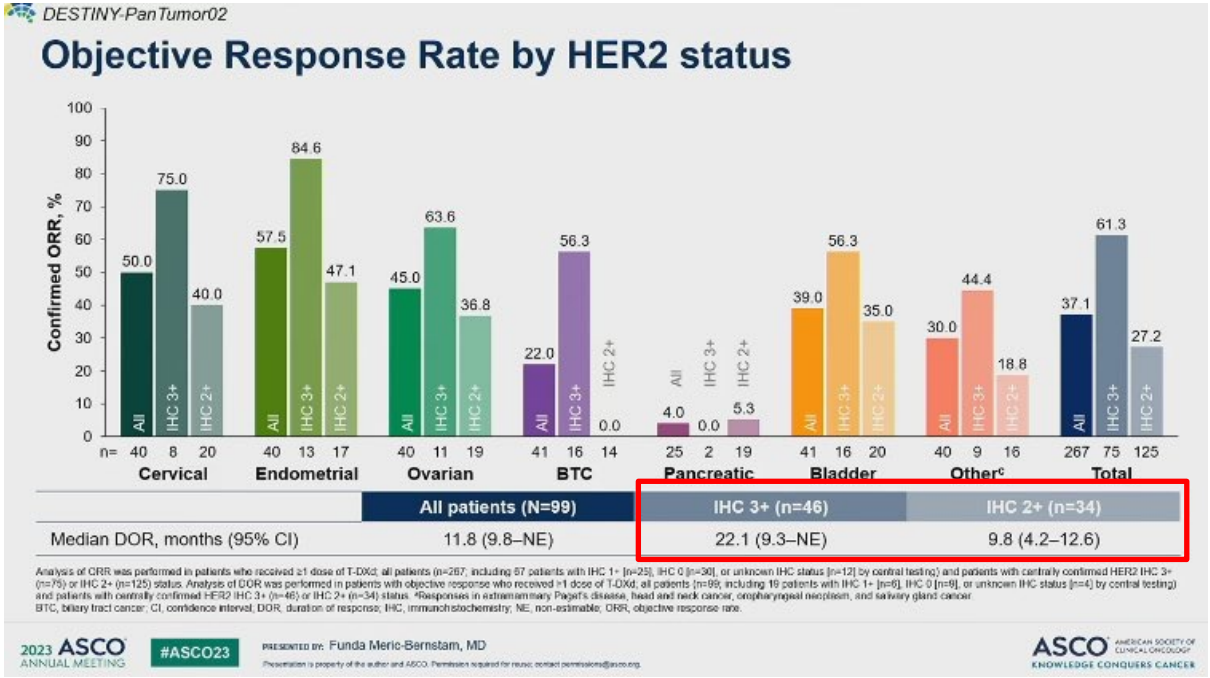
ADC Therapeutic Development

ADCs Hold Promise But Target Expression Dependence Limiting

ADCs are an exciting therapeutic modality that are changing the therapeutic landscape for many patients but even the best ADCs are still dependent on target expression for maximal benefit



Bispecific ADCs (BsADCs) at AACR 2024

Company	Asset ID	Target pair	Payload	Format	Additional tech.	Stage	Notes
Innovent	IBI3001	EGFR x B7H3	Topo	1+1	Fc silent Synaffix SS	Preclinical	• **NHP tolerability**: 90 mg/kg HNSTD (skin, GI)
Profound	PRO1286	EGFR x MET	Topo	1+1	-	Preclinical	• Looking to DAR optimize and take to clinic • **NHP tolerability**: >30 mg/kg (bone marrow)
LigaChem	LCB36	CD20 x CD22	Masked PBD	1+1	ConjuAll SS	Preclinical	• **NHP tolerability**: 0.5 mg/kg HNSTD (hematological)
VelaVigo	VBC103	Nectin4 x TROP2	Topo	2+1	-	Preclinical	• **NHP tolerability**: 36 mg/kg HNSTD (skin)
	VBC101	EGFR x MET	MMAE or Topo	2 (bip.) +1	-	Discovery	• Biparatopic MET
Hangzhou	DXC024	EGFR x TROP2	Tubulysin	1+1 (hybrid)	-	Discovery	
	DXC025	EGFR x MUC1	Tubulysin	1+1 (hybrid)	-	Discovery	
BiOneCure	BIO-201	HER2 x TROP2	Topo	2+2 (Fab/ScFv)	-	Discovery	• N+N term format, HER2 binding domains are scFvs
Celon	CPBT0976-MMAE	Axl x PD-L1	MMAE	2+2 (VHH)	-	Discovery	
Biotheus	PM1300	EGFR x HER3	Topo	1+1	-	Discovery	• Lack of monovalent binding
Biocytogen	DM002 (partner: Doma)	HER3 x MUC1	Topo	1+1	Common LC	Preclinical	• GLP **NHP** study ongoing • IND target EOY 2024
	BCG016	5T4 x MUC1	MMAE	1+1	Common LC	Discovery	
	BCG017	EGFR x PTK7	MMAE	1+1	Common LC	Discovery	
	BCG019	EGFR x HER3	Topo	1+1	Common LC	Discovery	
	BCG022	HER3 x MET	Topo	1+1	Common LC	Discovery	
	BCG023	FRa x MUC1	MMAE	1+1	Common LC	Discovery	
	BCG033	PTK7 x TROP2	Topo	1+1	Common LC	Discovery	• Reduced affinity TROP2 paratope

Two clinical BsADCs not discussed at AACR 2024: AstraZeneca (EGFR x cMET, 1+1) and Systimmune (EGFR x HER3, 2+2)

Target Heterogeneity is a Major Challenge for Targeted Therapeutics

- Present in patient population and in tumor mass
  - Targeting two antigens independently may provide greater coverage across an indication and within a tumor mass or lesions

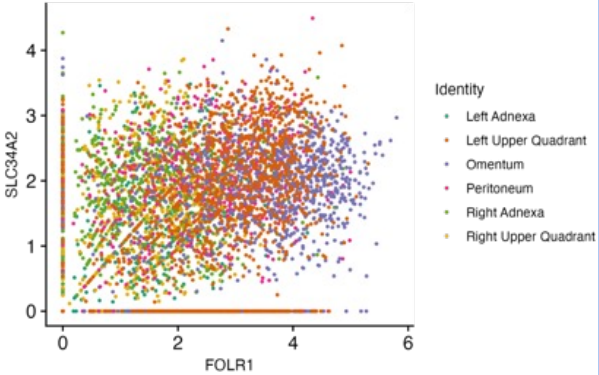
A hypothetical distribution of patients that express target A, target B, both targets, or neither target

Expression of FRα and NaPi2b in 101 HGS ovarian carcinoma samples

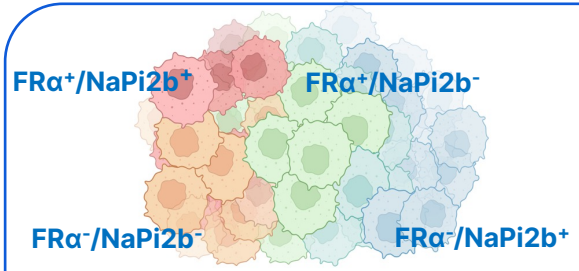
NaPi2b IHC Score	FRα 0	FRα 1+	FRα 2+	FRα 3+
3+	0	5	8	20
2+	0	6	12	12
1+	0	11	7	12
0	2	2	2	5

Immunohistochemistry score of FRα and NaPi2b in 101 high grade serous ovarian cancer (HGSOC) patient samples

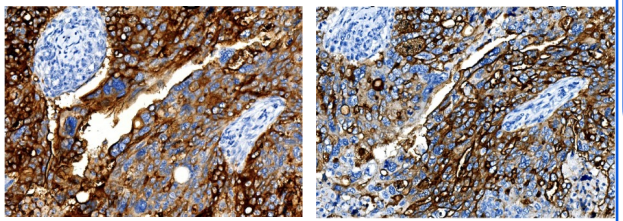
FOLR1 v NaPi2b expression within individual patient may vary dependent on tumor location



Single cell RNA analysis of treatment-naive high-grade serous ovarian cancer (HGSOC) patient tumor samples (Data extracted from Vazquez-Garcia et al 2022 Nature 612: 778)

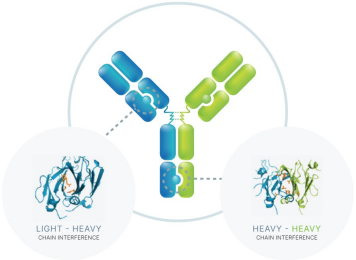


Cartoon of a tumor mass with cells expressing FRα, NaPi2b, both antigens, or neither antigen

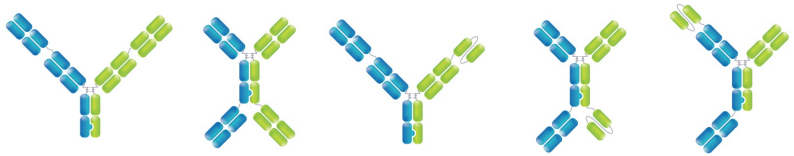


Immunohistochemistry staining of FRα and NaPi2b from the same patient sample and same region

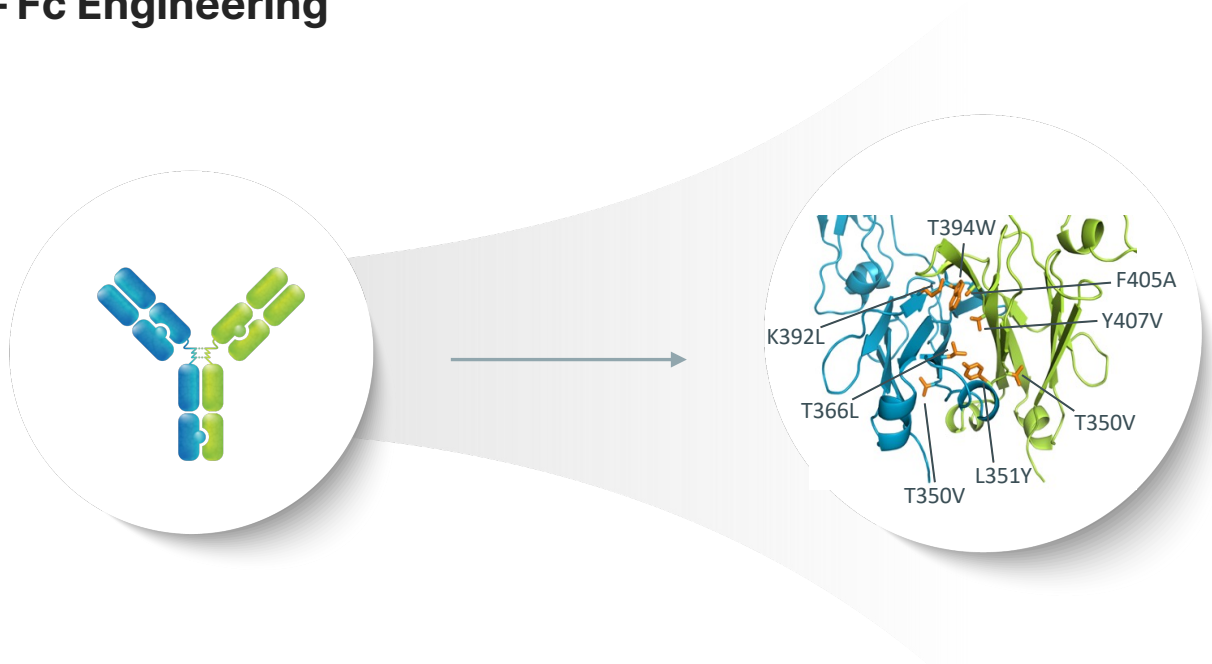
A Bispecific ADC May Overcome Target Heterogeneity- Azymetric™ Enables a Variety of Bispecific Formats



- Enables screening of antibodies with different valency and geometry
- Desirable drug-like features of IgG-based antibodies
- Compatible with standard manufacturing processes

1+1 Bispecific Format			*Enhanced specificity* *Reduced patient population*
2+1 Bispecific Format			*Enhanced ADC function* *Patient population is target1 + % overlap*
2+2 Bispecific Format			*Enhanced ADC function* *Enhanced patient population*

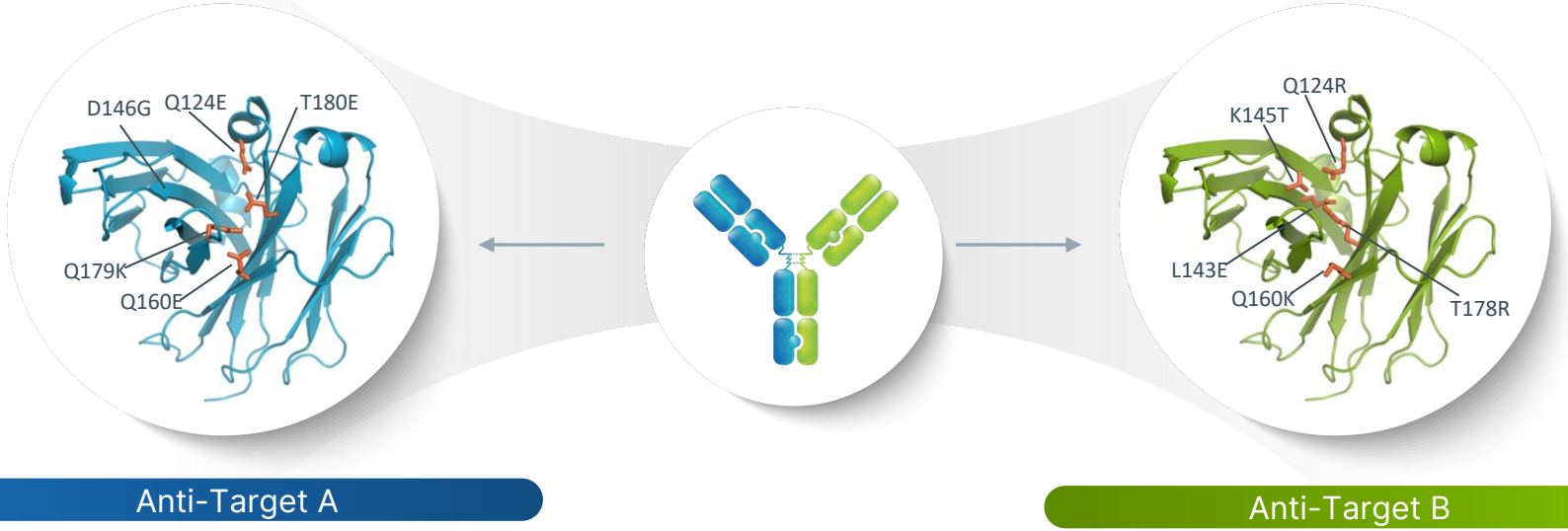
Azymetric™ – Fc Engineering



- Set of transferable mutations identified (4 per chain) that can successfully produce pure and stable Fc heterodimers with exclusive chain pairing during co-expression in mammalian cells
- Wild-type Fc properties; compatible with CH2 engineering (FcgR/FcRn) and glyco-engineering approaches
- Compatible with human (IgG1, IgG2a, IgG4) and mouse frameworks

Kreudenstein *et al. mAbs 5:5*, 646-654, 2013.
Kreudenstein *et al. Methods 65*, 77-94, 2014.

Azymetric™ – Fab Engineering

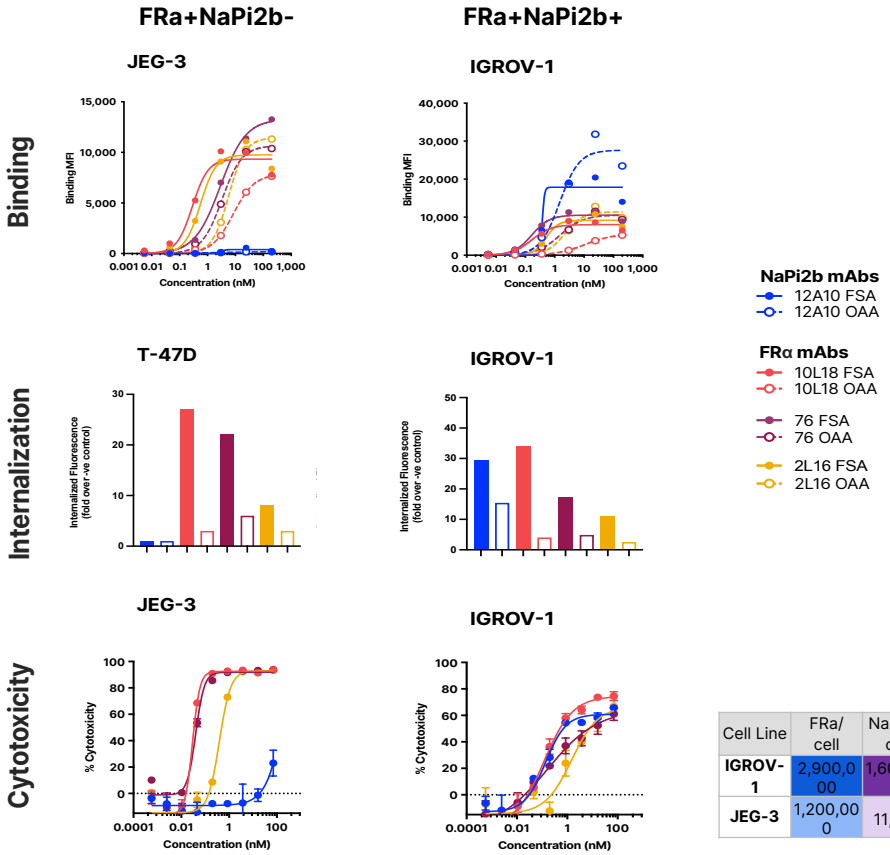


- Example of a set of constant domain Fab mutations that can selectively drive light chain pairing with its heavy chain partner upon co-expression
- This mutation set is representative of a small library of solutions
- Libraries available for both kappa/kappa & kappa/lambda bispecific LC combinations (currently top 2 lead solutions for each scenario are in use)

FRα x NaPi2b Bispecific ADC Library Screen Design

- Proof of concept system with tentative aim of targeting tumors that express either FRα, NaPi2b, or both targets (OVCA/NSCLC)
  - 48 bispecific ADCs produced, across
    - 3 different valencies (1+1, 2+1, 2+2)
    - 11 different formats (geometry and Fab/scFv components)
    - several paratopes
    - with ‘model’ payload (ZymeLink™ Auristatin)
  - Paratope diversity (affinity/avidity and epitope space) as well as the relative target expression (H/M/L) are factored into bispecific ADC designs
  - Evaluated for binding, internalization, and cytotoxicity (in cell lines representative of several expression scenarios)

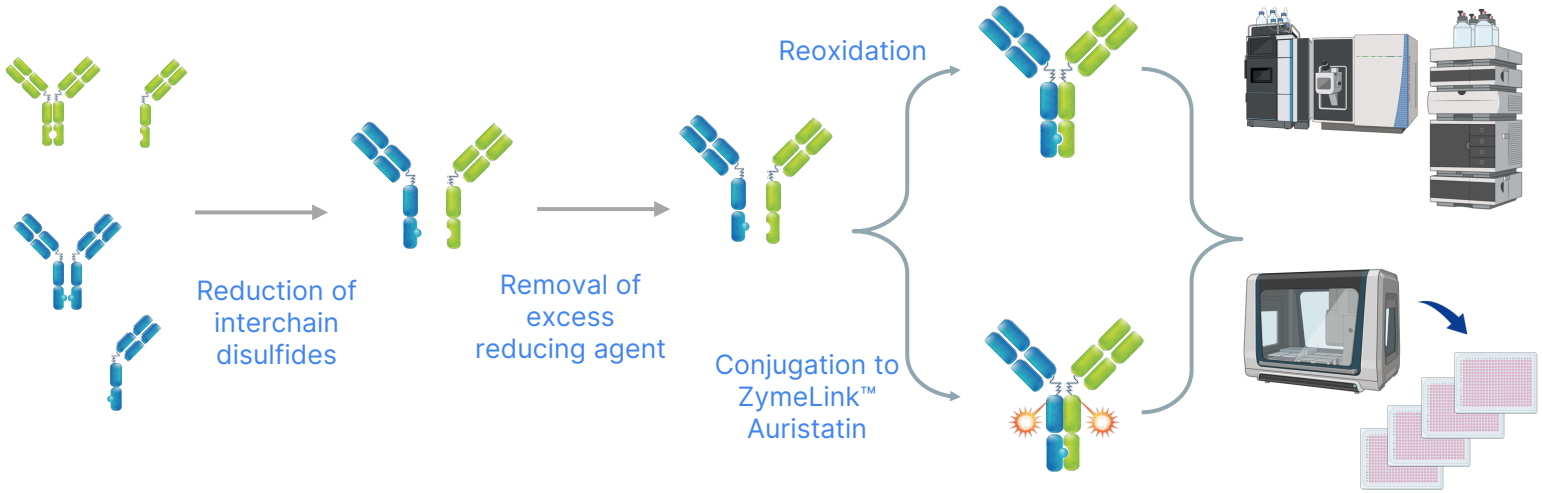
Diverse Anti-FRα and One Anti-NaPi2b Paratopes Were Explored in bsAb ADC



Cell Line	FRa/ cell	NaPi2b/ cell	FRa+ NaPi2b	FRa/NaPi2b
IGROV-1	2,900,000	1,600,000	**4,500,000**	++++/+++
JEG-3	1,200,000	11,000	**1,211,000**	+++/-

- Anti-FRα mAbs: 10L18, 76 and 2L16 bind to different epitopes
- Most of anti-FRα mAbs are avidity driven while anti-NaPi2b 12A10 is affinity driven mAb
- 10L18 is the most active anti-FRα paratope out of the three, followed closely by 76 and then 2L16

Bispecific Antibody and ADC Generation and Characterization Workflow

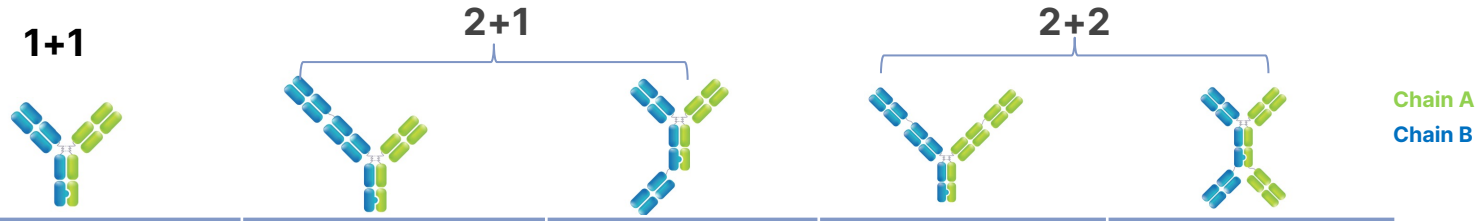


Half antibodies and homodimers are combined in equimolar amounts

Bispecific biophysical and functional high throughput screening

For exemplary purpose, schematic depicting 1+1 regular bispecific and ADC generation

48 Bispecific Antibodies Were Generated With High Purity



Chain A + Chain B paratopes	12A10+10L18/76/2L16	12A10+2x10L18/76/2L16, 10L18/76/2L16+2x12A10	12A10+2x10L18/76/2L16, 2x12A10+ 10L18/76/2L16	2x12A10+2x10L18/76/2L16	2x12A10+2x10L18/76/2L16
Monomer (%) (HPLC-SEC)	94-95	92-96	93-95	89-93	94-96
Reoxidized Bispecific (%) (Caliper)*	91-93	93-97	93-95, 4-7	89-97	2-4

Chain A + Chain B paratopes	12A10+76 scFv, 12A10+2L16 scFv	*10L18/76/2L16+2x12A10,2x10L18/76/2L16+ 12A10*	12A10+2x10L18/76/2L16, 2x12A10+ 10L18/76/2L16	2x12A10+2x10L18/76/ 2L16	2x12A10+2x10L18/76/ 2L16	2x10L18/76/2L16 (Fab+scFv)+ 2x12A10
Monomer (%) (HPLC-SEC)	91-94	86-95	84-95	91-96	85-94	89-95
Reoxidized Bispecific (%) (Caliper)*	47-51	2-51	2-33	1-17	0-4	13-15

*Expected mass detected for all the constructs, aside from the ones that contained a cloning error that prevented full formation of hinge disulfide bonds (in red), hinge disulfide bond formation upon re-oxidation was slower in scFv containing than in Fab containing bsAbs (in blue)

Fab Containing bsAb Species Were Successfully Formed

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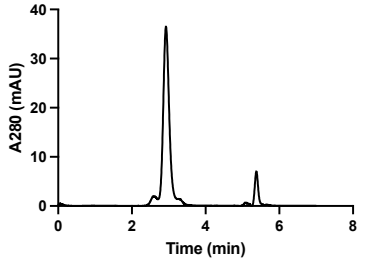
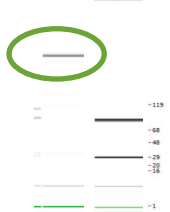
2+2 (10L18+ 12A10)

HPLC-SEC

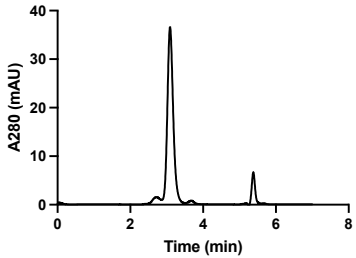


Caliper NR R

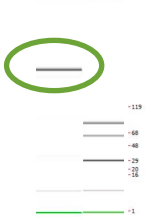


2(10L18)**+1** (12A10)

HPLC-SEC

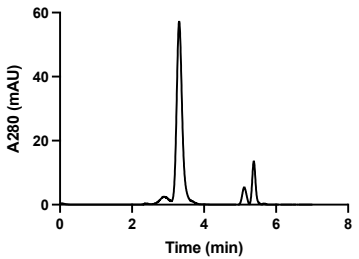


Caliper NR R

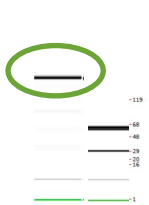


1+1 (10L18+12A10)

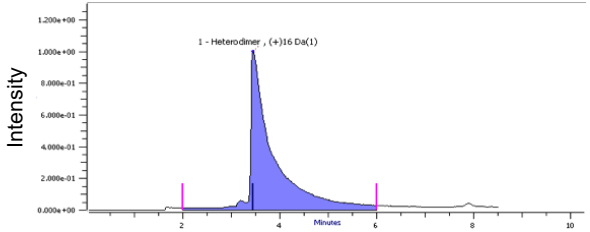
HPLC-SEC

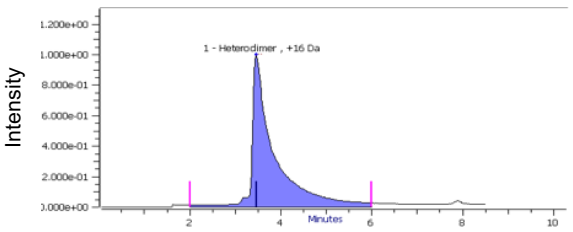


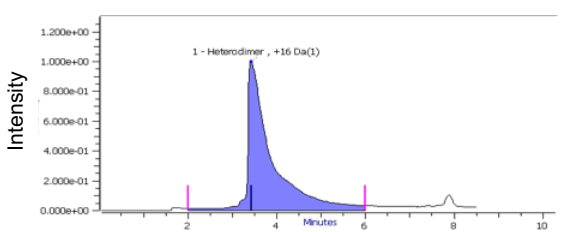
Caliper NR R



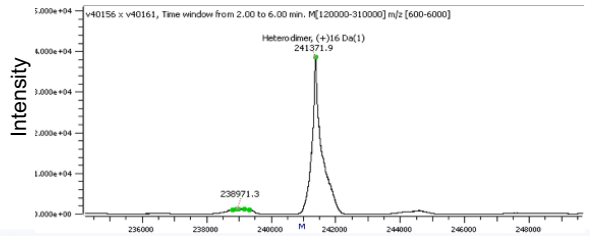
TIC

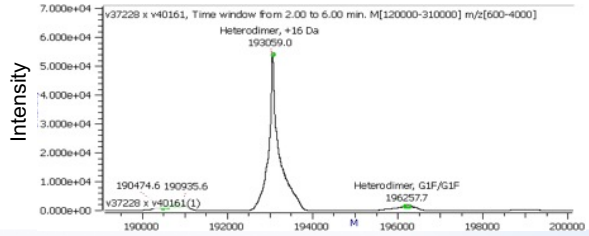


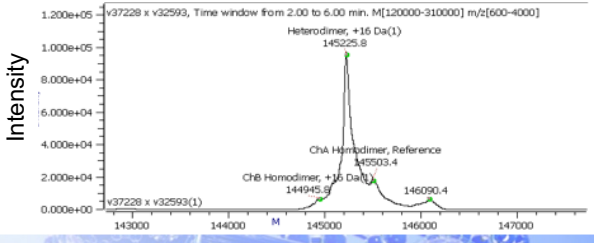




MS

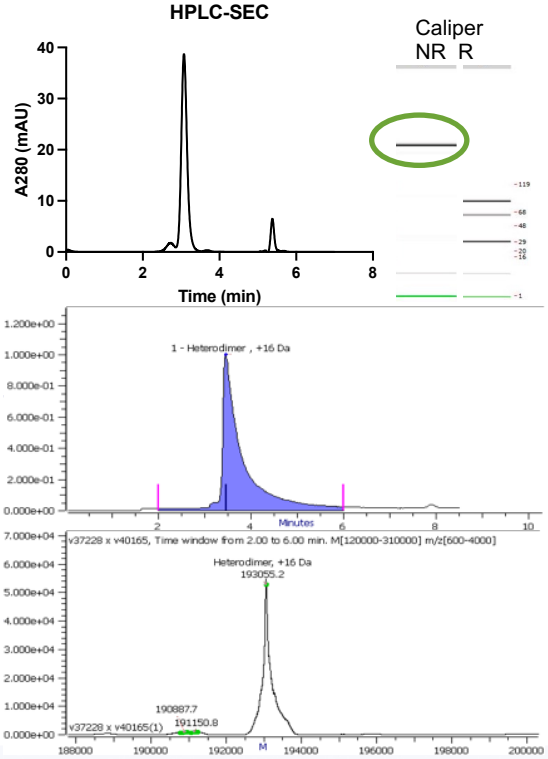




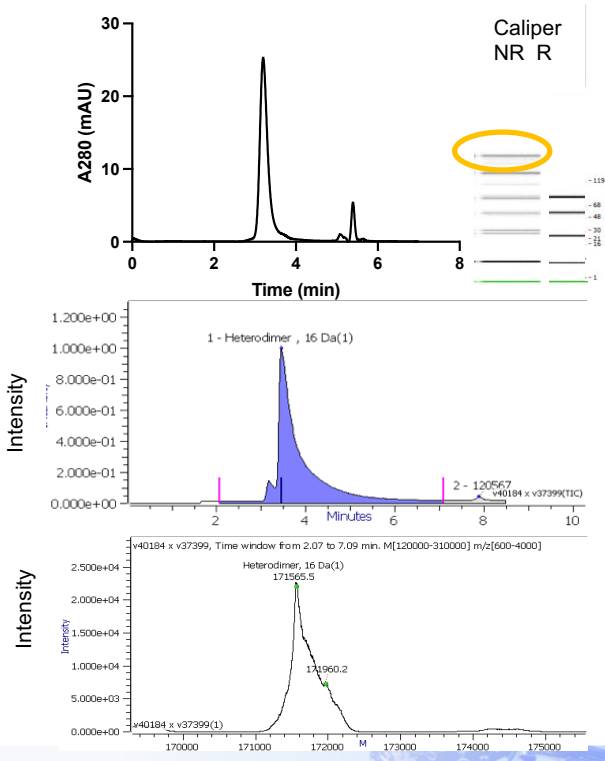


scFv Containing bsAb Species Were Formed (Full Re-oxidation is a Slower Process)

2(10L18)+**1** (12A10)

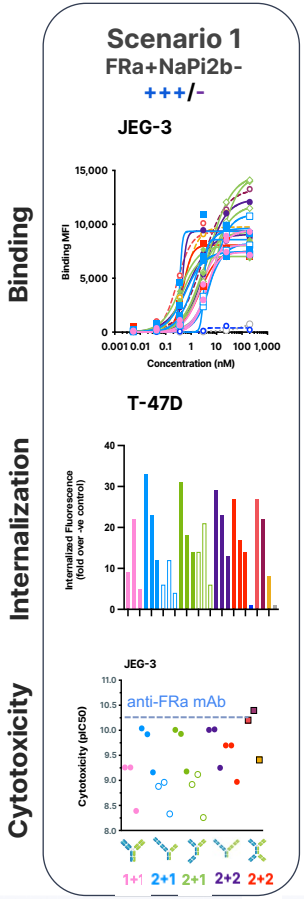


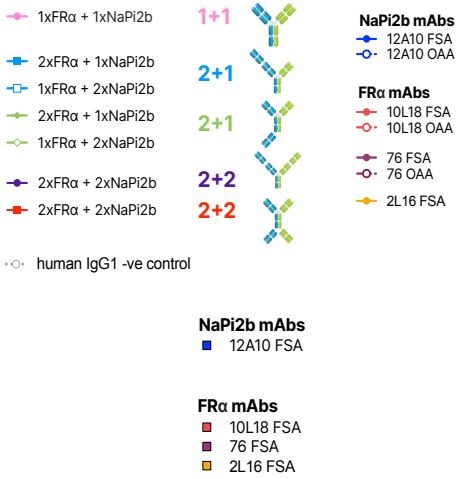
2(10L18-Fab +scFv)+**1** (12A10)



*LC/MS analysis performed at a later time point compared to Caliper analysis

2+1 and 2+2 bsAb Formats Were More Active in a Broader Range of Cell Lines Than 1+1

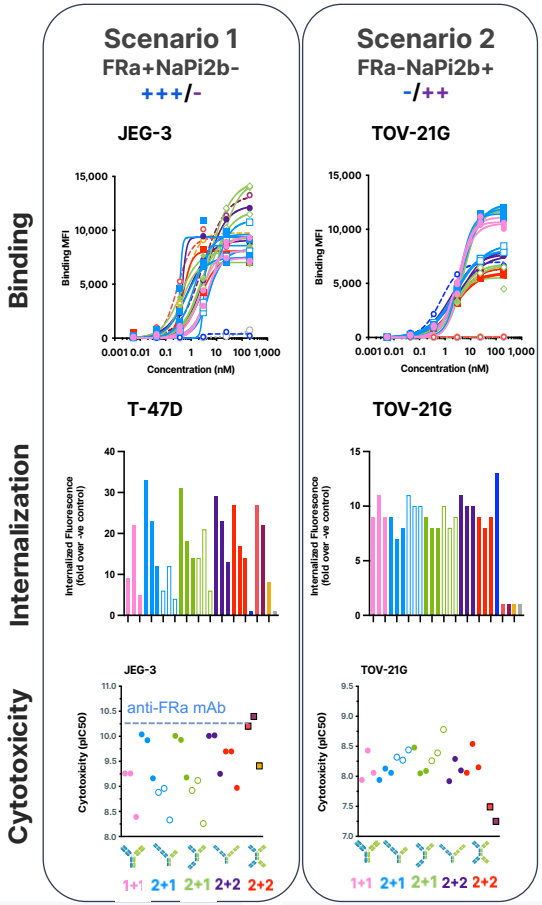


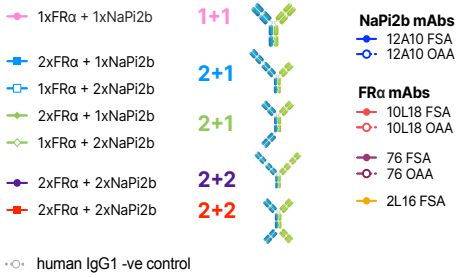


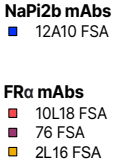
Cell Line	FRa/ cell	NaPi2b/ cell	FRa+ NaPi2b	FRa/NaPi2 b
JEG-3	1,200,00 0	11,000	**1,211,000**	**+++/-**

- Scenario 1
  - Activity of 2+1 and 2+2 bsAb >1+1
- Some differentiation between 2+2 (‘N-term’) and 2+2 (‘N+C-term’) bsAb formats

2+1 and 2+2 bsAb Formats Were More Active in a Broader Range of Cell Lines Than 1+1



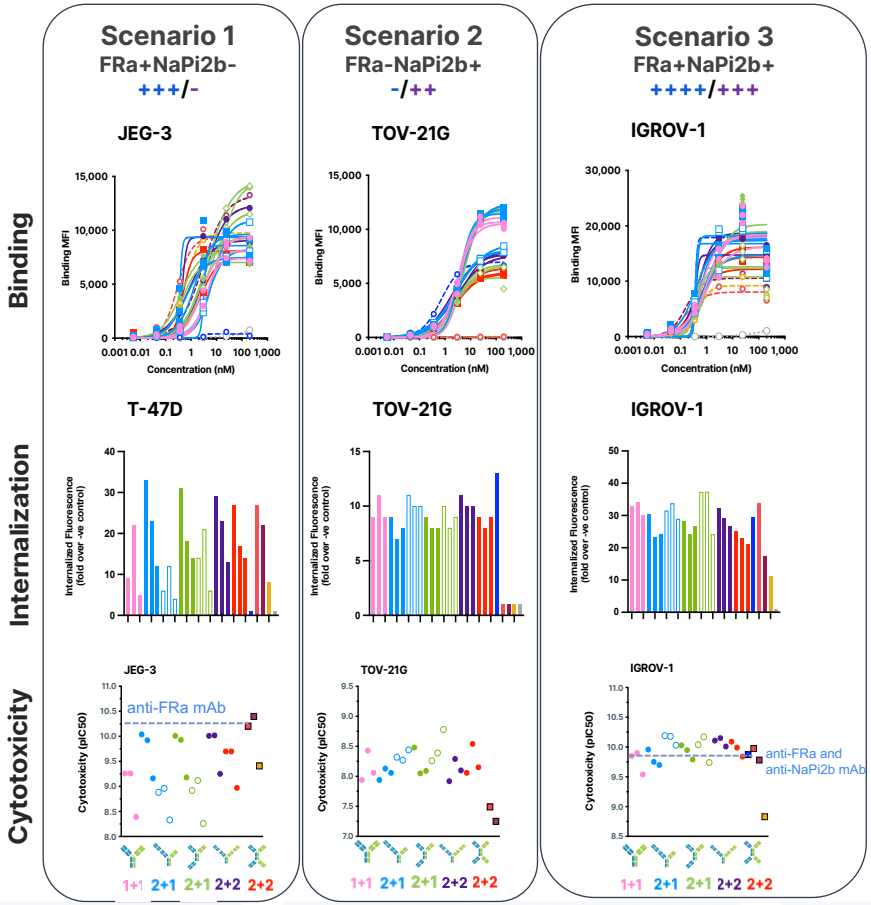


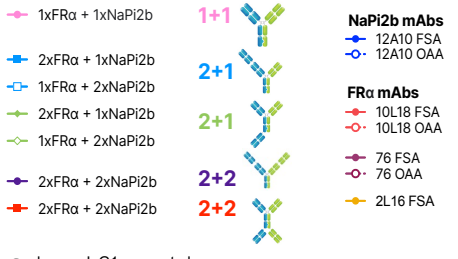


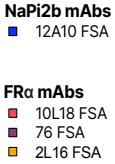
Cell Line	FRa/ cell	NaPi2b/ cell	FRa+ NaPi2b	FRa/NaPi2 b
JEG-3	1,200,00 0	11,000	1,211,000	+++/-
TOV21G	6,000	350,000	356,000	-/++

- Scenario 1 and potentially Scenario 2:
  - Activity of 2+1 and 2+2 bsAb >1+1
- Some differentiation between 2+2 ('N-term') and 2+2 ('N+C-term') bsAb formats

2+1 and 2+2 bsAb Formats Were More Active in a Broader Range of Cell Lines Than 1+1



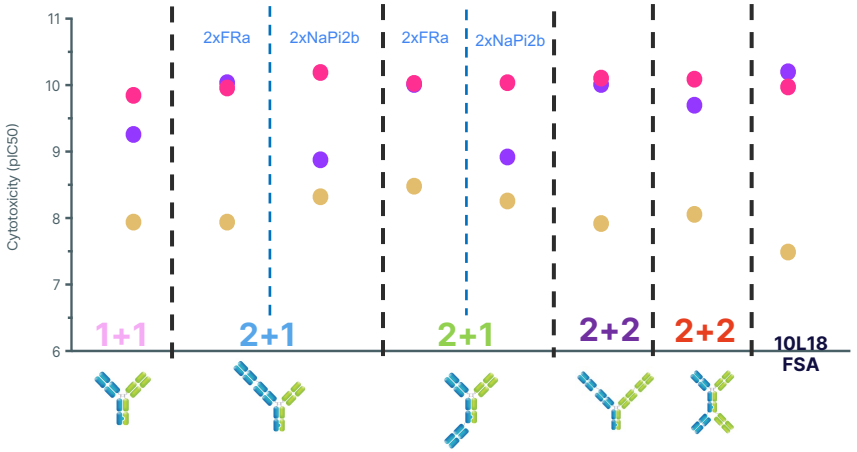




Cell Line	FRa/ cell	NaPi2b/ cell	FRa+ NaPi2b	FRa/NaPi2 b
IGROV-1	2,900,000	1,600,000	4,500,000	++++/+++
JEG-3	1,200,000	11,000	1,211,000	+++/-
TOV21G	6,000	350,000	356,000	-/++

- Scenario 1 and potentially Scenario 2:
  - Activity of 2+1 and 2+2 bsAb >1+1
- Scenario 3:
  - Activity of 2+1 and 2+2 bsAb > or ~1+1
- Some differentiation between 2+2 ('N-term') and 2+2 ('N+C-term') bsAb formats

Scenario Expression Specifics May Determine Which 2+1 bsAb Format Provides Activity Advantage and its Extent Over 1+1 bsAb



IGROV-1 Scenario 3 F+ N+ ++++/+++

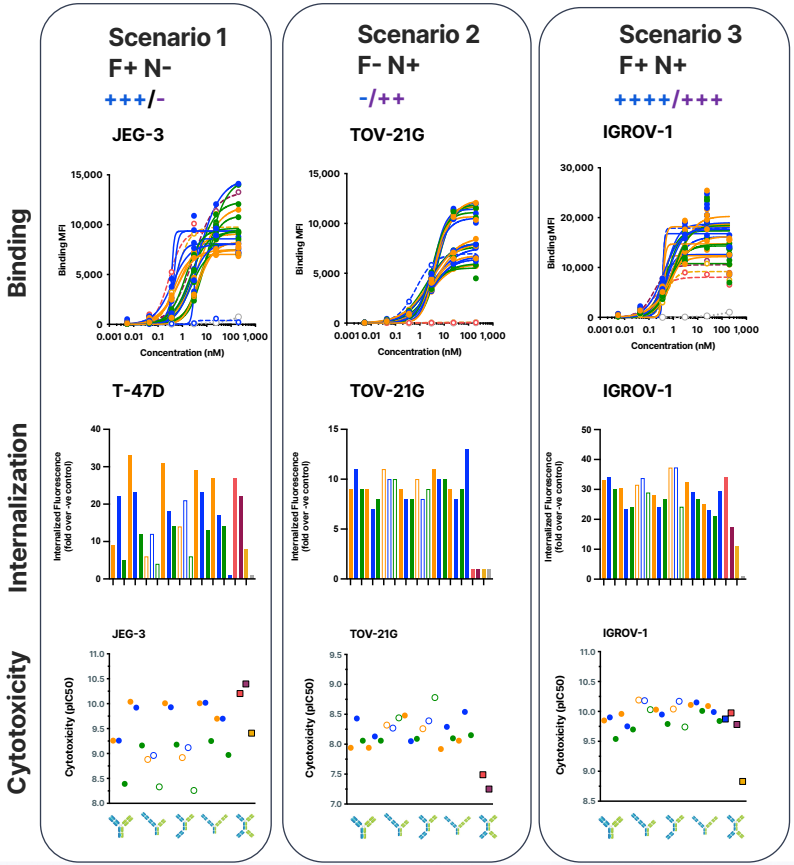
JEG-3 Scenario 1 F+ N- +++/-

TOV-21G Scenario 2 F- N+ -/++

- 2+1 bsAb of type 2xFRa but not 1xFRa provides improved activity over 1+1 in Scenario 1
- 2+1 bsAb of type 2xNaPi2b in some cases can provide improved activity (compared to 1xNaPi2b) over 1+1 in Scenario 2
- In heterogenous tumor scenario 2+2 bsAb would be expected to provide the best activity benefit

*10L18 Fab-only containing bsAbs example

10L18 and 76 Containing bsAbs Were Mostly Superior to Formats Containing 2L16 FRα Paratopes



10L18 containing bsAbs
76 containing bsAbs
2L16 containing bsAbs

NaPi2b mAbs
- 12A10 FSA
- 12A10 OAA

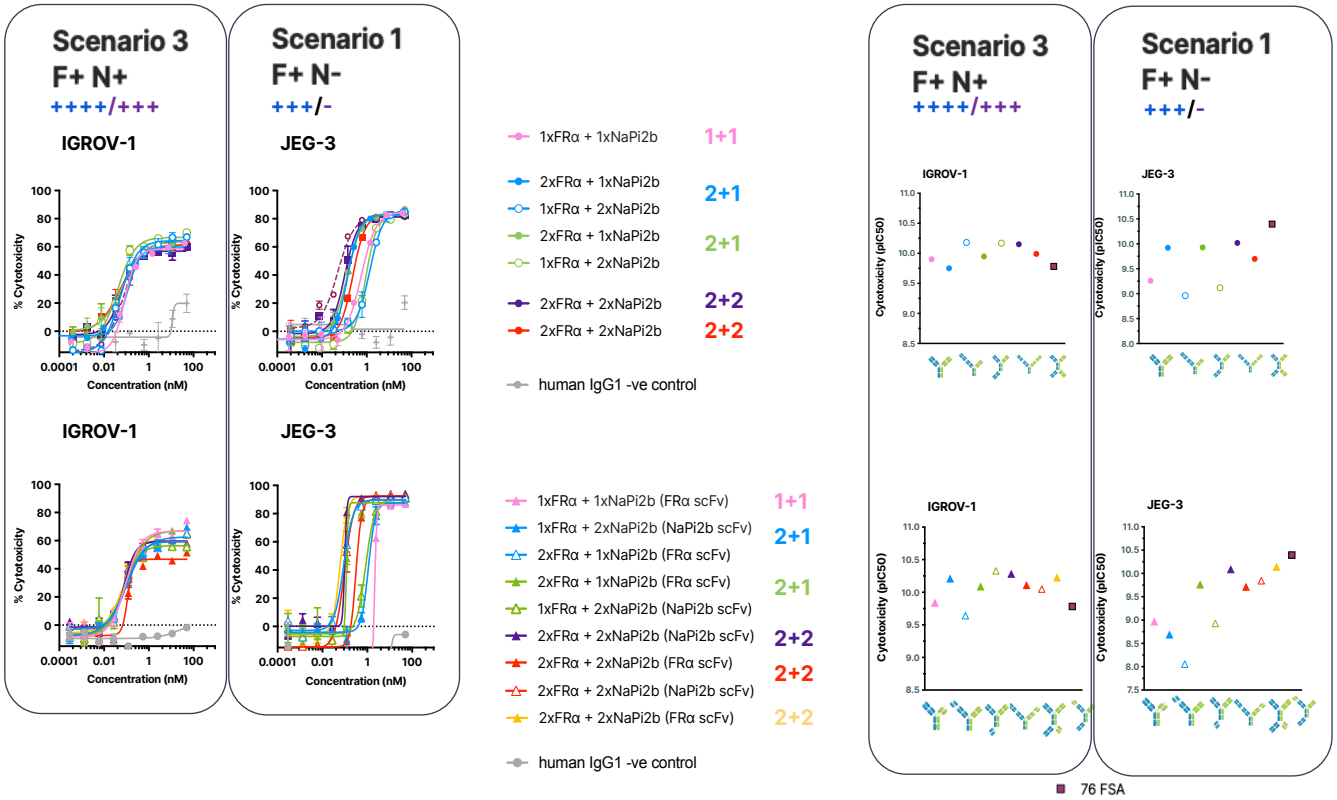
FRα mAbs
- 10L18 FSA
- 10L18 OAA
- 76 FSA
- 76 OAA
- 2L16 FSA
- 2L16 OAA

NaPi2b mAbs
- 12A10 FSA

FRα mAbs
- 10L18 FSA
- 76 FSA
- 2L16 FSA

- In general, paratope functional trends observed in regular mAb format hold in various bispecific formats as well
  - 10L18~76> 2L16
- These trends were more pronounced in Scenario 1

Similar Functional Trends Were Observed for Similar Formats Across Fab-only and scFv-containing Bispecific Antibodies



- Additional 2+2 scFv-containing bsAb format (compared to Fab-only bsAbs) was explored
  - 2+2 activity ~ 2+2/2+2

*76 containing bsAb example

Conclusions

- 48 bispecific antibodies and ADCs were generated using an Azymetric™ workflow employing 4 different paratopes and 11 formats
- 2+2 and 2+1 bispecific formats were more active in a broader range of cell lines compared to 1+1 bispecific formats
- 2+2 ‘N-term’ Fab and 2+2 ‘N+C-term’ containing bispecific formats show some distinctive activity
- Bispecific formats containing the 10L18 FRα and 76 FRα paratope were mostly superior in activity compared to formats containing 2L16 FRα paratope
- Similar functional trends were observed for similar formats across Fab-only and scFv-containing bispecific antibodies

Next Steps

- 10 bispecific antibody ADCs were selected for production as ‘4-chain’ Abs and further evaluation
  - PK assessment
  - In vivo study efficacy

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