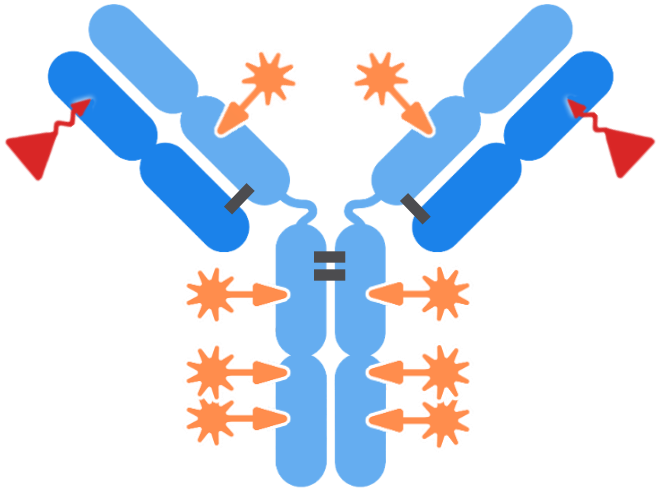


Developing Next-Generation Immunostimulatory Dual-Payload ADCs

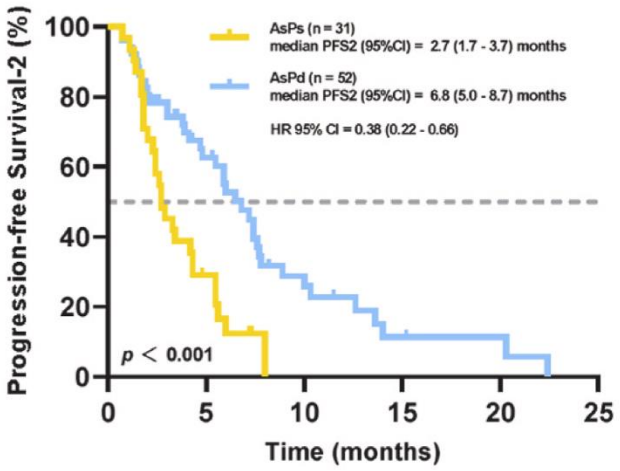
Gang Yin, Ph.D.

SUTRO BIOPHARMA

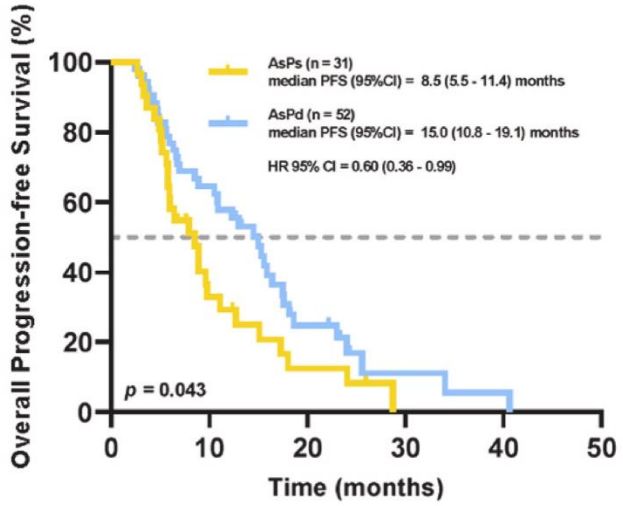
16th ANNUAL WORLD adc SAN DIEGO 2025

Optimal Sequential Strategies (ADC1→ADC2) for ADCs Highlight the Potential of Payload Diversity to Overcome Resistance

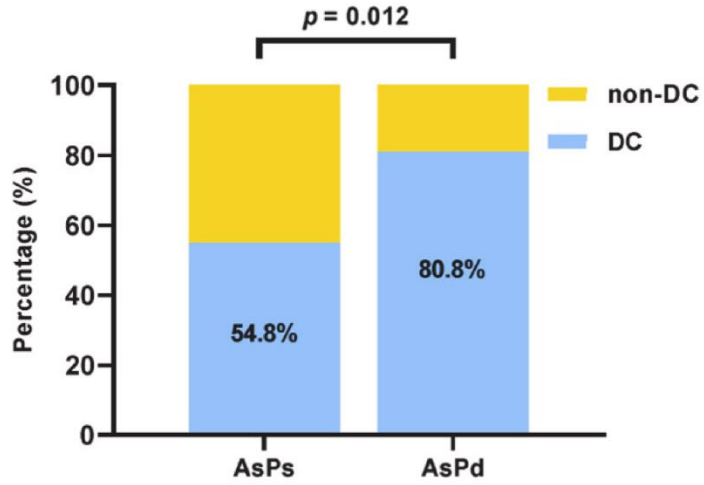
PFS2



Overall PFS



Disease Control

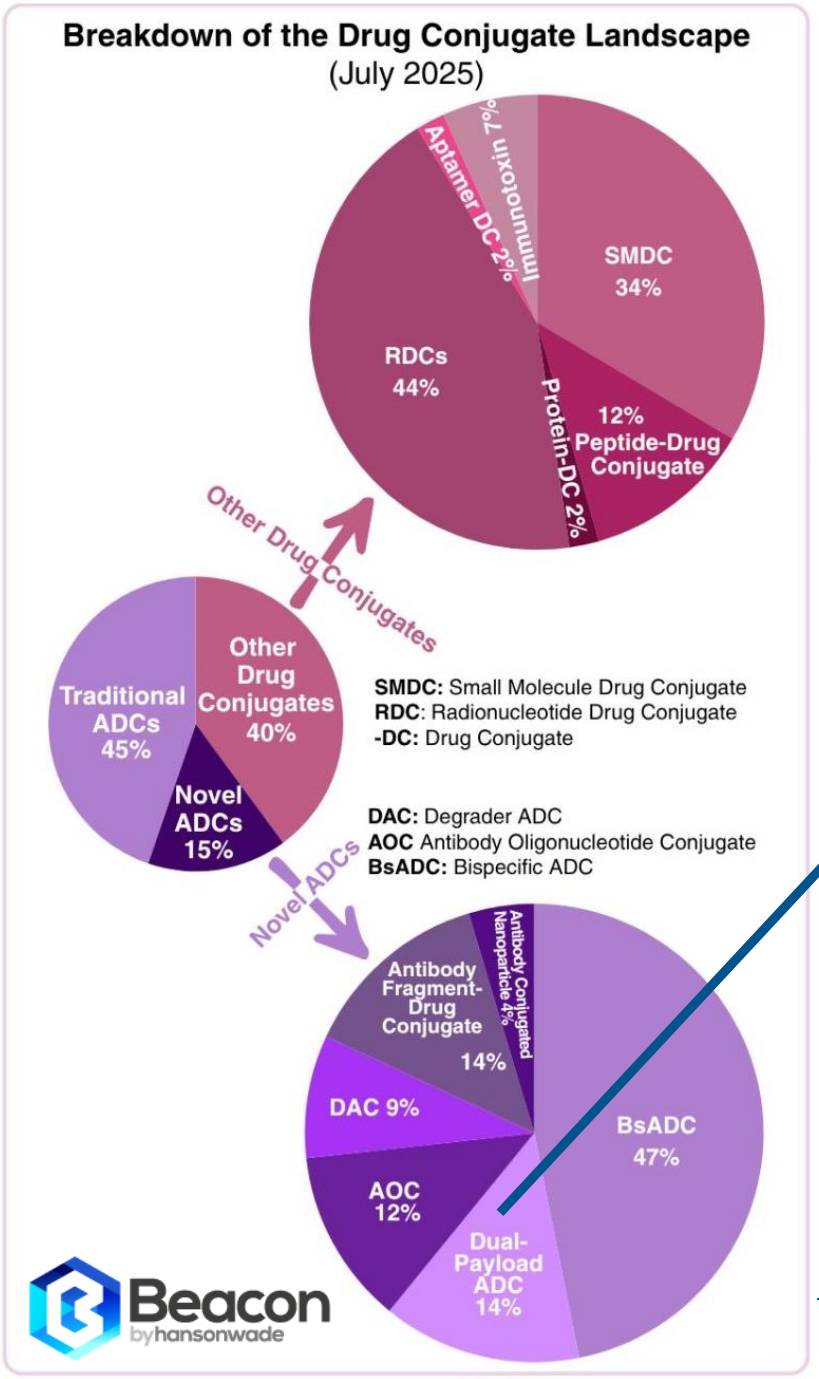


ADC2 with a different payload from ADC1 exhibited significantly longer PFS2

HER2-expressing Metastatic Breast Cancer

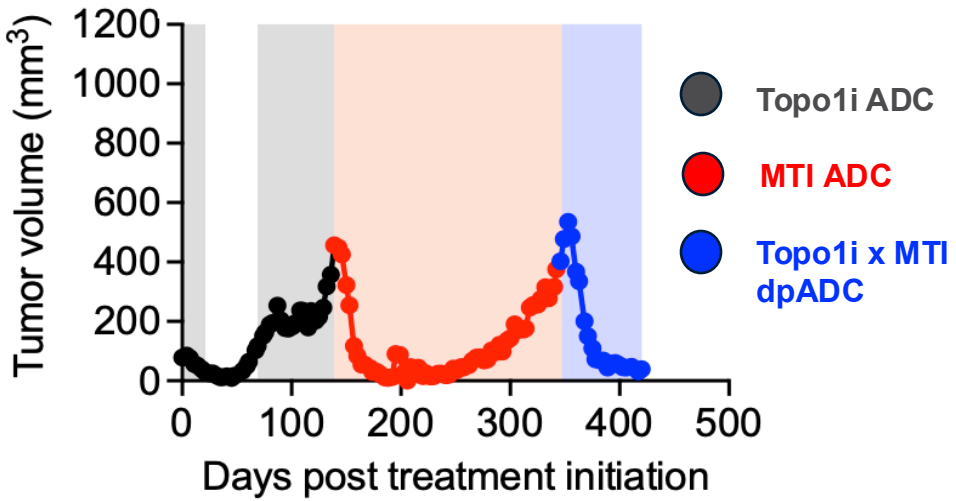
ADC2	PFS2	PFS1+PFS2	DC
With Similar Payload (MTi→MTi; Top1i→Top1i)	2.7 months	8.5 months	54.8%
With Different Payload (MTi→Top1i; Top1i→MTi)	6.8 months	15 months	80.8%

The Breast 81 (2025): 104470



Dual-payload ADCs have the potential to overcome ADC resistance with payload diversity.

Nov 3rd, Novel Conjugates Day, Dr. Dan Calarese



There is a rising trend in ADC development for dual-payload ADCs: Two dual-payload ADCs have entered the clinic, with dozens more in the pipeline.

Strategies for Payload Combination in Dual-Payload ADCs to Overcome Resistance and Activate Anti-Tumor Immunity

Two Distinct Cytotoxins

Combine cytotoxins with distinct MOAs.

- Overcome drug resistance
- Example: Top1i + MTi e.g. exatecan + MMAE

Nov 3rd, Novel Conjugates Day, Dr. Dan Calarese

Cytotoxin + Synergizer

The second payload to enhance the efficacy of the primary payload

- Re-sensitizing resistant tumors after single-payload ADC failure
- Example: Top1i + DDRi e.g. exatecan + ATRi

Nov 5th, Discovery Chemistry, Dr. Krishna Bajjuri

Cytotoxin + Immune Modulator

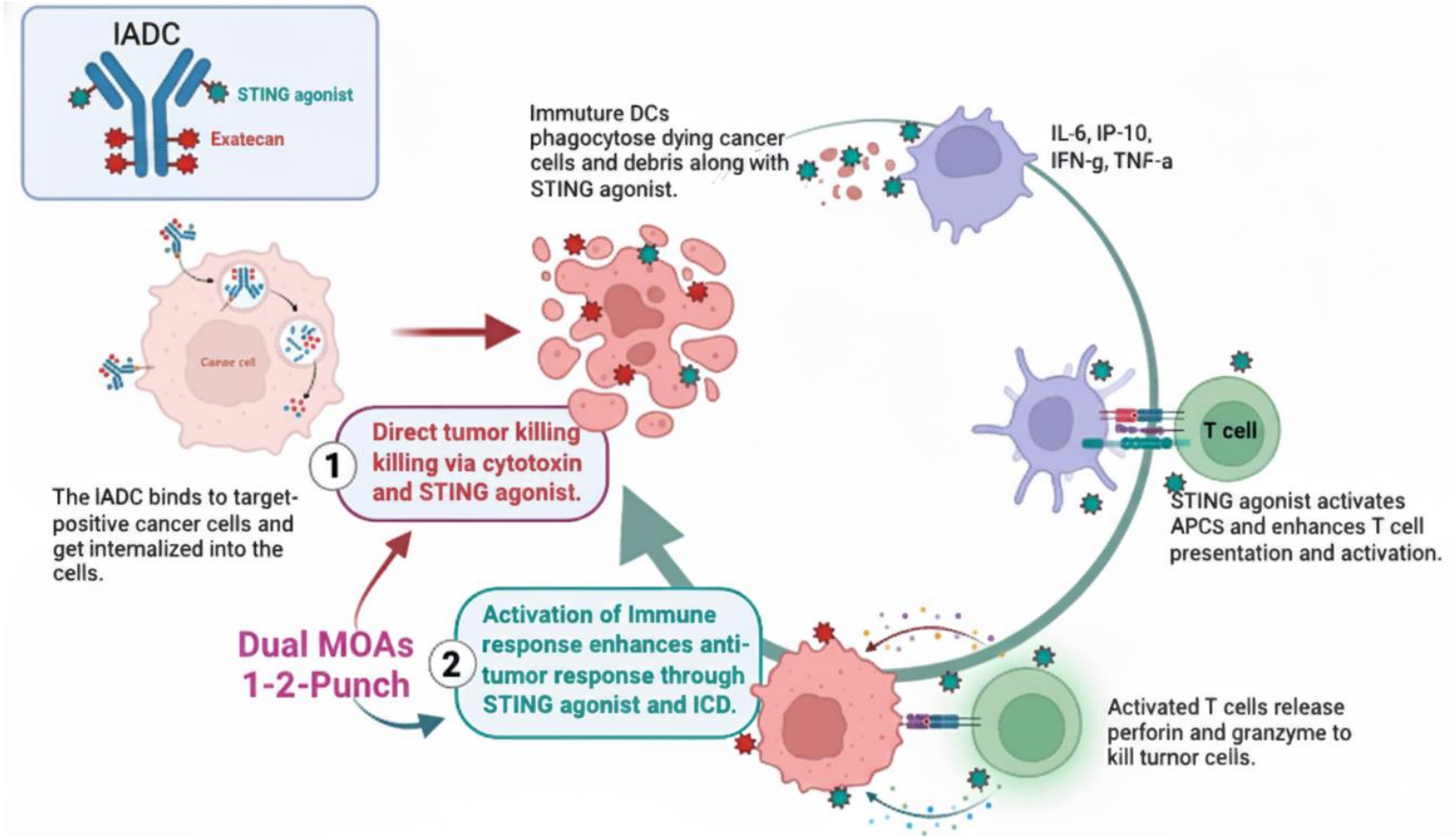
Delivery of both cytotoxin and immune modulator straight into tumors

- Directly kill tumor cells and activate immune response
- Example: Top1i + immune agonist e.g. exatecan + STING

Nov 3rd, ADCs in Combination Day, Dr. Gang Yin

New Modality for Cold Tumors: Immunostimulatory Antibody Drug Conjugate (iADC)

- Combining **exatecan** and **STING agonist** enabled by Sutro's dual-payload ADC platform
- Sutro and Astellas are co-developing two iADC programs.
- Sutro retained option to develop iADCs in other targets
  - The HER2 iADC is wholly owned by Sutro and available for partnering, along with other potential target programs.

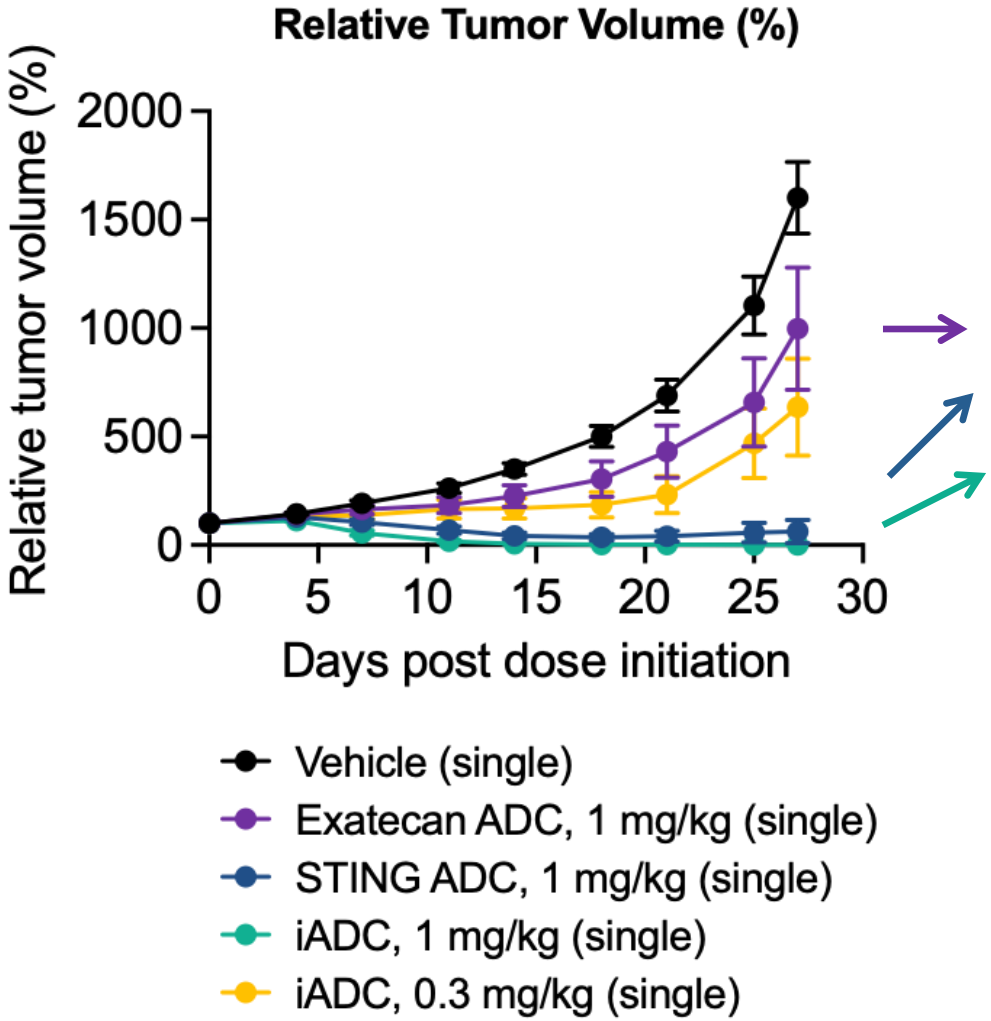


Immune Agonists Conjugates: Common Concerns and Sutro's Approaches to Mitigation

Common Concerns*	Cause	Sutro's Approach to Mitigation
Limited Efficacy	• Low potency of TLR agonist to avoid ADA; This trade-off can compromise therapeutic benefit	• Higher potency STING agonist • Combine cytotoxin and immune agonist into a single Ab to achieve dual MOAs in TME
Anti-drug Antibody (ADA) Generation	• TLR agonist-Ab conjugate induces ADA • TLR broadly expressed on B cells	• STING agonist, primarily expressed in myeloid and less immunogenic • Non-cleavable linker • Measure ADA generation in NHP
Systemic Toxicity (cytokine release syndrome)	• FcγR mediated uptake into immune cells • Cleavable linker • Unstable conjugation chemistry • High DAR of immune agonist • Fast clearance of the conjugate	• Eliminate FcγR function • Non-cleavable linker • Stable conjugation chemistry • Low DAR of STING agonist (DAR=2) • Site selective approach for desirable PK • Assess safety and cytokine release in NHP

*Journal for immunotherapy of Cancer, 2025: 13 (8), Where does ISAC go from here?

HER2 iADC Treatment Results in More Complete Responses than Both Exatecan ADC and STING ADC Treatment in the MC38-hHER2 Tumor Model

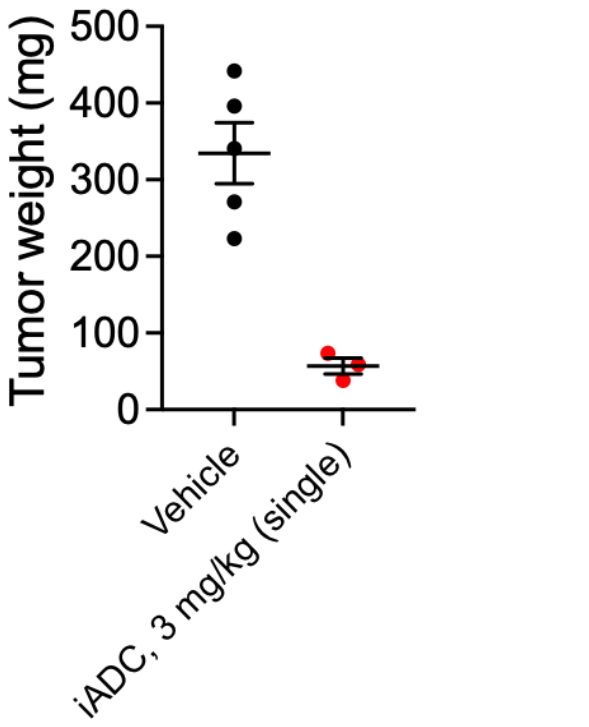


Group	% TGI (Day 27)	CR/Total
Vehicle	--	0/8
Exatecan ADC, 1 mg/kg	40	1/8
STING ADC, 1 mg/kg	102	5/8
iADC (SP13016), 1 mg/kg	107	8/8
iADC (SP13016), 0.3 mg/kg	64	0/8

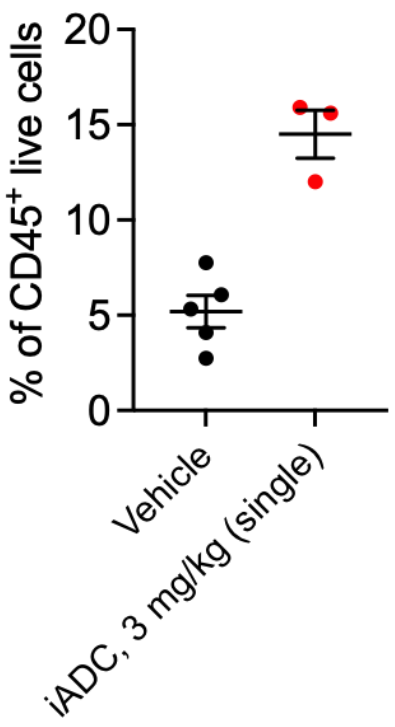
- iADC demonstrates dose-related efficacy
- iADC treatment results in a greater complete response rate (100%) than the STING ADC (63%) and Exatecan ADC (13%)

HER2-iADC Treatment Increases Proportion of NK Cells and Monocytes Infiltrating the Tumor

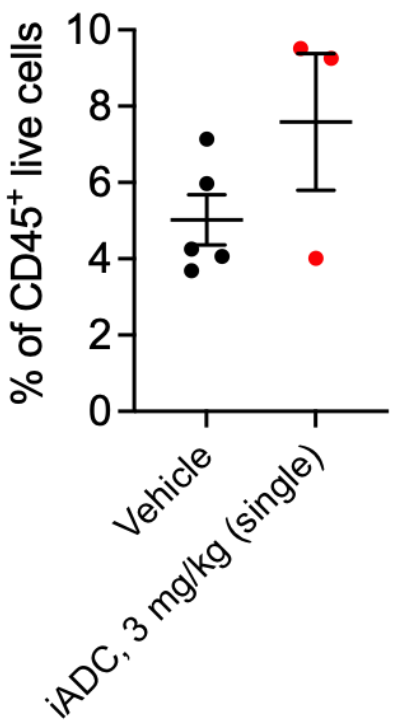
Tumor weight (Day 5)



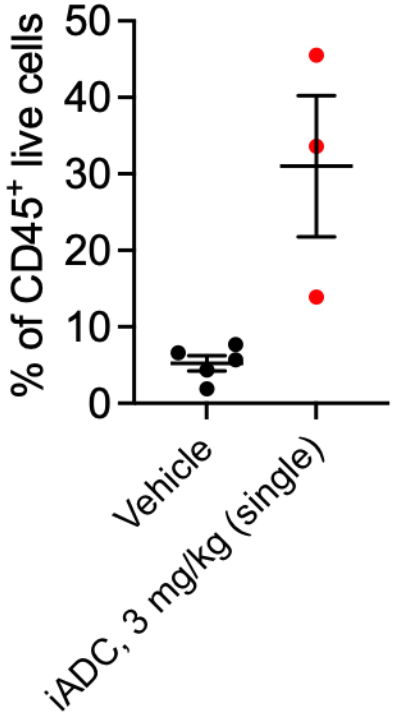
NK cells



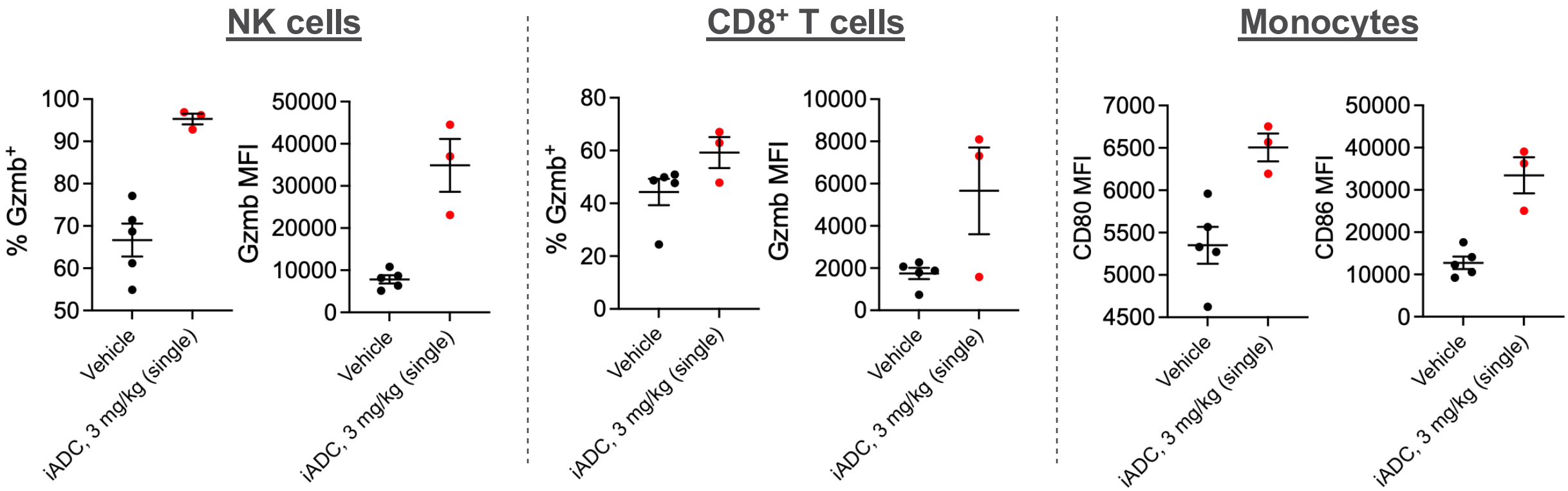
CD8+ T cells



Monocytes

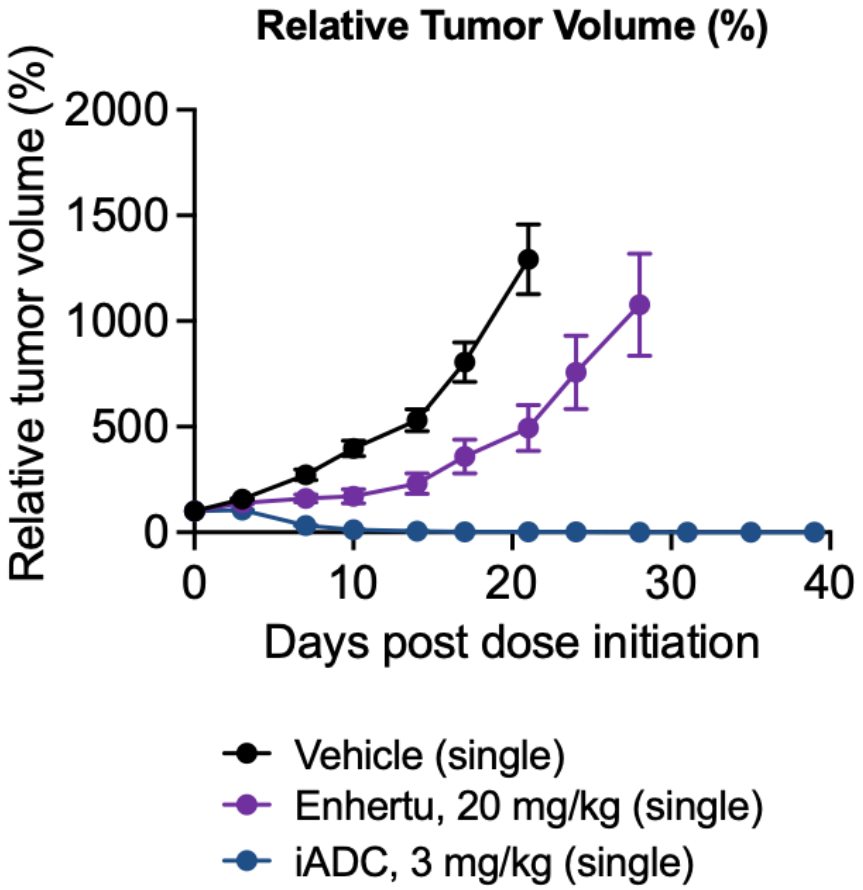


HER2-iADC Treatment Increases the Cytotoxic Potential of NK Cells and CD8+ T Cells and Stimulates Monocyte Activation in Tumors



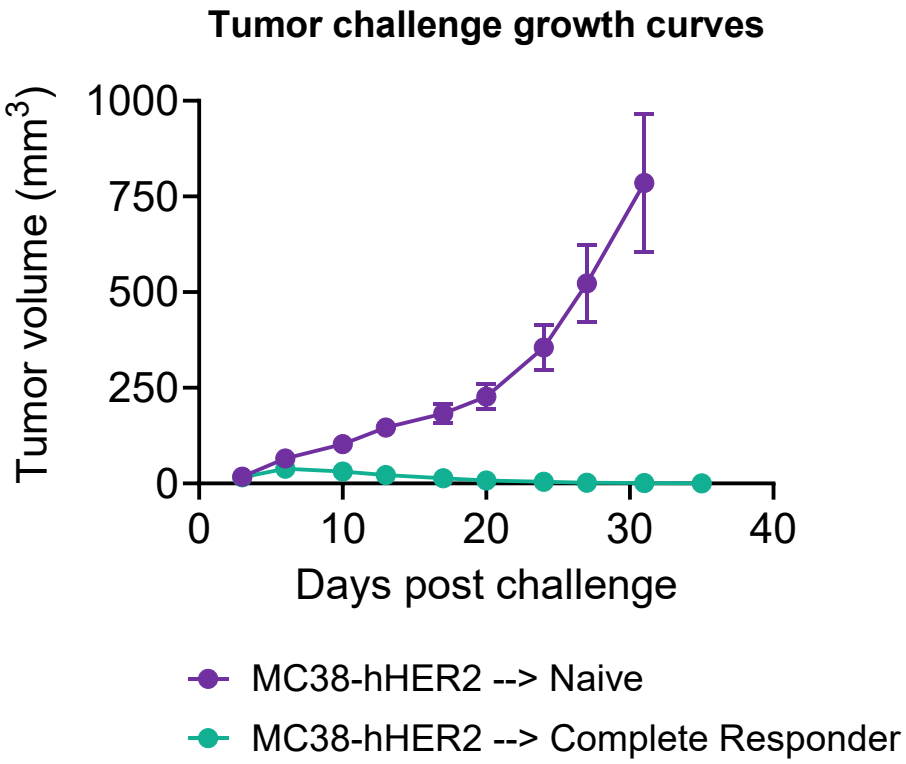
iADC treatment leads to increased cytotoxicity (granzyme B) of NK and CD8+ T cells and increased activation (CD80/86) of monocytes

HER2-iADC Treatment Shows Superior Efficacy Compared to Enhertu, Achieving a 100% Complete Response Rate in the MC38-hHER2 Tumor Model



Group	% TGI (Day 21)	CR/Total
Vehicle	--	0/8
Enhertu, 20 mg/kg	67	0/8
iADC, 3 mg/kg	108	8/8

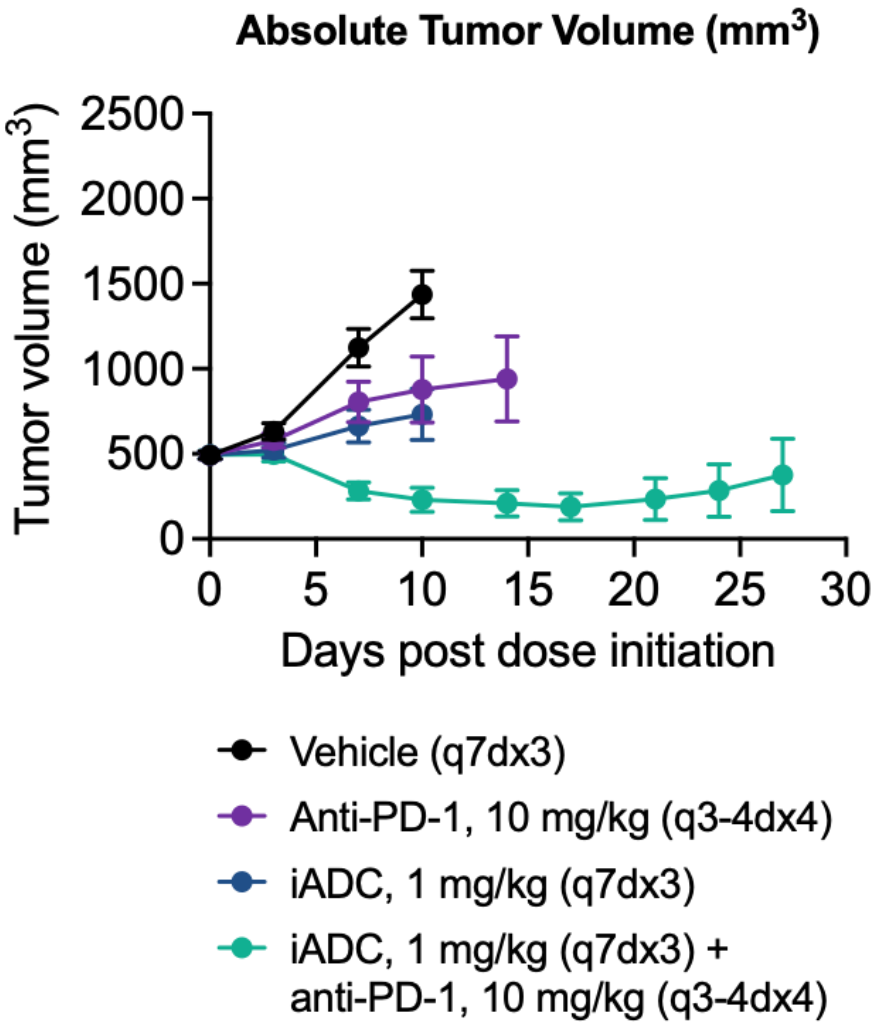
Complete Responders Remain Tumor-Free Following MC38-hHER2 Rechallenge Suggesting Development of Long-Term Immunological Memory



Mouse	Challenge	Tumor-free mice (Day 35)
Naïve C57BL/6	MC38-hHER2-#11	0/10
iADC (SP13016) - treated Complete Responder C57BL/6	MC38-hHER2-#11	11/12

Mice that eliminated MC38-hHER2 tumors following iADC (SP13016) treatment exhibit protection against MC38-hHER2 rechallenge

HER2-iADC Plus Anti-PD-1 Combination Enhances Therapeutic Response in Large MC38-hHER2 Tumors Compared to Monotherapy



Group	% TGI (Day 10)	CR/Total
Vehicle	--	0/8
Anti-PD-1, 10 mg/kg	62	0/8
iADC, 1 mg/kg	78	0/8
iADC, 1 mg/kg + anti-PD-1, 10 mg/kg	130	1/8

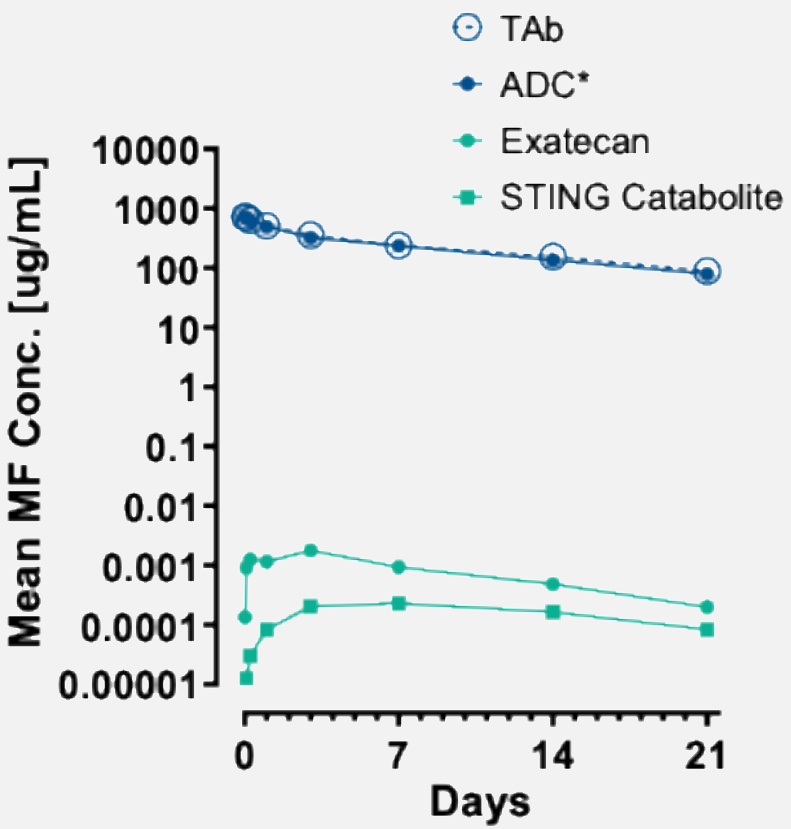
HER2-iADC was Well Tolerated in Exploratory NHP Toxicity Study (Q3W x2)

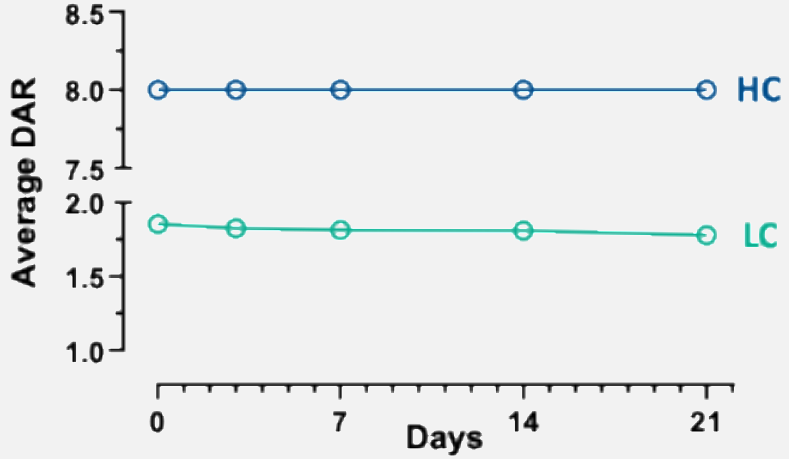
Study:

Two doses of 12.5 or 25 mg/kg given to 1M/1F per group on Days 1 and 22; Day 43 necropsy

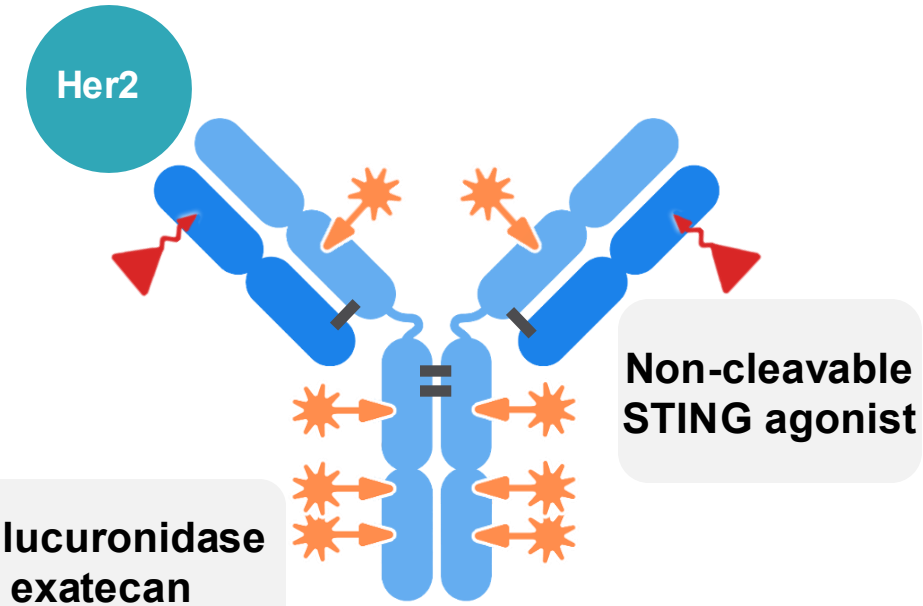
Findings:

- Both doses well tolerated with no abnormal clinical signs
- No increases in cytokines indicative of potential for cytokine release syndrome such as IL-2, IL-6 and TNFα
- Exhibited low clearance, long half-life ($T_{1/2}$ ~9.5d, DAR stability, and negligible ADA formation
- MTD = 25 mg/kg; toxicity profile similar to other exatecan containing ADCs and to HER2-exatecan DAR8 single conjugate

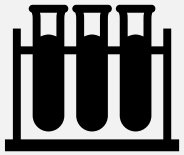




Summary of Her2 iADC Preclinical POC Data



iADC



In vitro

- Specific cytotoxicity toward Her2+ tumor cells
- Activated monocyte, DC, NK, B and T cells, as well as cytokines production in Her2+ tumor cells /PBMCs co-culture.

Pharmacokinetics

- Desirable PK profiles in Tg32 mice

Pharmacodynamics in syngeneic mouse models

- Enhanced anti-tumor activity compared to mono-payload ADCs and Enhertu.
- iADC treatment led to greater NK cell, CD8+ T cell, and monocyte activation
- iADC and anti-PD-1 combination benefit observed in the MC38-hHER2 large tumor model
- Rechallenge study confirmed long-lasting anti-tumor immunity

Safety assessment in NHP

- Well tolerated at highest dose tested, 25 mpk, 2XQ3W
- Minimal effects on clinical chemistry or clinical signs

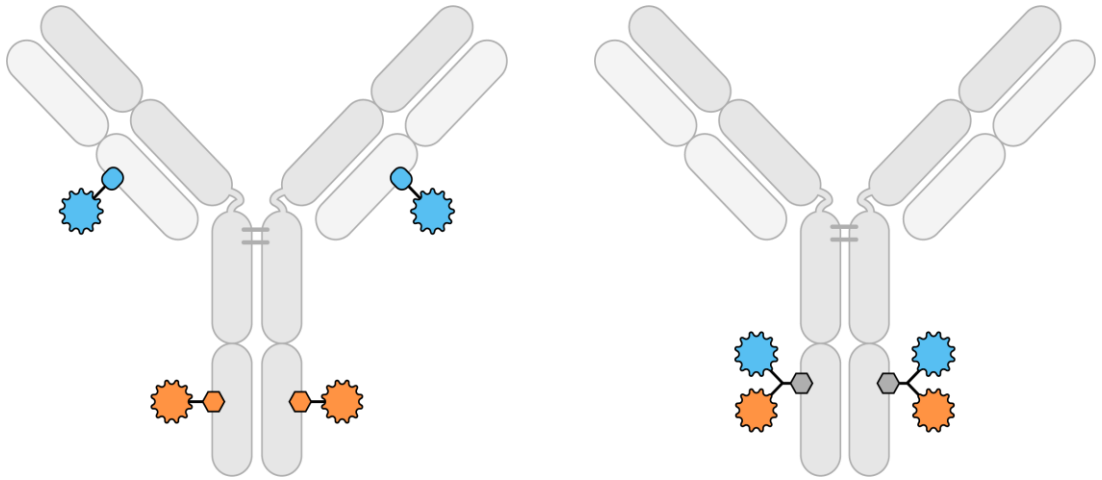
Manufacturability

- Desirable developability

Sutro's iADCs Combine Innate and Adaptive Immune Mechanisms to Enhance Antitumor Immunity, Resulting in Superior Protection Against Tumor Progression

	Sutro iADC	STING / TLR	ISAC	PD-1 / PDL-1	CAR-T Cells	Vaccine
Molecule	Targeted and homogeneous	Chemo	Mixed ADC	Ab	Biologic	Biologic
Opportunity: Risk	Combine ICD with innate agonists (TLR, STING, etc.)	Non-targeted, issues with TI	requires Fc effector	Limited tumor types, small tumors	Safety concerns	Ag selection challenge
Avoids FcγR meditated uptake into immune cells	■	NA	■	NA	NA	NA
Mechanisms to achieve anti-tumor immunity: Direct tumor cell killing	■				■	
Tumor antigen presentation	■		■			■
Priming and activation of Antigen Presenting Cells	■	■	■			■
T-cell recruitment to tumor	■	■	■	■	■	

Dual Conjugation Can Be Achieved by Combining Any Two Orthogonal Chemistries or by Using A Branched Linker w/ A Single Chemistry



- **DAR and payload ratio** should be driven by **pharmacology and safety** profiles, not by technological constraints
- **PK and physicochemical properties** of the resulting ADC must be **maintained** without compromise
- **Manufacturability** should be **practical, scalable, and efficient**

~10,000 views/downloads in 5 months

MABS
2025, VOL. 17, NO. 1, 2498162
https://doi.org/10.1080/19420862.2025.2498162

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REVIEW

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Homogeneous antibody-drug conjugates with dual payloads: potential, methods and considerations

Miao Wen, Abigail Yu, Young Park, Daniel Calarese, Hans-Peter Gerber, and Gang Yin

Sutro Biopharma Inc, South San Francisco, CA, USA

ABSTRACT

The development of site-specific dual-payload antibody-drug conjugates (ADCs) represents a potential advancement in targeted cancer therapy, enabling the simultaneous delivery of two distinct drugs into the same cancer cells to overcome payload resistance and enhance therapeutic efficacy. Here, we examine various methodologies for achieving site-specific dual-payload conjugation, including the use of multi-functional linkers, canonical amino acids, non-canonical amino acids, and enzyme-mediated methods, all of which facilitate precise control over payload attachment while ensuring homogeneity. We explore the implications of different conjugation techniques on drug-to-antibody ratios and the ratios of the two payloads, as well as their impact on process complexity and manufacturability. Additionally, we address the potential advantages of dual-payload ADCs compared to ADCs combined with traditional chemotherapy or single-payload ADC/ADC combinations. By evaluating these innovative methods, we aim to provide a comprehensive understanding of the current landscape in dual-payload ADC development and outline emerging directions necessary for further advancement of this promising therapeutic strategy.

ARTICLE HISTORY
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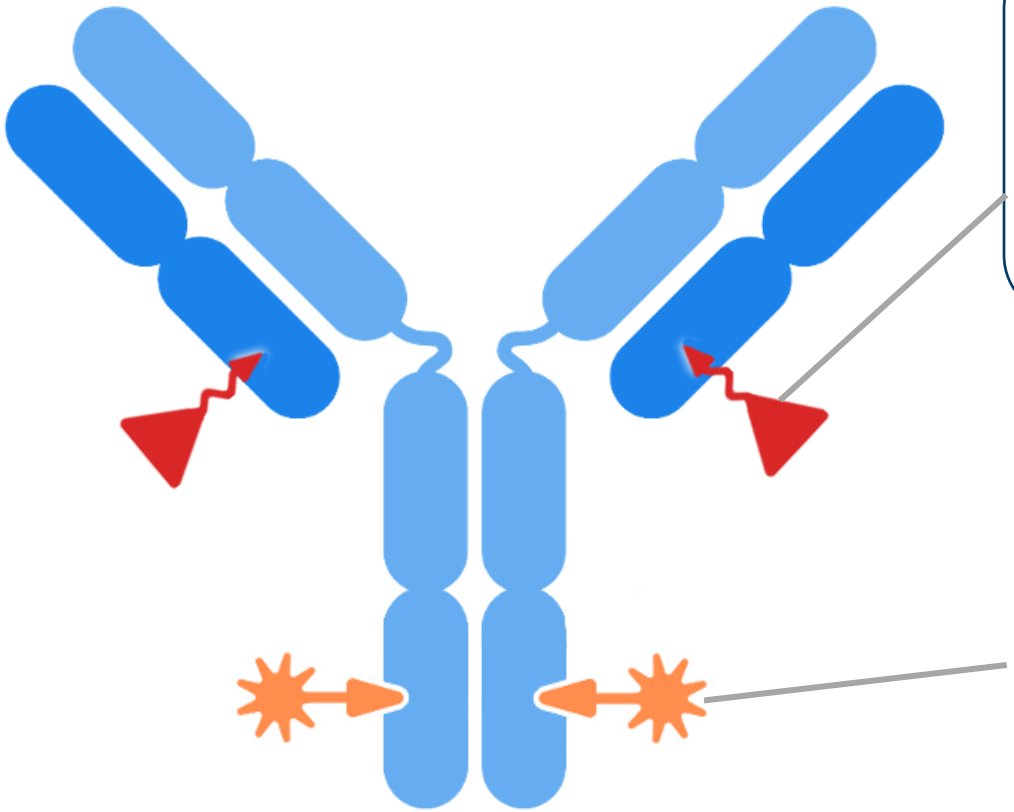
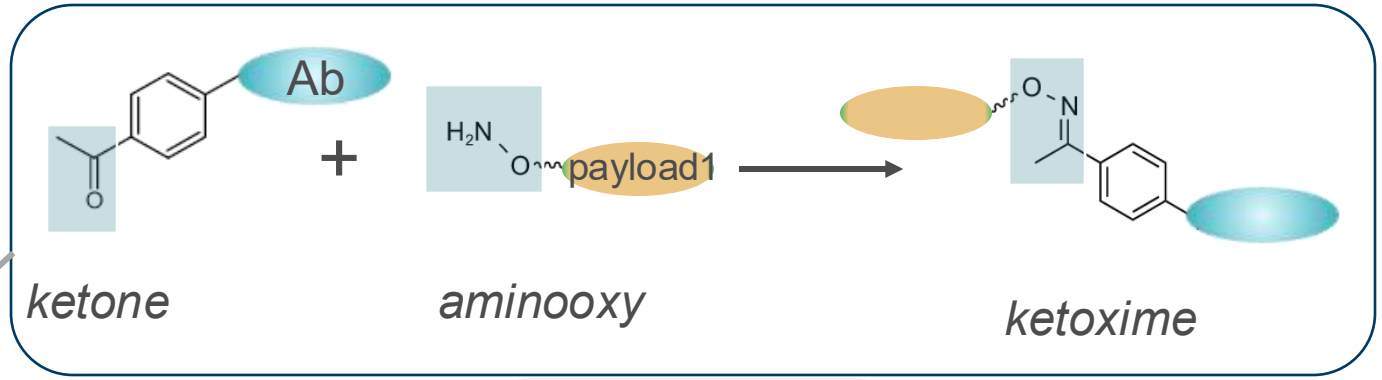
KEYWORDS
Antibody drug conjugate; conjugation; drug resistance; dual-payload; homogenous; manufacturability; next generation cancer therapy; process complexity

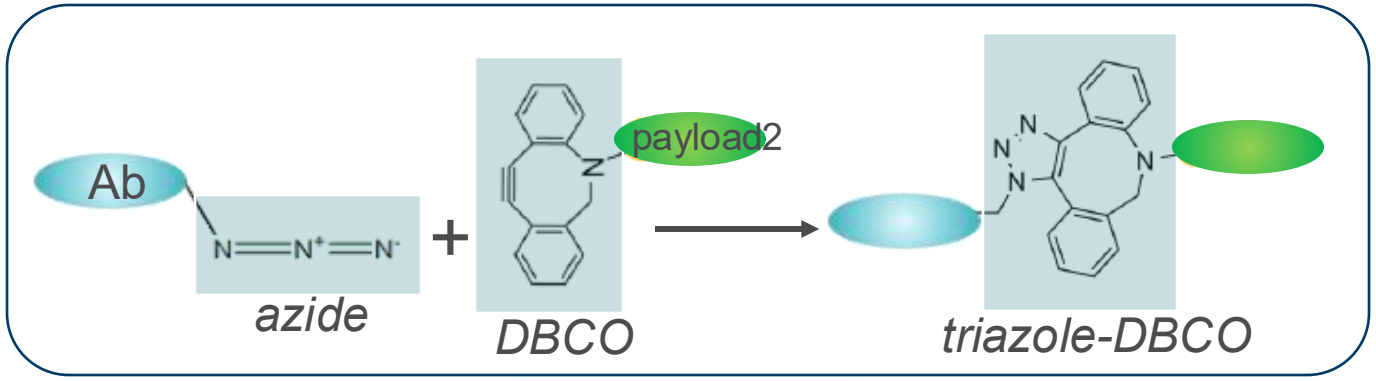
https://www.tandfonline.com/doi/full/10.1080/19420862.2025.2498162

Sutro's Platform Enables Orthogonal Conjugations in a Single-Pot Reaction – No Enzymes or Post-Conjugation Purification Needed; Desirable Physicochemical Properties Achieved for Dual-Payload ADCs



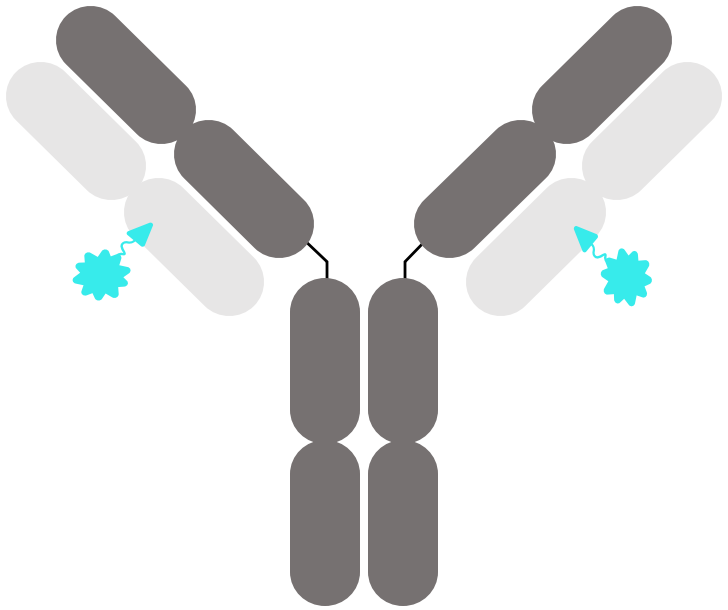


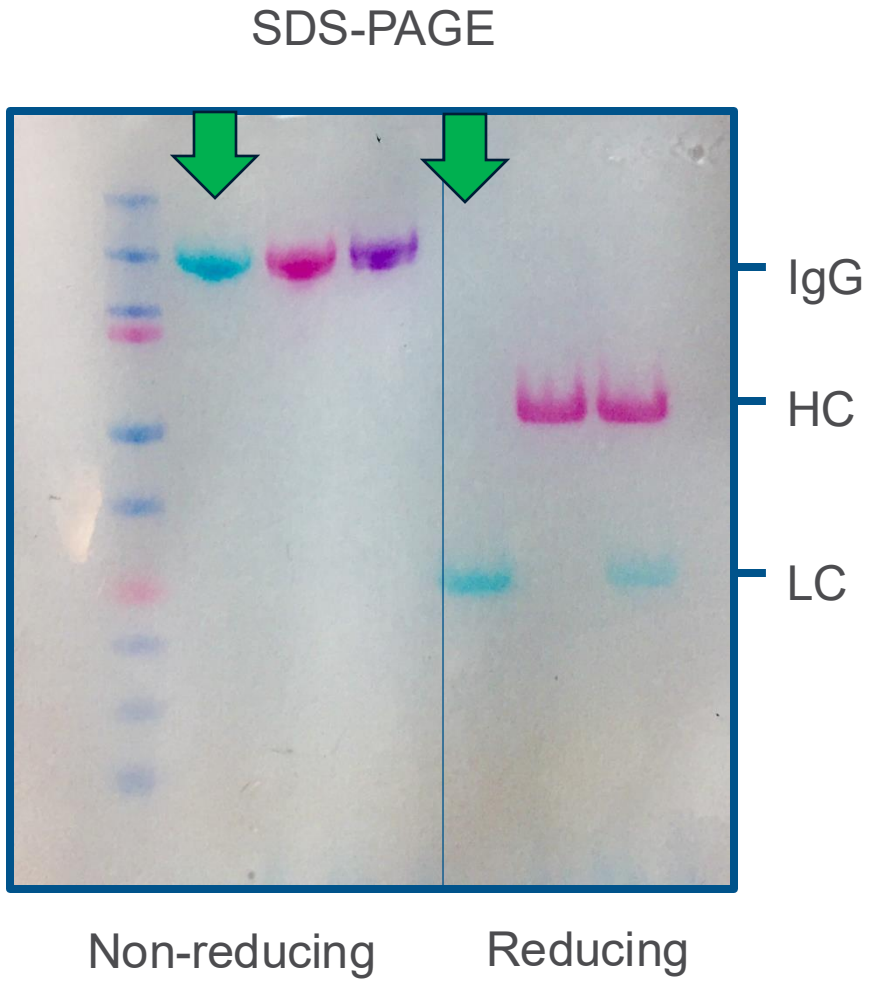
Oxime Ligation



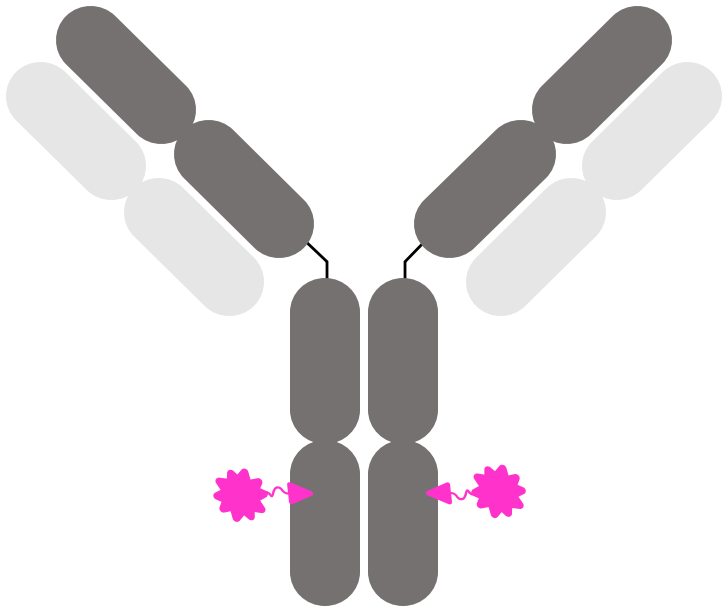
SPAAC

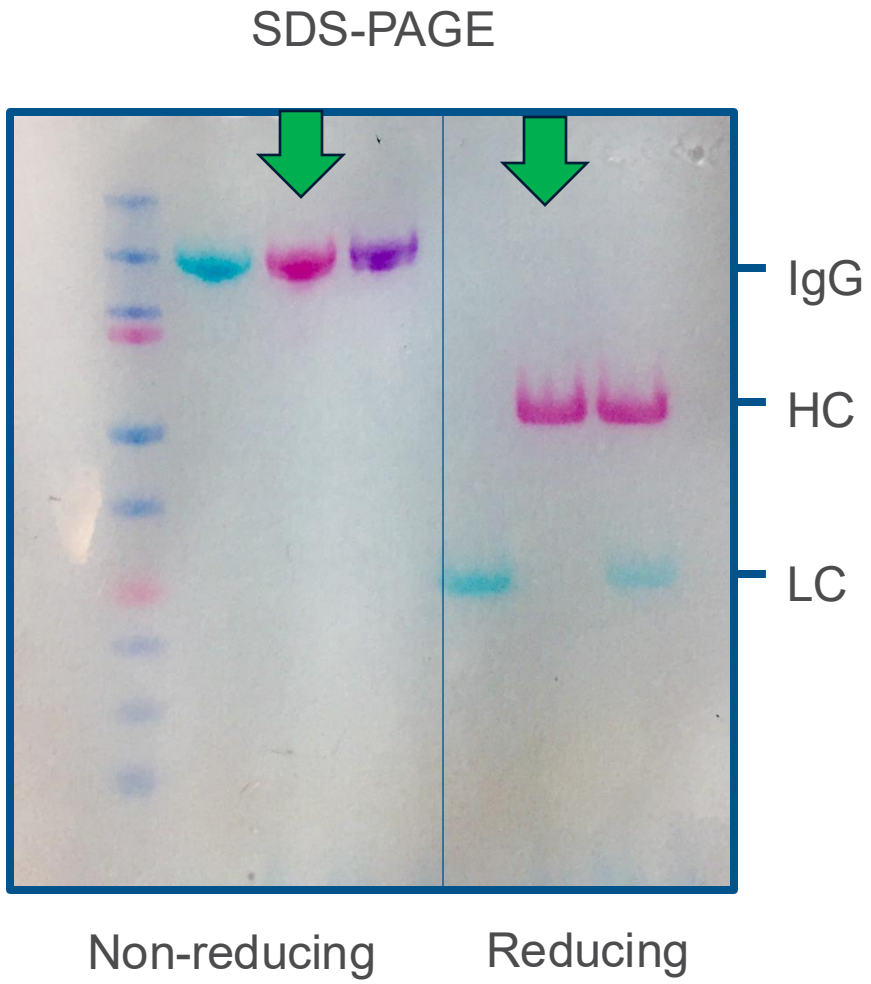
Visualization of Precision Dual-Conjugation on SDS-PAGE





Visualization of Precision Dual-Conjugation on SDS-PAGE





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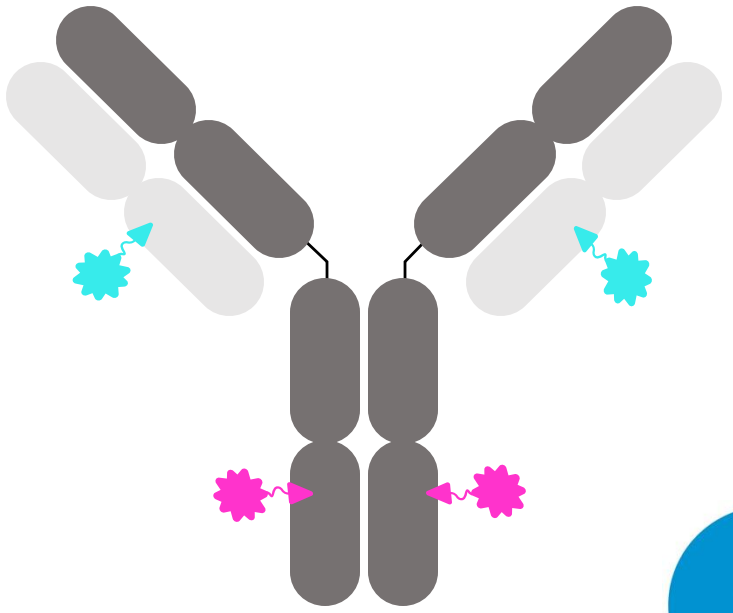
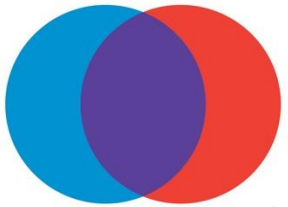
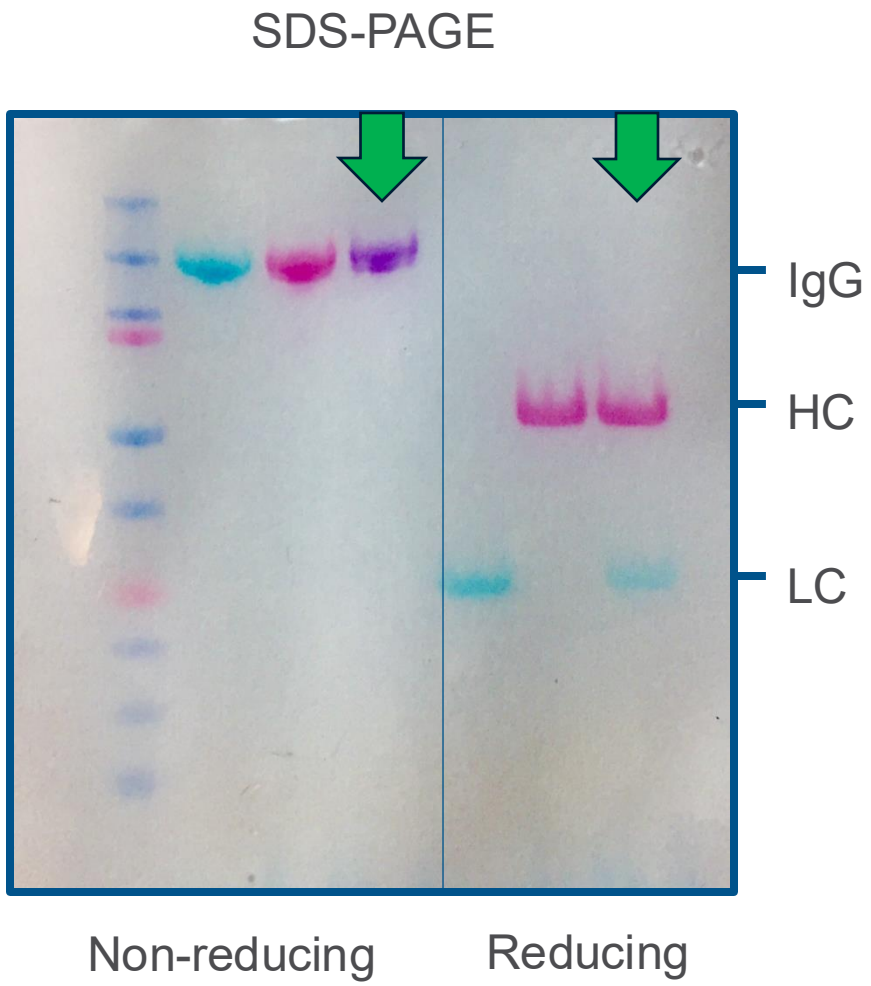
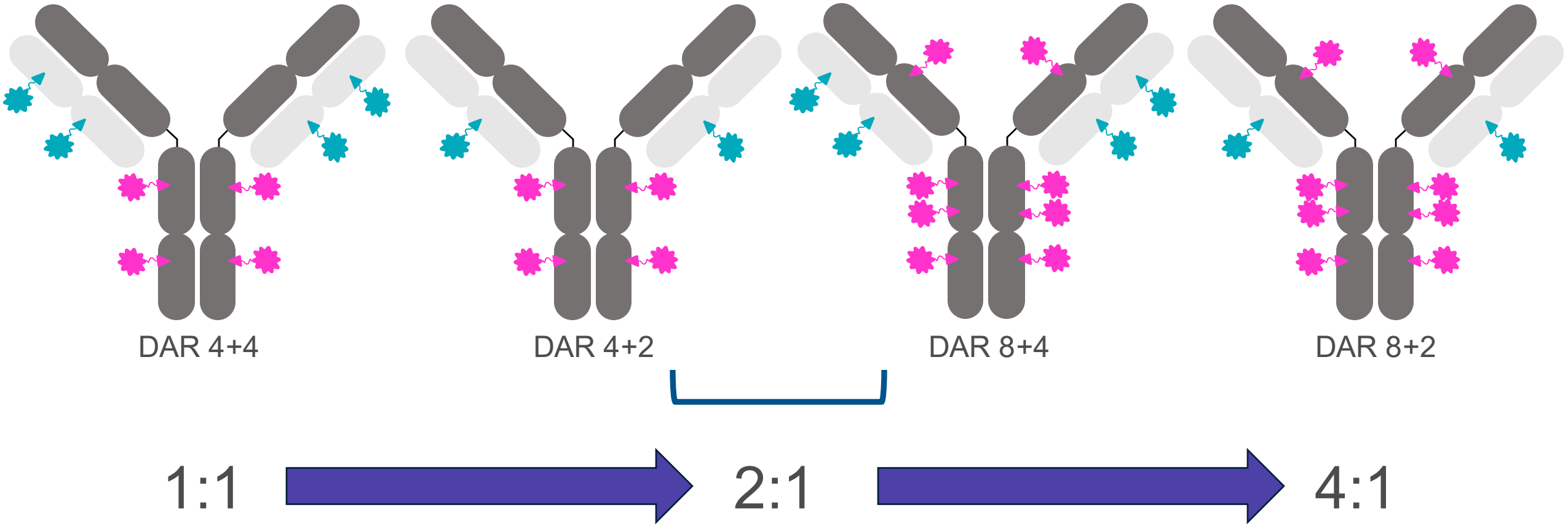






Precise Tuning of the DARs and Ratio of Two Payloads Are Critical for Optimal Synergy of Two Mechanisms of Action

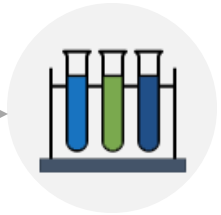


Sutro's ADC Platform is Fundamentally Different: Manufacturing of Proteins in Cell-Free Extracts

DNA Vector Engineering → Genome Sequencing → Strain Engineering

Prokaryotic Cells

1977: Somatostatin
1978: Insulin
1979: HGH



Enzymes
Proteases
Cytokines

Sutro Cell Free

2010: 100L CF demonstration
2011: mAbs production
2013: nnAA incorporation and ADCs
2014: bsAb and ADC
2016: CF GMP manufacturing
2024: 4,000L GMP run
2025: Dual-payload ADCs GMP manufacturing

Advantages to Improve Therapeutic Index of ADCs

- Site Specific Conjugation
- High DAR (>8)
- Dual-Payload ADCs

1976

Genentech: Boyer (UCSF)
Purpose: Manufacture Protein Therapeutics

2003

Sutro: Swartz (Stanford)
Purpose: Manufacture in Prokaryotic Cell Extract

2025

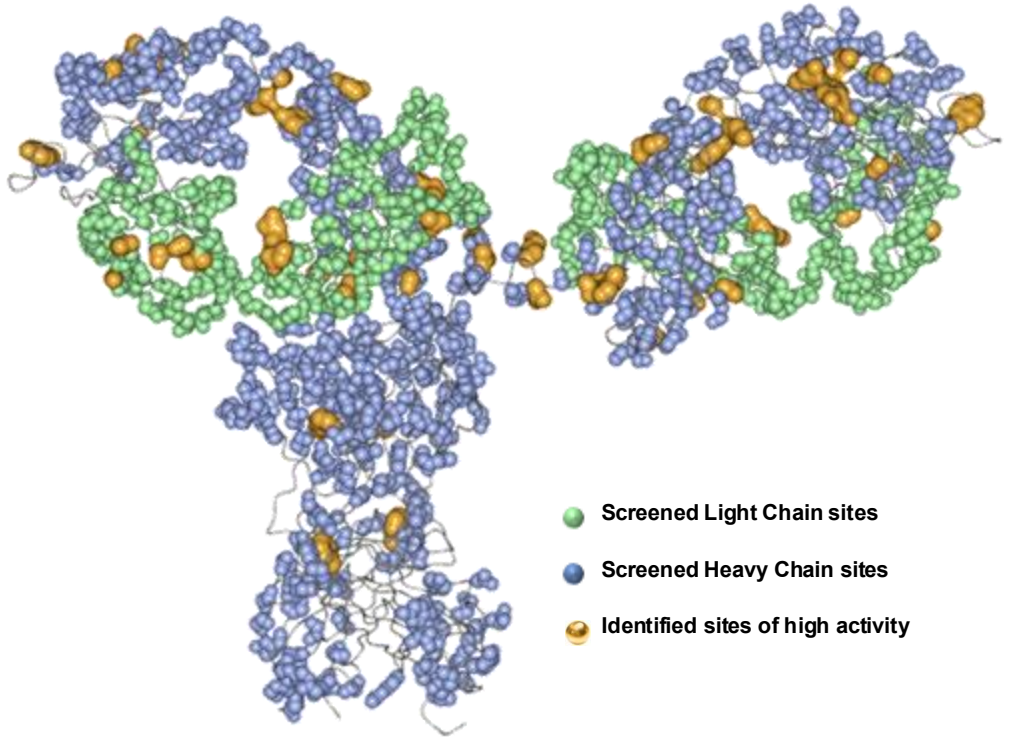
Sutro Milestones
Manufacture Cheaper, Faster, Better

nnAA – non-natural amino acids; CF – cell-free; bsAb – bispecific antibody; GMP – good manufacturing practice

XpressCF+® Screening Platform Allows for Rapid Empirical Evaluation and SAR Analysis to Identify the Best Conjugation Sites for ADCs

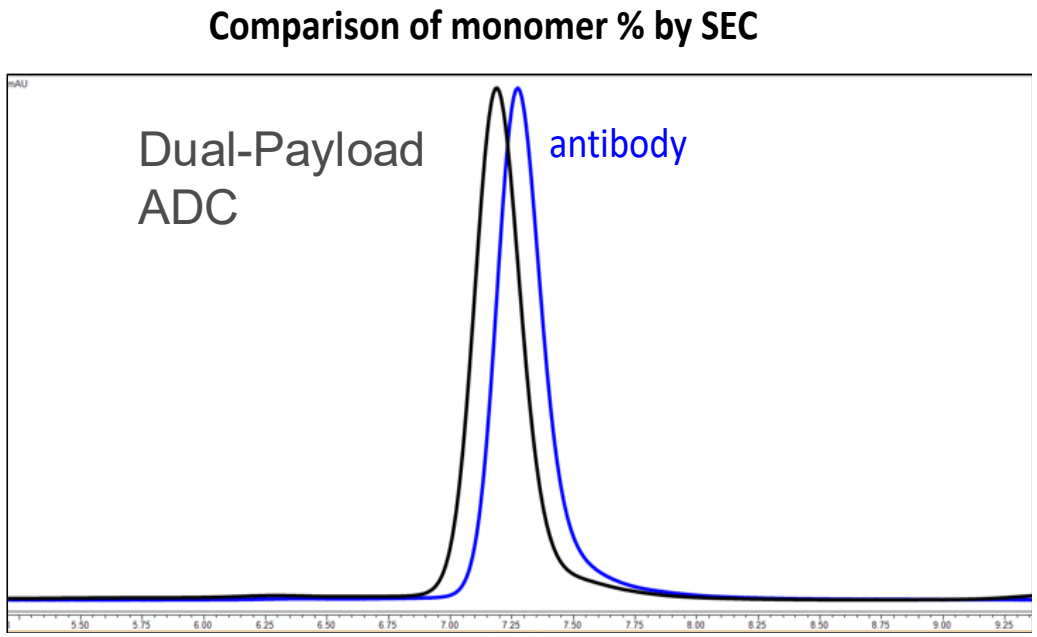
- Extensive screening of ~400 sites and site combinations conducted to identify sites that exhibit favorable characteristics: stability, conjugation efficiency, PK, efficacy & safety
- All conjugation sites were selected based on empirical data.
- These proprietary sites are utilized across various ADC programs at Sutro and may not be accessible through other conjugation technologies.
- Developing best-in-class dual-payload ADCs

Surface Sites Screened on an IgG during an ADC Campaign:



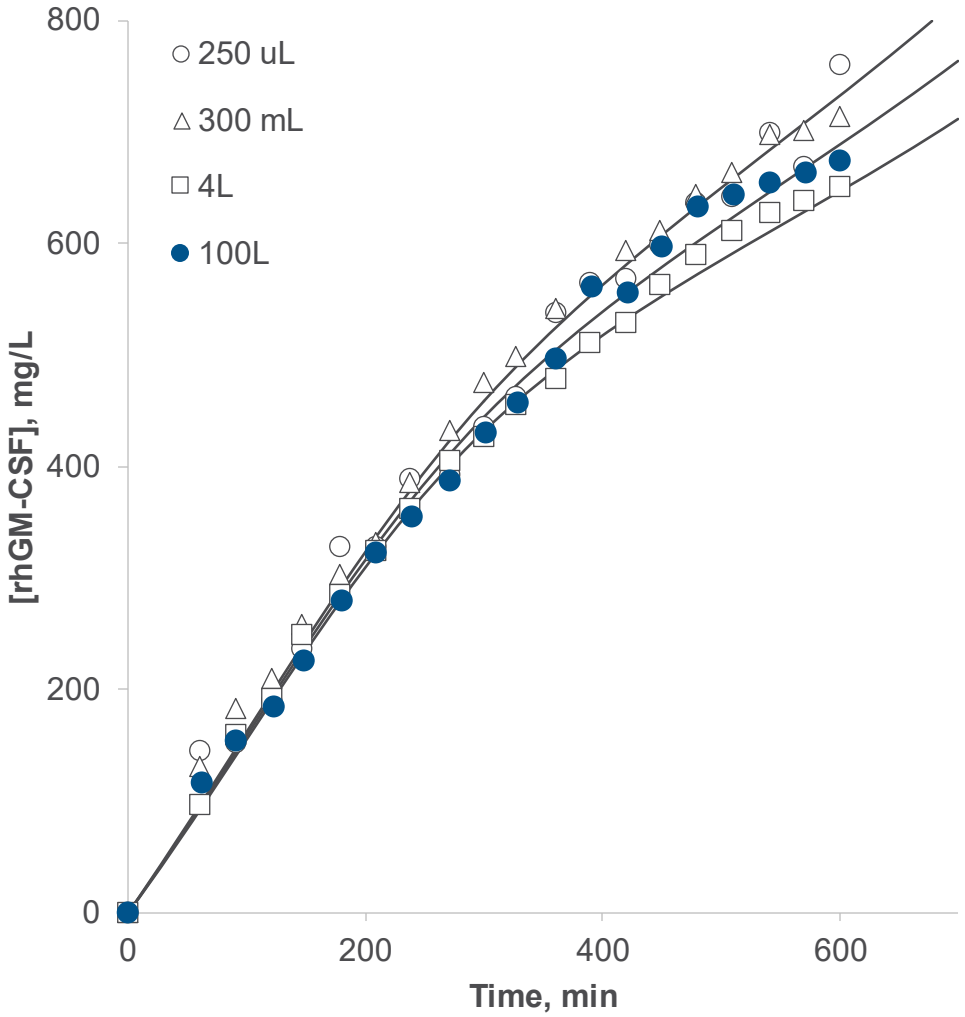
Scientific Reports volume 7: 3026 (2017)

Conjugating 8+4 Payloads Achieved >95% Conj. Efficiency with Desirable Physicochemical Properties w/o Aggregation or Precipitation



- ✓ High expression titer independent of DAR;
- ✓ High conjugation efficiency independent of DAR;
- ✓ Highly homogenous; no post conjugation purification needed
- ✓ Demonstrated favorable thermal, freeze/thaw, accelerated and long-term stability;
- ✓ Favorable developability
- ✓ Doesn't impact binding affinity to tumor antigen or FcRn
- ✓ Desirable PK profile

XpressCF® Enables Rapidly Scalable Expression: 250uL→100L→4,000L



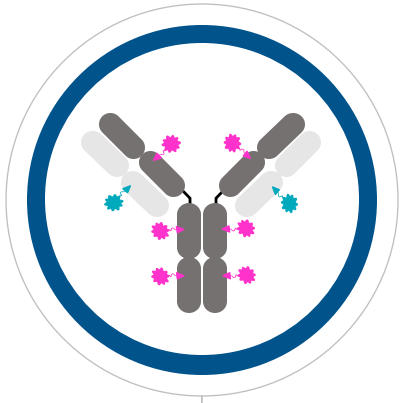
4,000L GMP Manufacturing Successfully Demonstrated with ADC Clinical Assets.

GMP Manufacturing Successfully Demonstrated for **Dual-Payload ADC** Programs

Biotechnol Bioeng. 2011 Jul;108(7):1570-8.

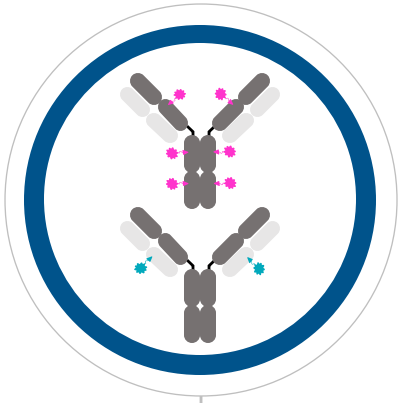
Regulatory Advantages of Dual Payload ADCs vs ADC Combination Trials

Dual Payload ADC Clinical Trial



SOC	ADC²
Arm 1 Standard of Care	**Arm 2** Dual Payload ADC

ADC Combo Clinical Trial



SOC	ADC X	ADC Y	ADC X & Y
Arm 1 Standard of Care	**Arm 2** ADC X	**Arm 3** ADC Y	**Arm 4** ADC X & Y Combo

SUTRO BIOPHARMA

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