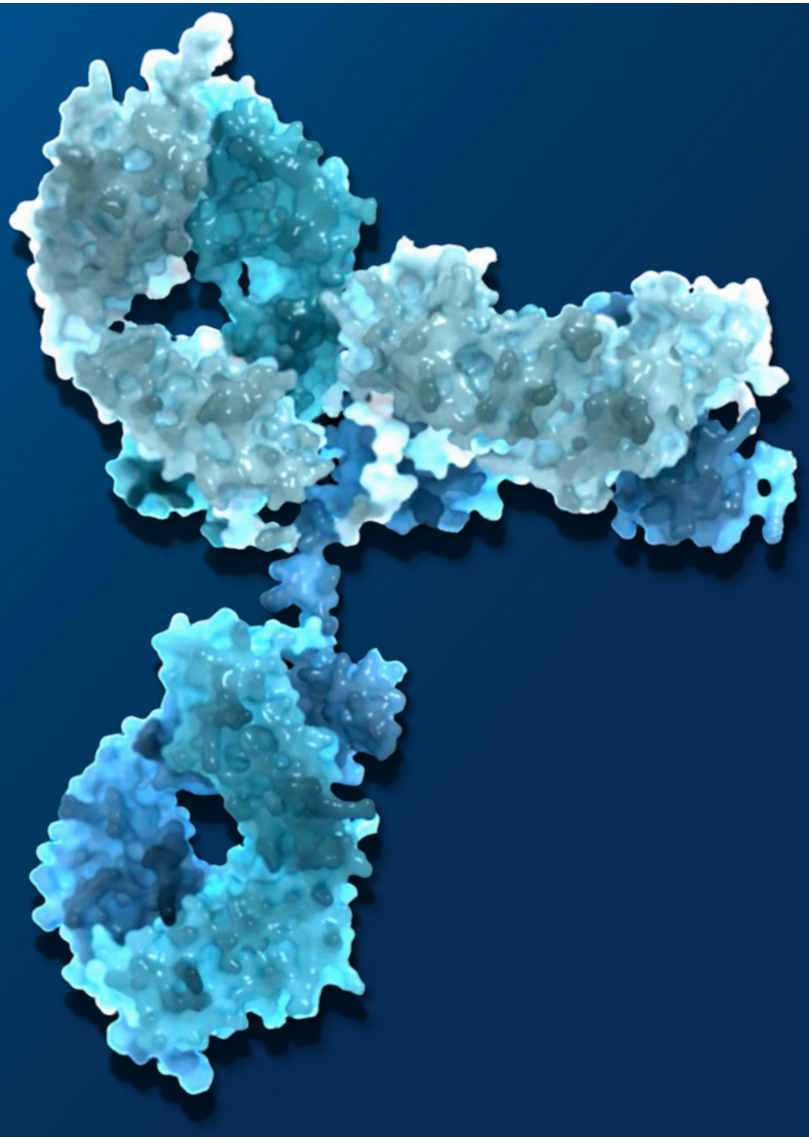
SUTRO BIOPHARMA

Leveraging Cell-Free Protein Synthesis for Site-Specific Conjugation to Enhance ADC Therapeutic Index

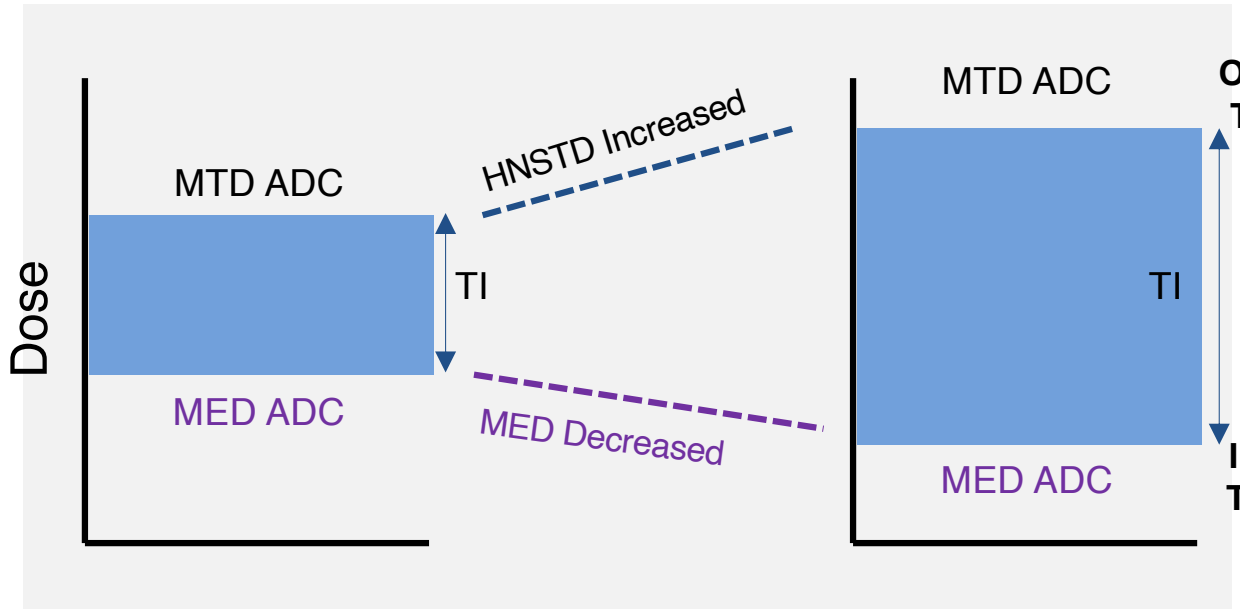
Gang Yin, PhD

VP, Platform Engineering & Process Research

15th ANNUAL WORLD adc SAN DIEGO 2024



Improving the Therapeutic Index by Reducing the Platform Toxicity and Enhancing the Efficacy

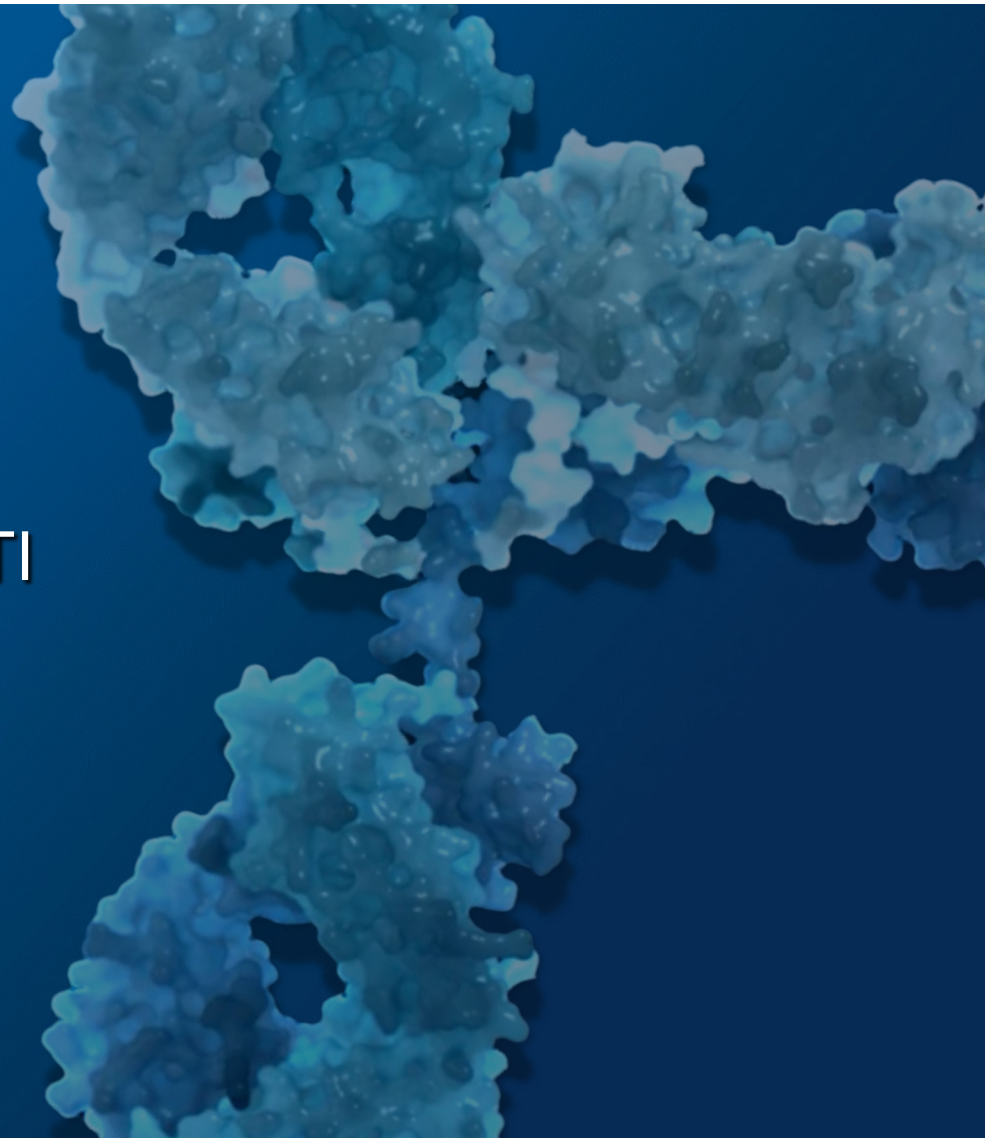


- Payload potency
- Linker payload stability
- Linker cleavage specificity
- Fc effector function
- Physicochemical properties

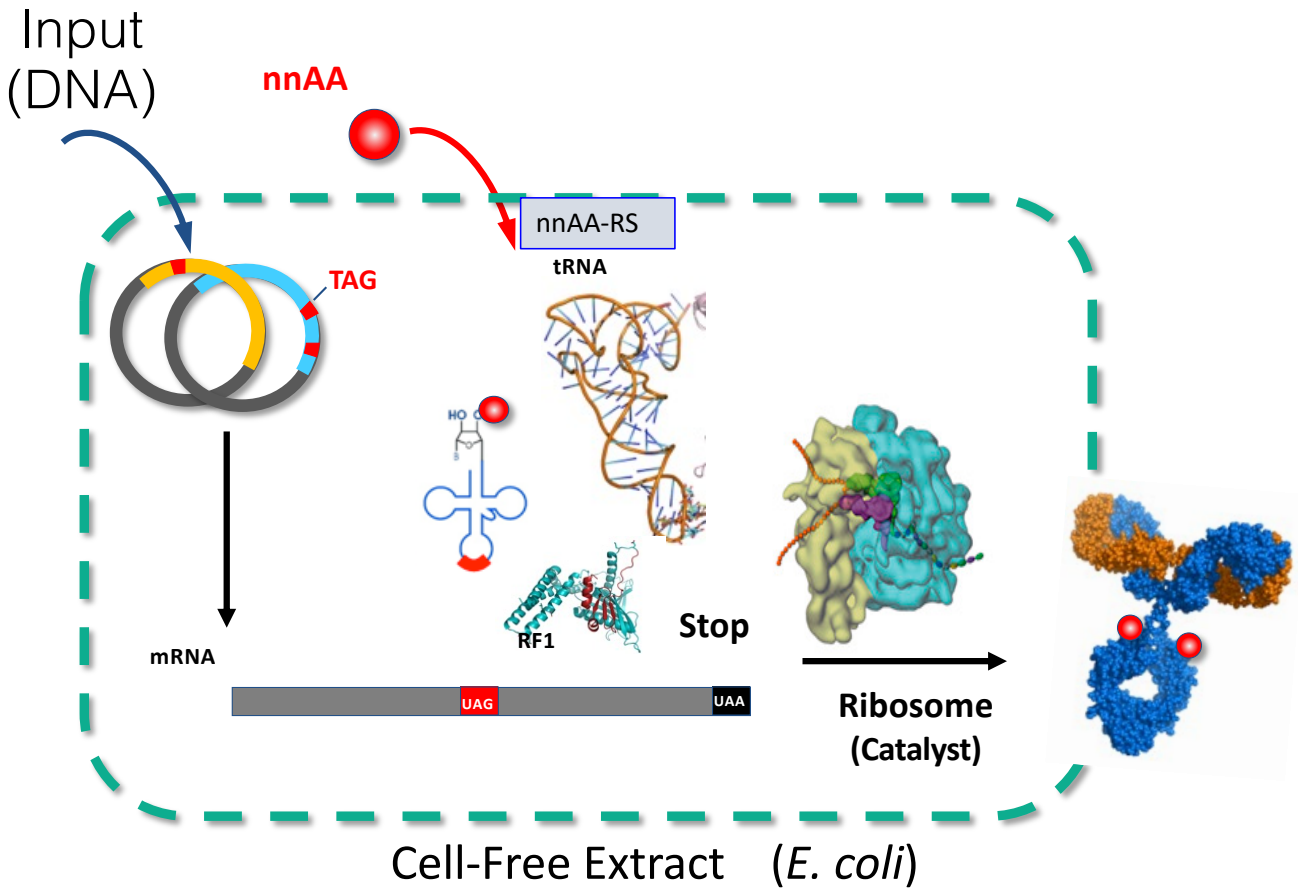
- Higher DAR → target-low tumor
- Dual-payload ADC → MOA combination

SUTRO BIOPHARMA

CF Expression Platform and Precise Conjugation Enables TI Improvement



Engineered Cell-free Protein Synthesis Enables Highly Efficient Incorporation of nnAA



RF-1

- Recognize stop codon and release polypeptide
- Essential to living cells

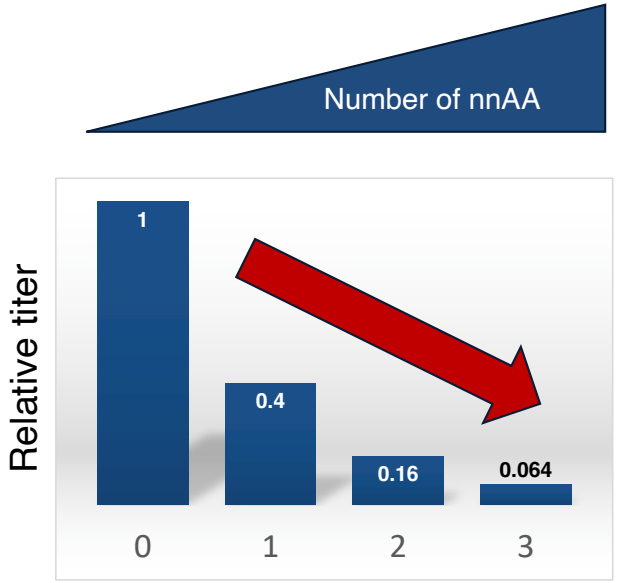
in vivo Expression

- Hard or impossible to engineer RF-1
- Incorporating multiple nnAA is challenging.

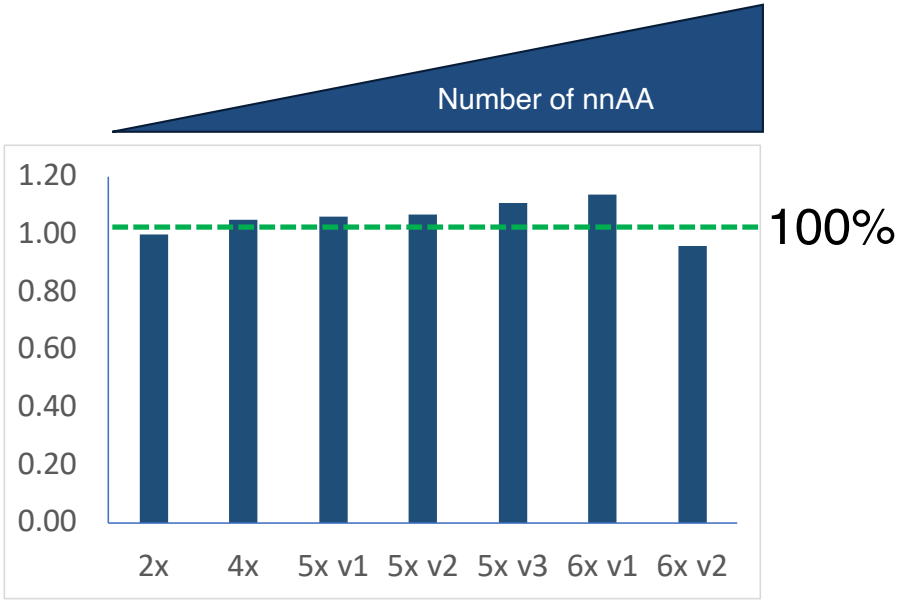
Sutro XpressCF+®

- Conditional inactivation of RF-1
- Incorporating multiple nnAA does not affect the titer.

Incorporation of pAMF Does Not Affect the Expression Titer in XpressCF+®



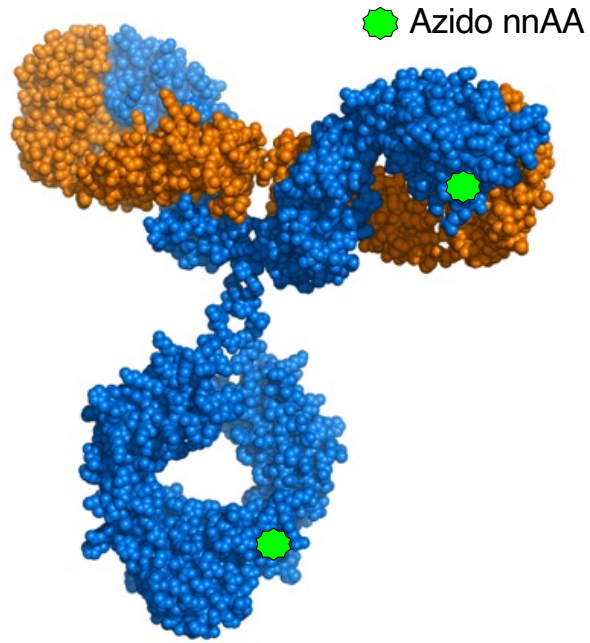
Other *in vivo* expression systems

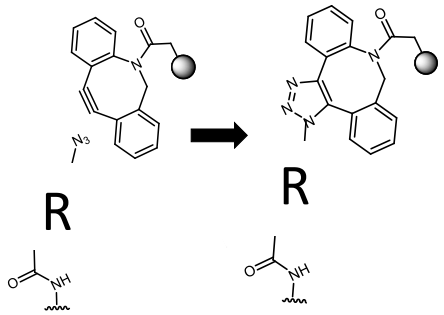


XpressCF+®

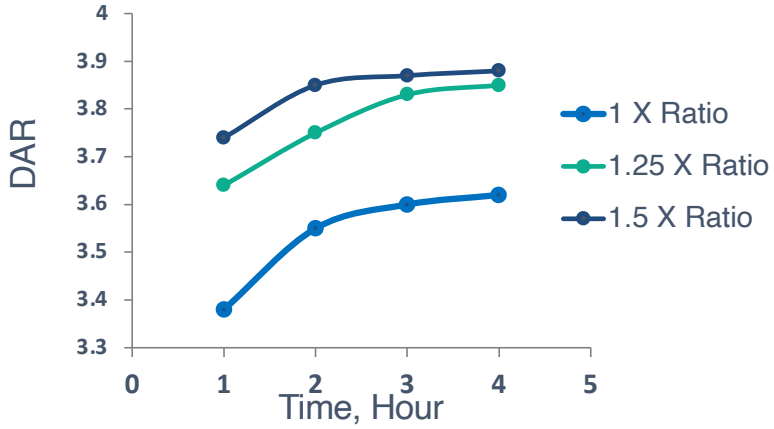
SUTRO BIOPHARMA

Azide Containing nnAA Enables Highly Efficient Cu free Click Conjugation Chemistry





1.25 X Molar Ratio results in conjugation (DAR=4) completion by 4 hr

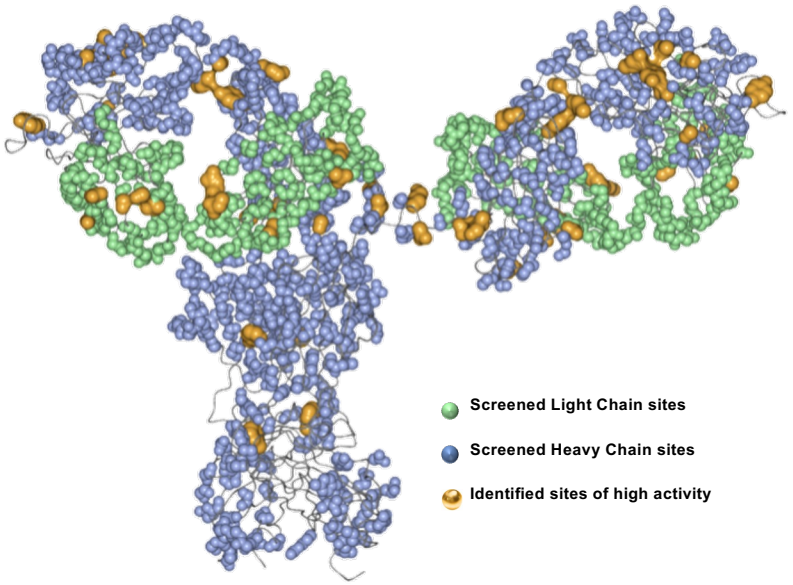


Conjugation technology is specific, irreversible, highly reactive and efficient in manufacturing

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

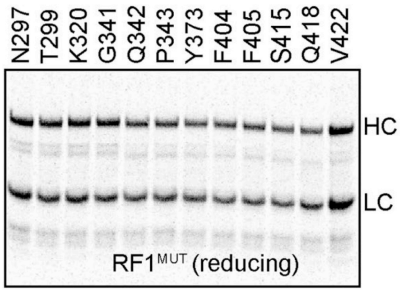
- Extensive screening of ~400 sites and site combinations conducted to identify sites that exhibit favorable characteristics
- These proprietary sites are utilized across various ADC programs at Sutro and may not be accessible through other conjugation technologies.
- Developing best-in-class ADCs

Surface Sites Screened on an IgG during an ADC Campaign:



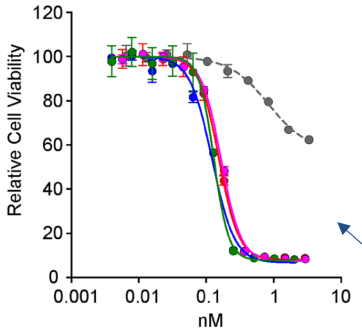
Site Selective, Not just Site Specific: Site Scan Selects Optimal Sites for Conjugation for Homogenous ADCs

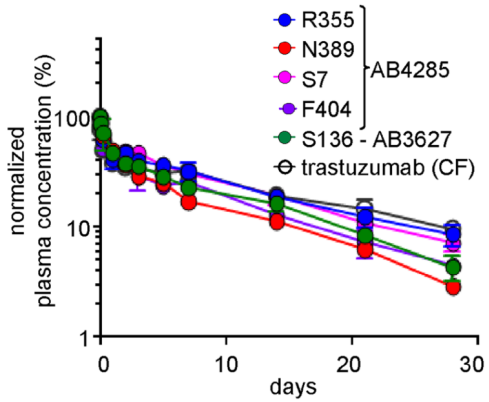
pAMF incorporation via amber suppression

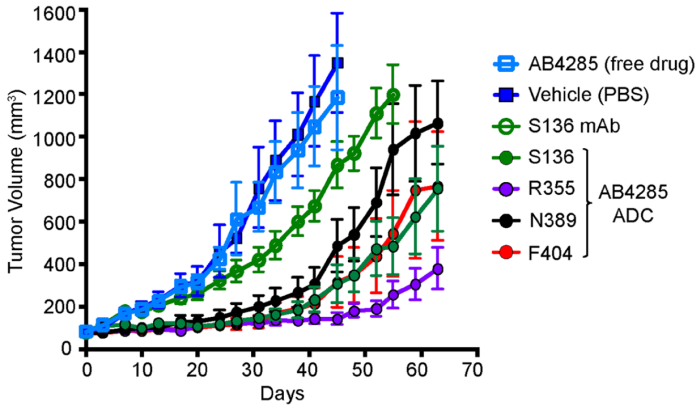


Trastuzumab ADCs conjugated to DBCO-MMAF

HER2+ cells





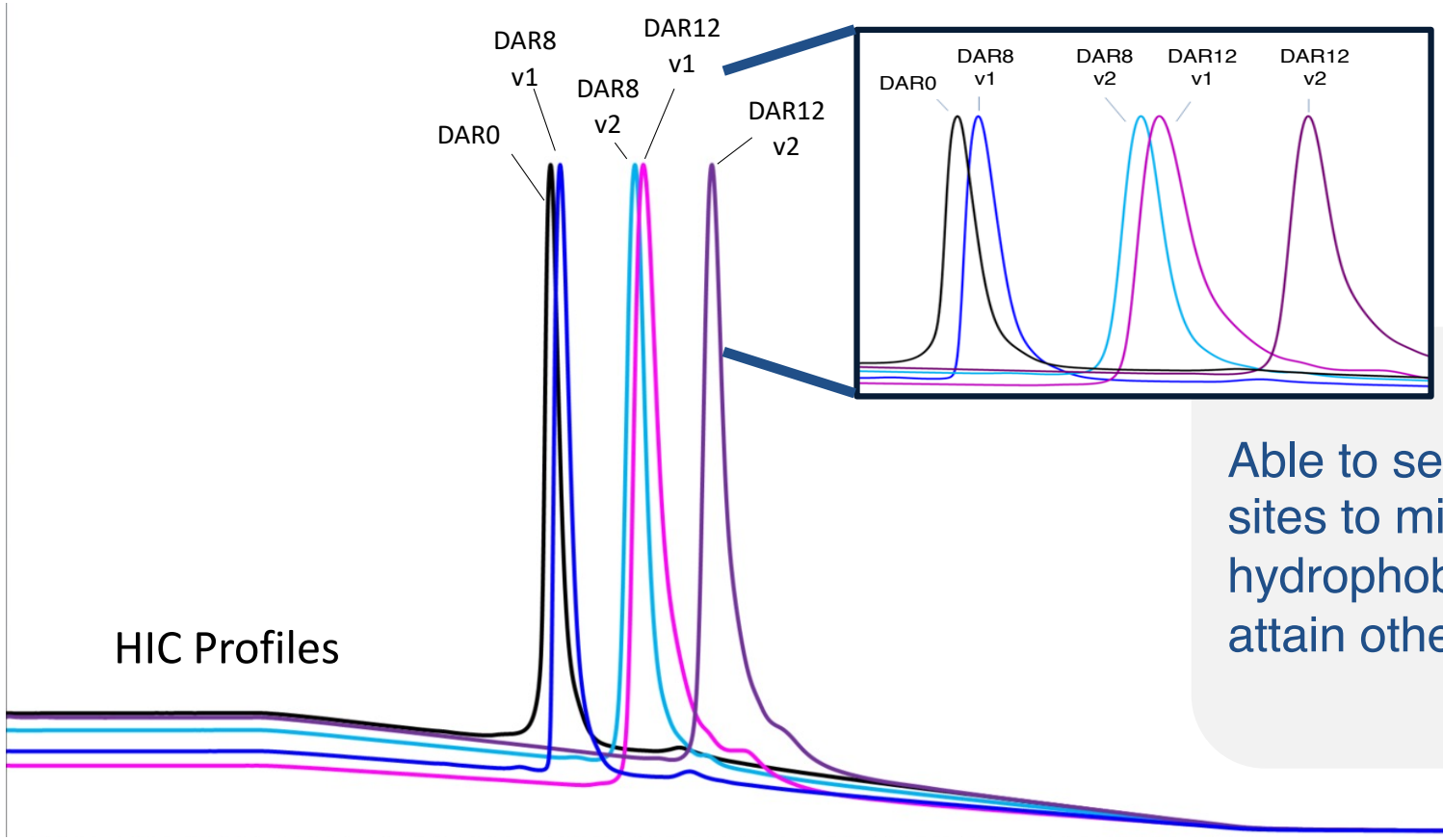


Even among the top sites identified from cell-killing assays, additional differentiation is possible in vivo.

Yin et al, Sci Rep **7**, 3026 (2017).

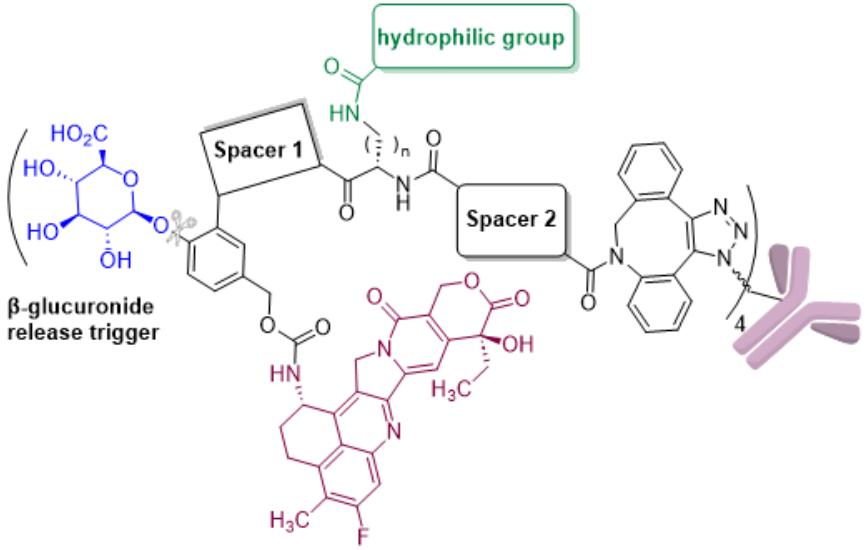
SUTRO BIOPHARMA

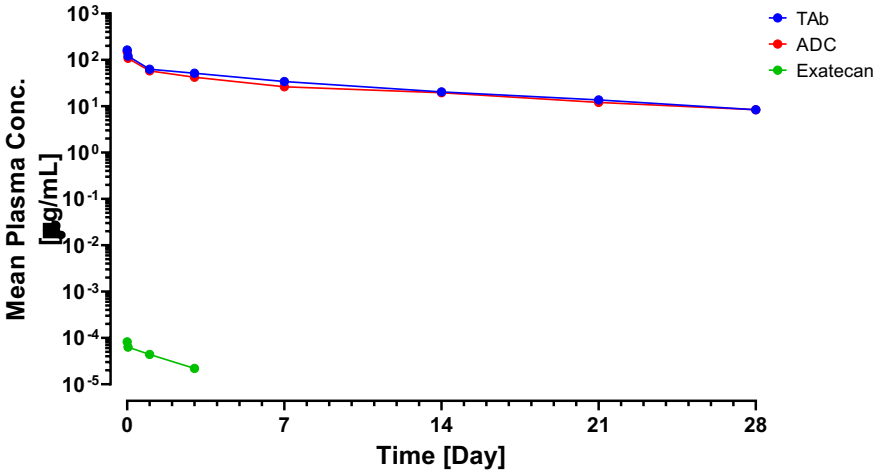
Choosing Conjugation Sites for Optimal Physicochemical Properties



Able to select conjugation sites to mitigate the hydrophobicity of ADCs and attain other desired attributes

Sutro's β-glucuronidase Exatecan Linker Designed for Enhanced PK

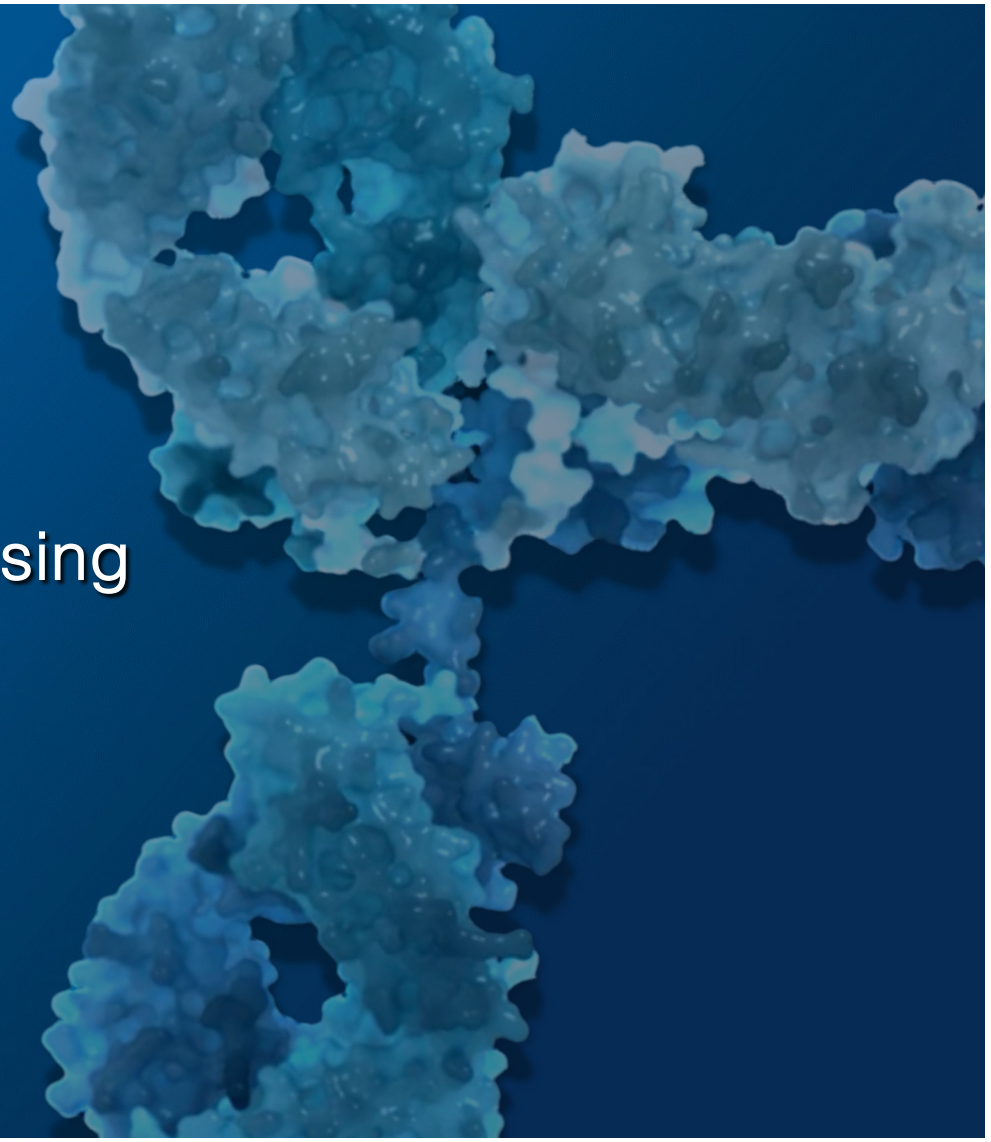




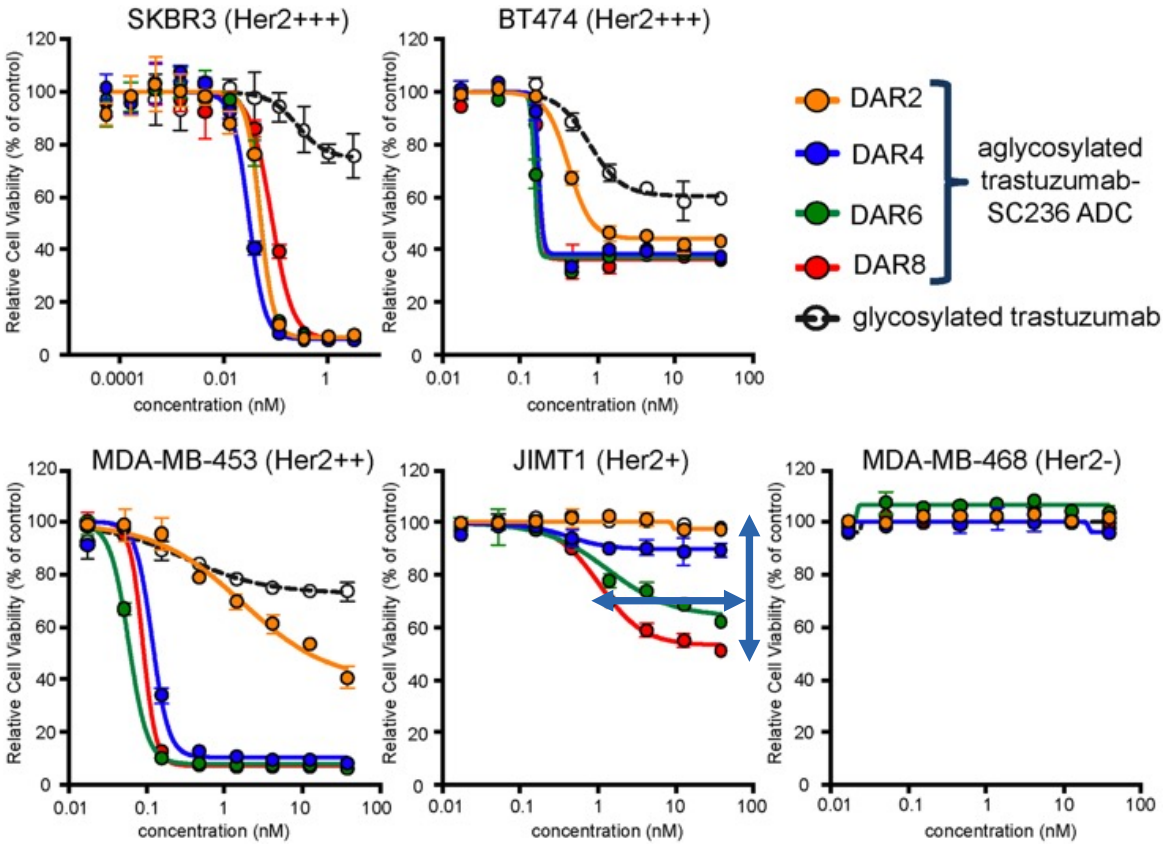
DAR8 ADC in Tg32 (5 mg/kg)

SUTRO BIOPHARMA

Higher DAR is Required for Targeting Low Antigen Expressing Cells



Lower Antigen Flux Requires Higher DAR for Cell-killing Activities

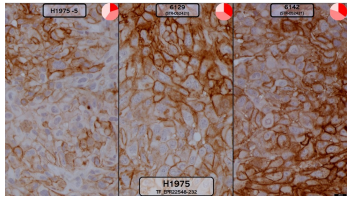


In higher expressing cell lines, lower DAR can achieve similar EC50 and Emax.

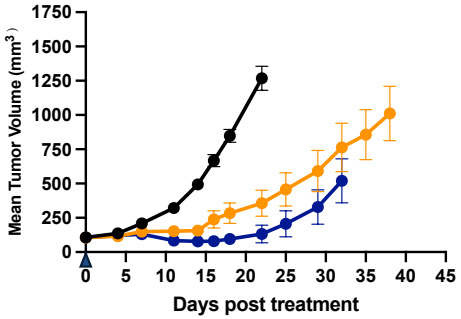
In lower expressing cell lines, higher DAR is required for delivering sufficient payload to drive PD.

Lower Antigen Expression Requires Higher DAR for Anti-tumor Activities

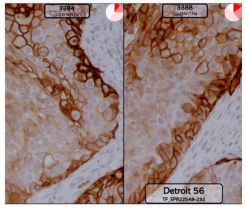
TF-high
(H1975, lung)



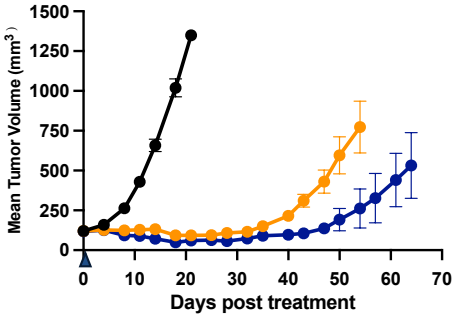
H-score = **200**
~65,000 copies/cell



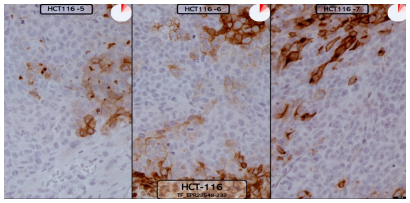
TF-medium
(Detroit562, HNSCC)



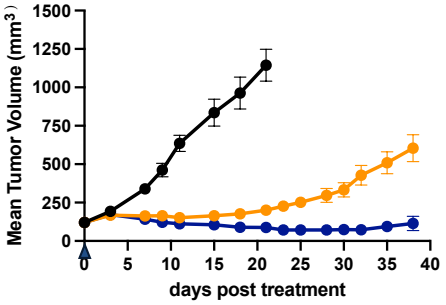
H-score = **118**
~80,000 copies/cell



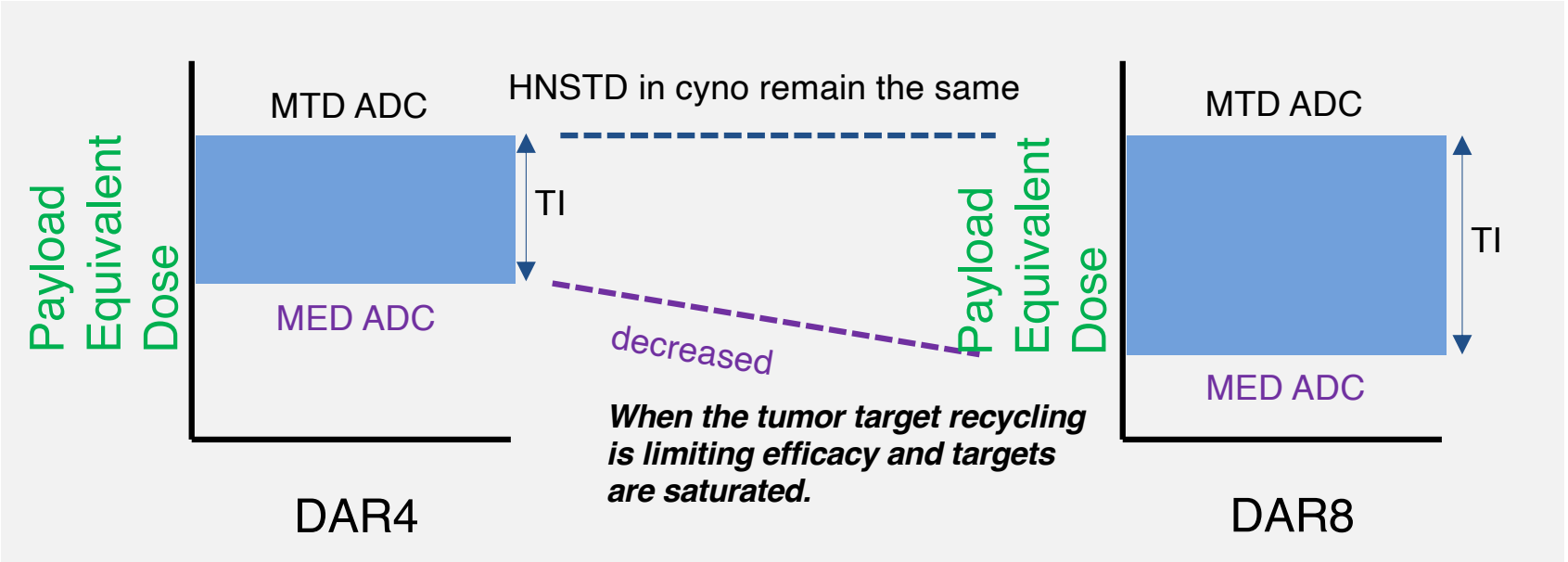
TF-low
(HCT116, CRC)



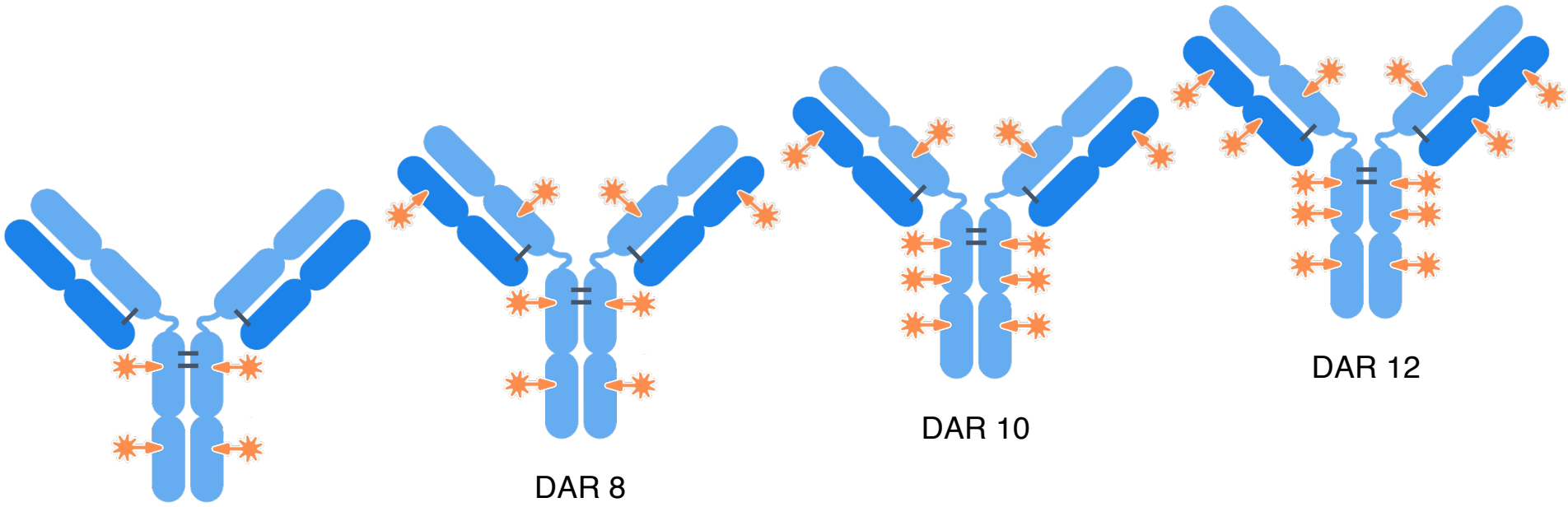
H-score = **37**
~20,000 copies/cell



Increased TI by Switching DAR4 to DAR8, Exemplified by Sutro ADC Programs

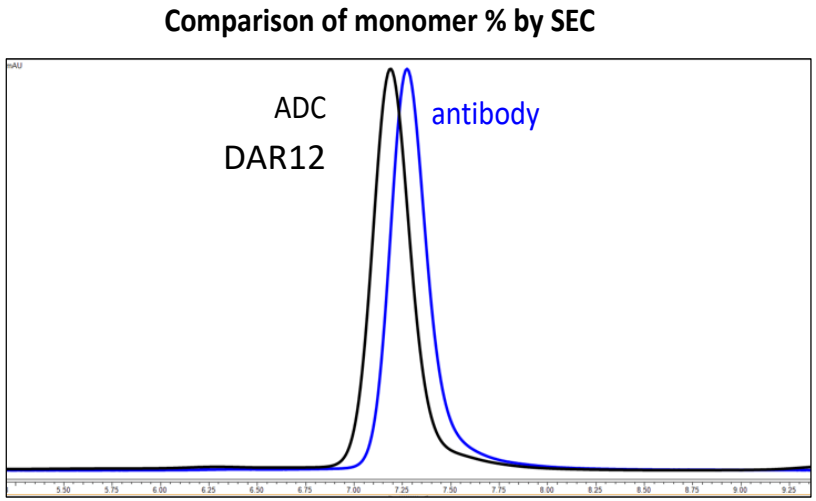


Pushing the Envelope: CF Platform Facilitates Adjustable DAR Values Ranging from 2 to 8+



Enhancing efficacy in target-low and heterogeneous tumors

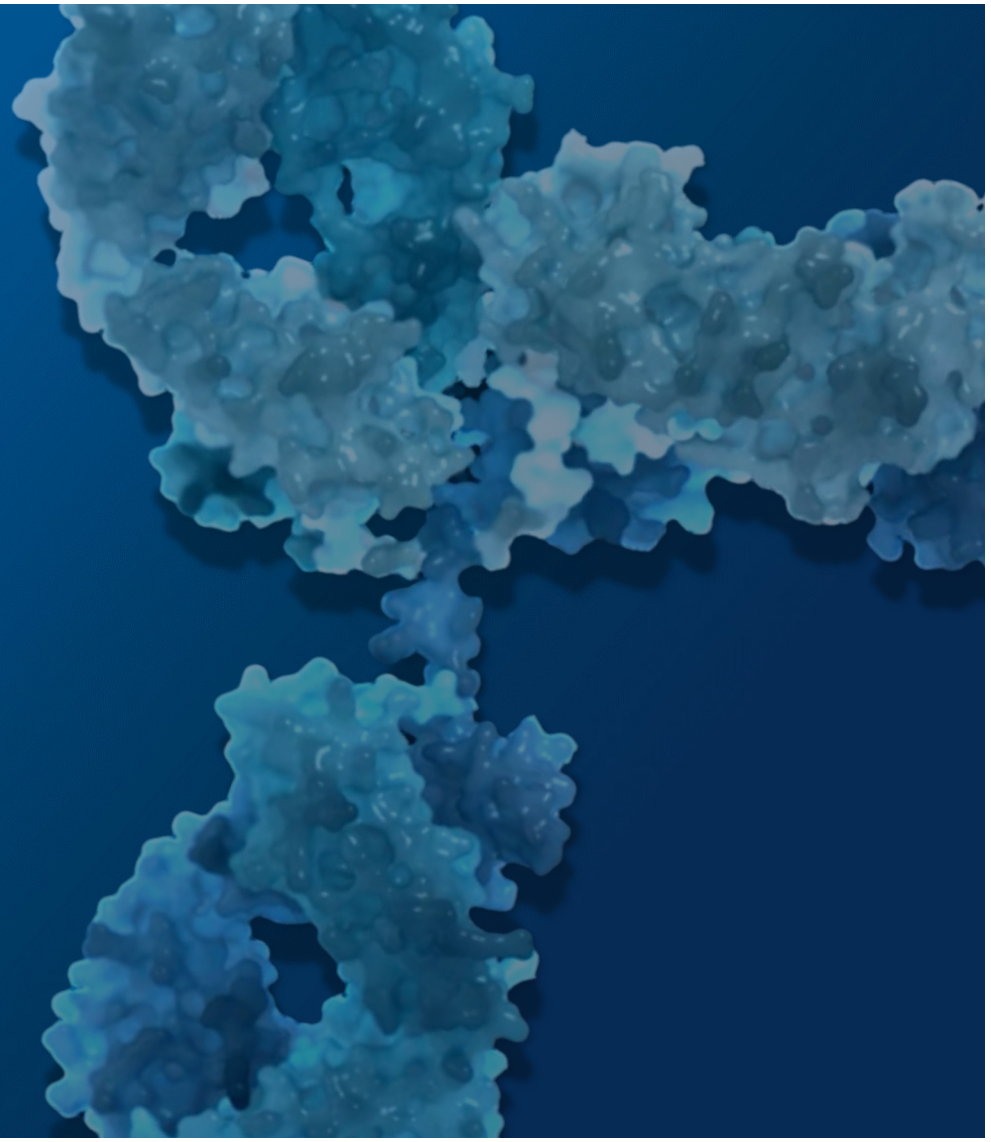
Conjugating 12x Payloads Achieved >95% Efficiency with Desirable Physico-chemical Properties w/o Aggregation or Precipitation



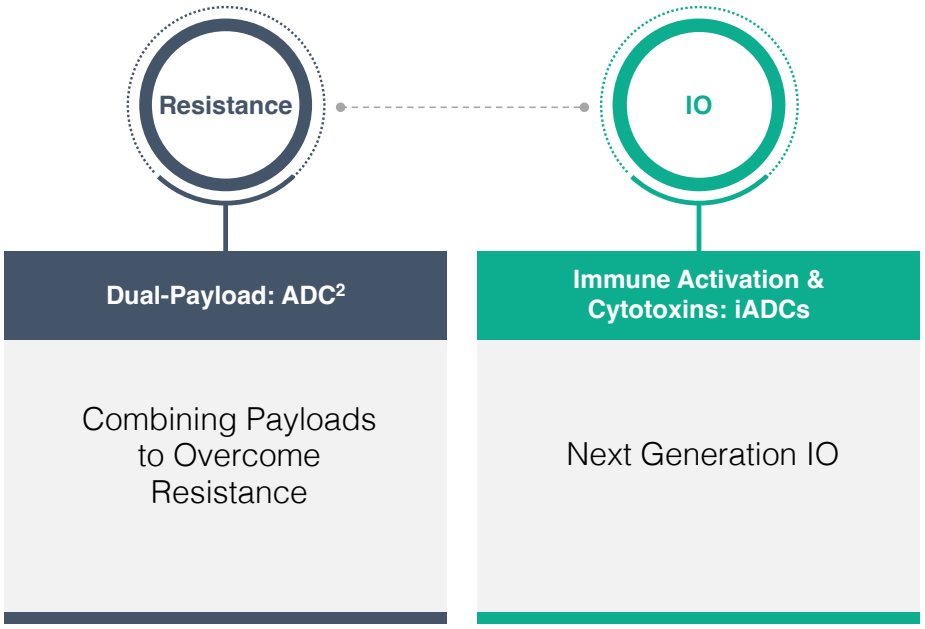
- ✓ High expression titer independent of DAR;
- ✓ High conjugation efficiency and rapid kinetics independent of DAR;
- ✓ Highly homogenous; no post conjugation purification needed
- ✓ Demonstrated favorable thermal, freeze/thaw, accelerated and long-term stability;
- ✓ Favorable developability
- ✓ Doesn't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

SUTRO BIOPHARMA

Enhancing ADC Efficacy by Payload Combination

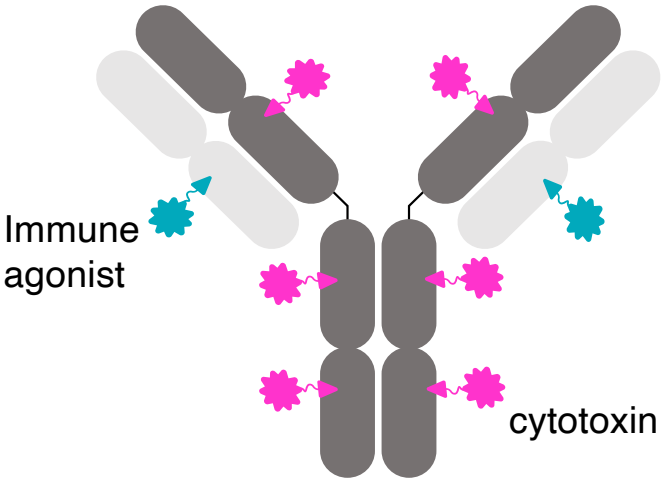


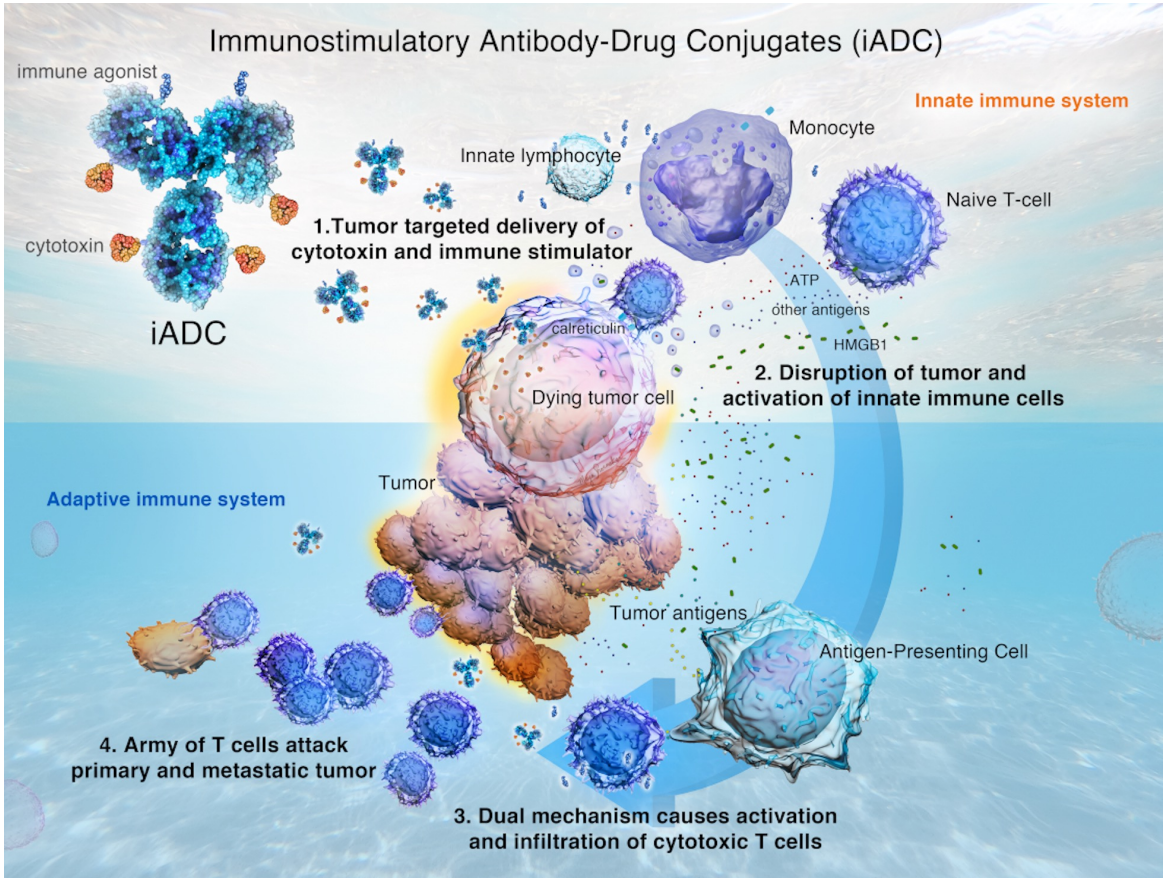
Dual-Payload ADC to Overcome Resistance or Activate Anti-tumor Immunity



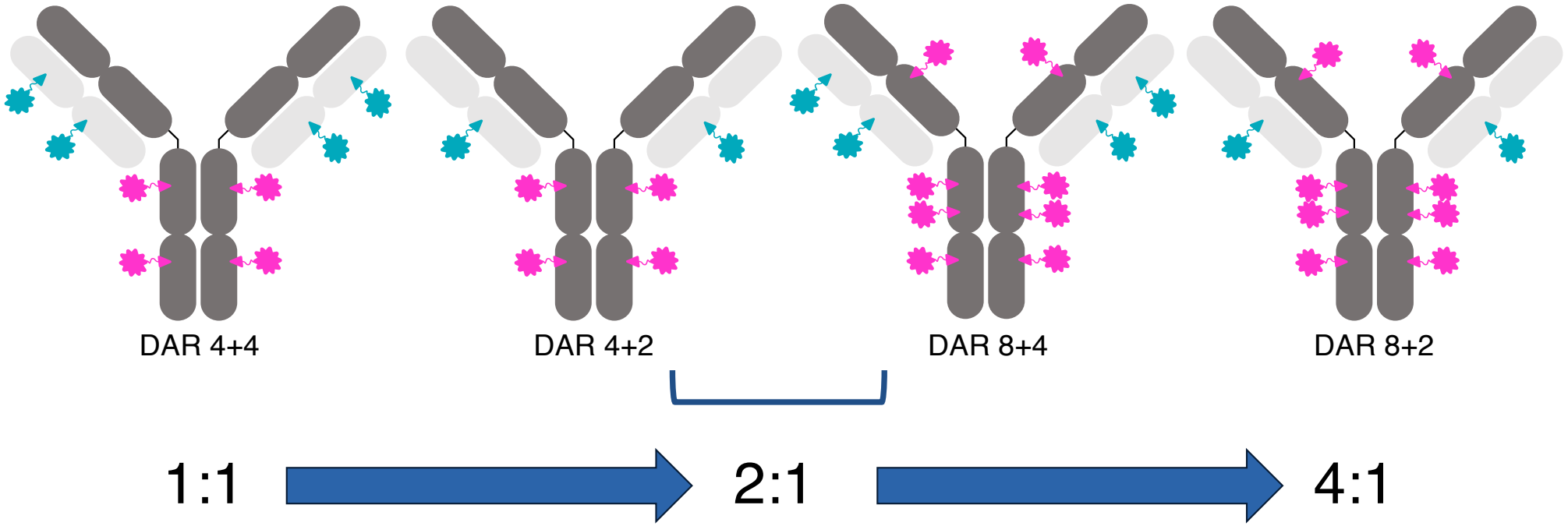
MTi: Microtubule inhibitor
DDRi: DNA damage response inhibitor

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





The Platform Facilitates Precise Tuning of the DARs and Ratio of Two Payloads Critical for Optimal Synergy of Two Mechanisms of Action

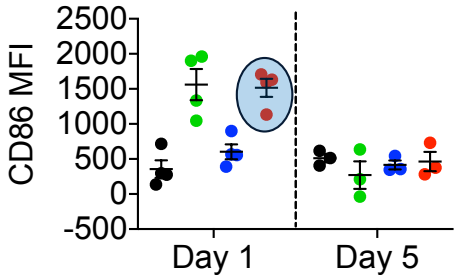


iADC Engaged Both Innate and Adaptive Immune Compartments in hTAA-MC38 Tumor Bearing Mice

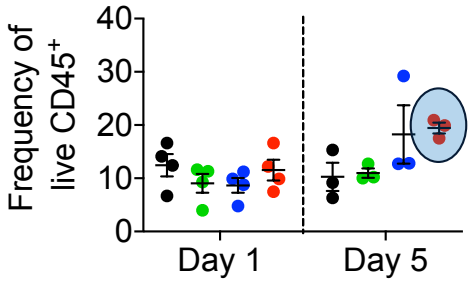
Innate | **Adaptive**

Tumor

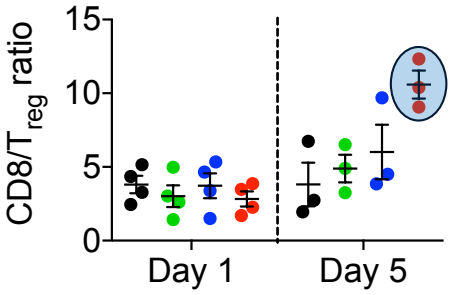
pDC



CD8⁺ T cells



CD8/T_{reg} ratio



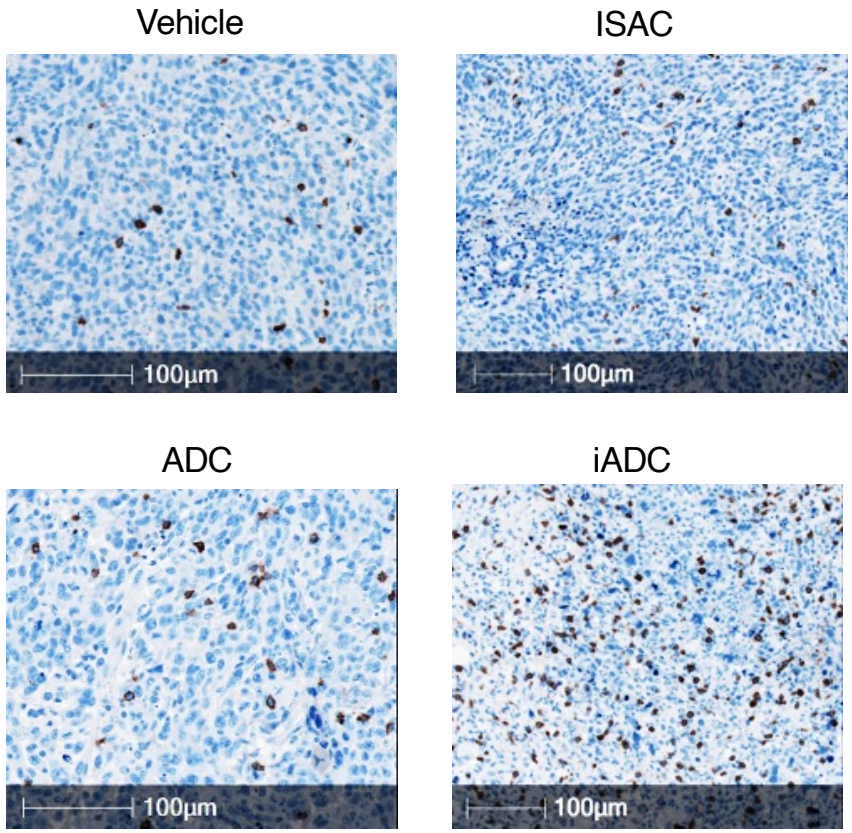
- Vehicle
- ISAC
- ADC
- iADC

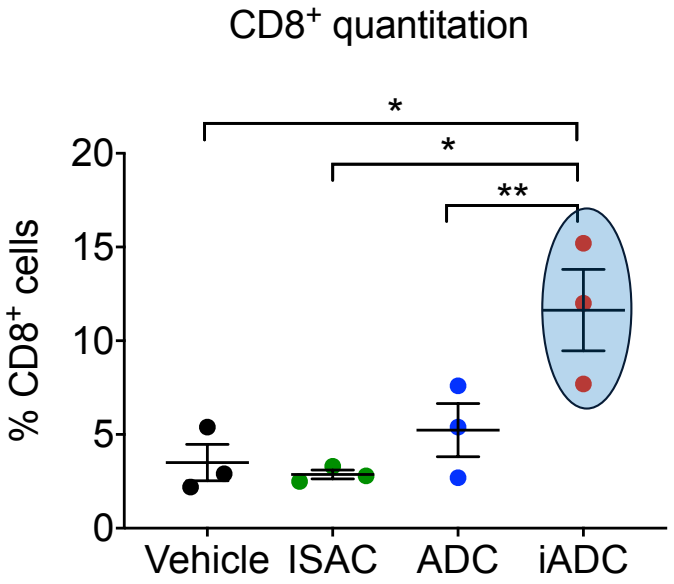
Early activation of pDCs following iADC and ISAC treatment

Followed by increased infiltration of CD8⁺ T cells and increased CD8/Treg ratio following iADC treatment

Single 10 mg/kg dose
Data Presented at FOCIS Meeting June 2022

iADC Increased CD8+ T cells in Tumor Microenvironment

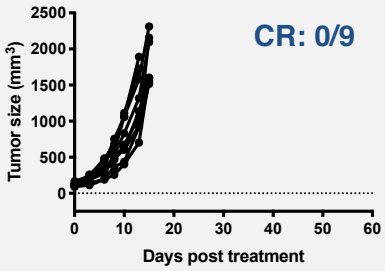




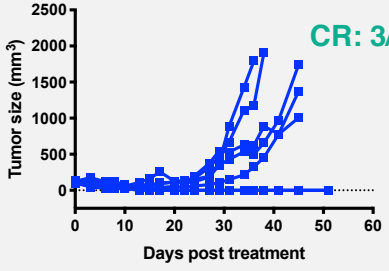
Data Presented at FOCIS Meeting June 2022

Superior and Durable Anti-Tumor Response with Single Dose of iADC vs. ADC Alone

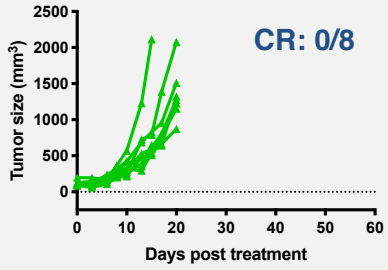
Vehicle



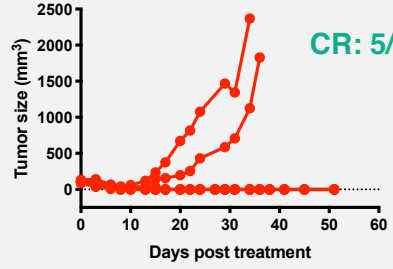
ADC
DAR4 CatB-Hemiasterlin



ISAC
DAR2 CatB-TLR7 Agonist

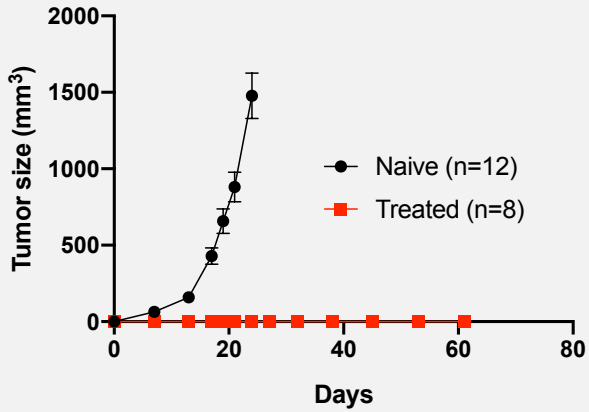


iADC
Hemiasterlin DAR4 + DAR2 TLR7



All CRs re-challenged with MC38-hTAA cells

Rechallenge



Data Presented at FOCIS Meeting June 2022

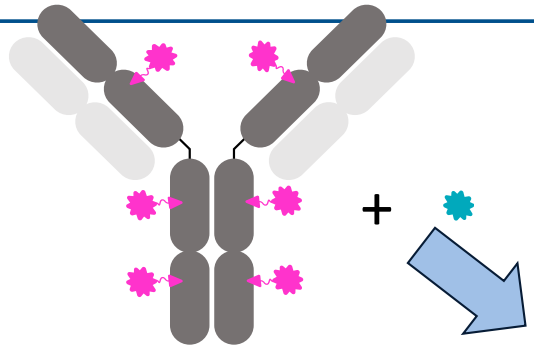
Novel Mechanism of Action Differentiates iADC from Other Immunotherapies

Sutro iADCs bridge innate and adaptive immunity to provide broad protection in a single molecule

	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
FcgR meditated uptake into myeloid			■			
Direct tumor cell killing	■				■	
Tumor antigen presentation	■		■			■
Priming and activation of Antigen Presenting Cells	■	■	■			■
T-cell recruitment to tumor	■	■	■	■	■	

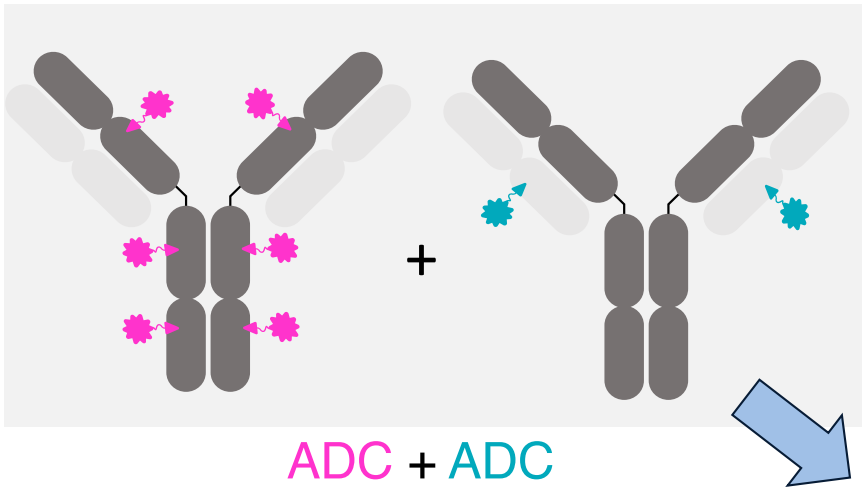
Mechanisms to achieve anti-tumor immunity

Dual-payload ADC Exhibits Potential Advantages over ADC/Chemo or ADC/ADC Combination



ADC + Chemo

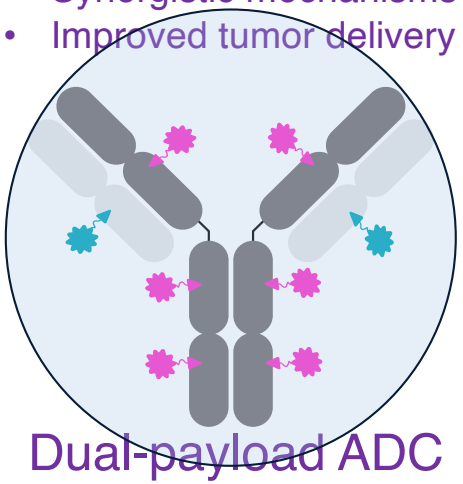
- Lacks tumor-targeted delivery
- Increased toxicity
- Narrower therapeutic window



ADC + ADC

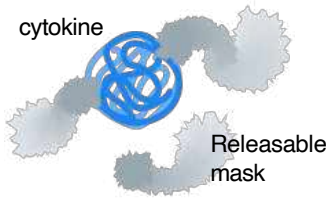
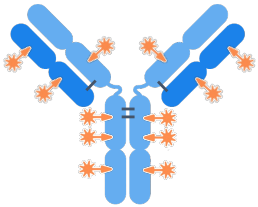
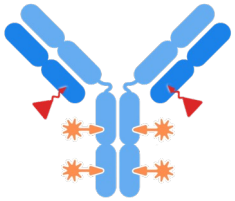
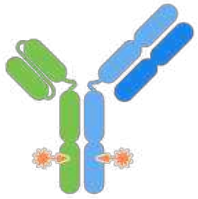
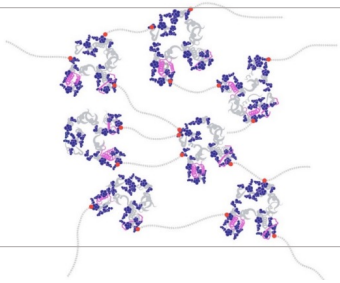
- Require separate
  - Pre-clinical & clinical dev.
  - Manufacturing
  - Regulatory
  - Treatment regimen

- Focused tumor targeting
- Synergistic mechanisms
- Improved tumor delivery

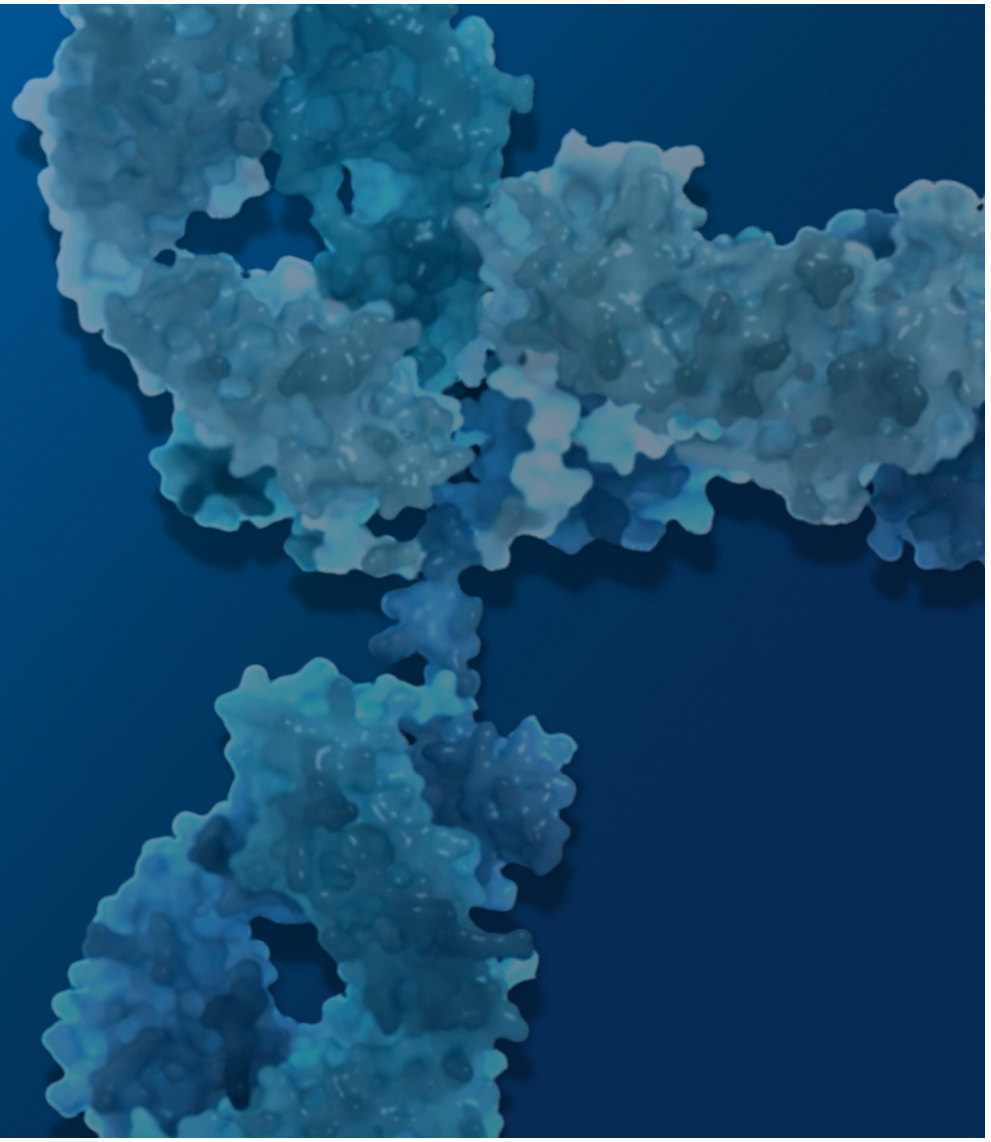


Dual-payload ADC

Drug Discovery Platform Can Enable Multiple Modalities

	Cytokine Derivative	Conjugated Antibody			Conjugate Vaccines
Modality	*Prodrug Cytokine Derivative*	*ADC*	*iADC* ***ADC²***	*Bispecific ADC*	*Multi-valent Conjugate Vaccine*
Target	Tumor Selective Mask	Tumor Antigen	Tumor Antigen	Dual Tumor Antigens	T-cell / B-cell Antigens
Structure					
Drug Properties	Prodrug cytokine targeting functional cytokine to tumor	ADC: targeting novel payloads with high DAR option	Site-specific dual drug conjugate with complementary modalities	Enhanced tumor targeting of cytotoxic payloads	Precise, site-specific conjugation sites on protein carrier, conjugated to polysaccharide antigens

SUTRO BIOPHARMA



Headquarters
111 Oyster Point Blvd
South San Francisco, CA 94080
P: 650.881.6500
F: 650.553.9659
W: www.SutroBio.com