SUTRO BIOPHARMA

Discovery of Novel Linker Payloads for Site-Specific ADCs with Improved Efficacy and Therapeutic Index

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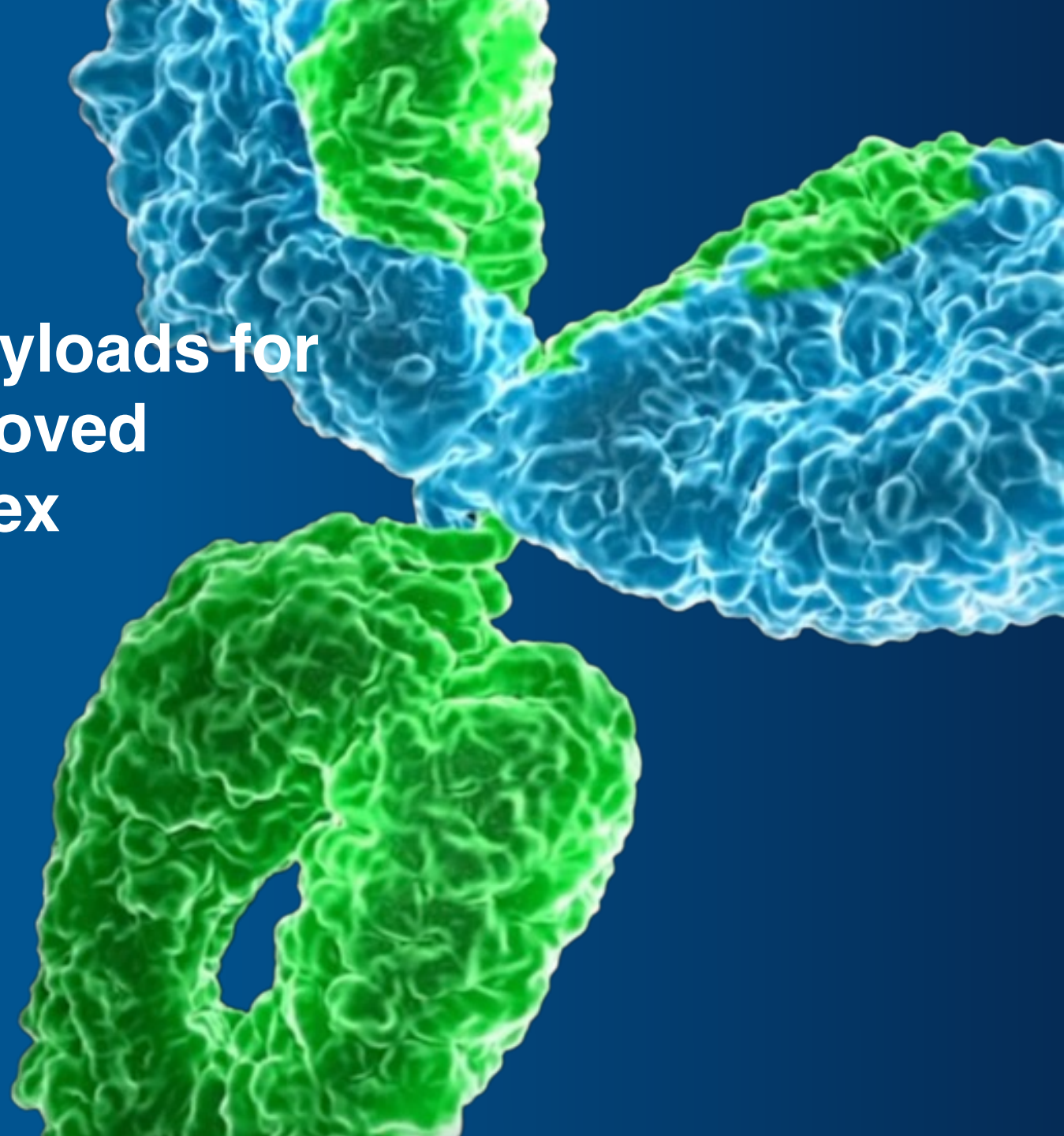
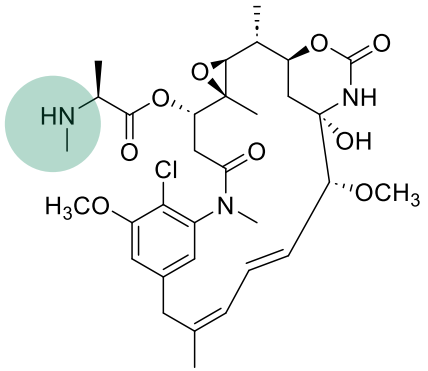
14th Annual WADC, San Diego 2023



Presentation Outline

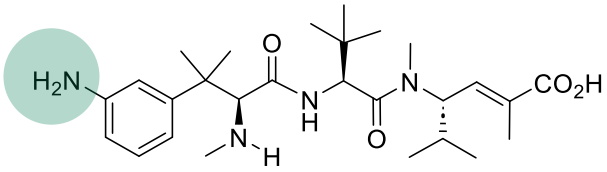
- Brief overview of the diverse classes of Sutro's linker payloads platform employed in the discovery and development of DAR4/8 site-specific ADCs
- Discovery of novel tumor-selective linkers and payloads explored in enhancing the efficacy and therapeutic index of α-ROR1 ADC (STRO-003)
- Highlighting Sutro's Site-Specific TAA ADCs utilizing the novel hydrophilic β-glucuronidase cleavable Exatecan linker payload

Expanding Sutro's Various Classes of Payload Platform for ADCs



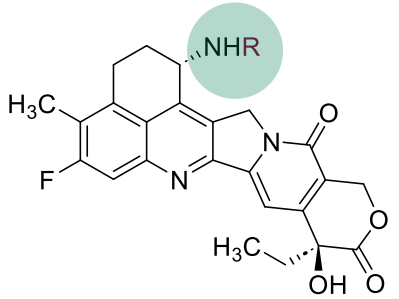
Maytansine

- Used on two Sutro clinical programs
- Tubulin inhibitor
- Can be used with cleavable and non-cleavable linkers
- Extensive clinical track record
- IC_{50}: 22 - 64 nM



Hemiasterlin Analog

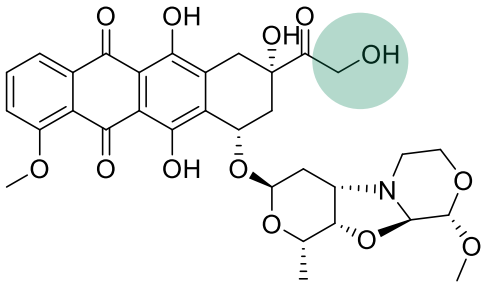
- Sutro novel payload asset used in two clinical programs
- Tubulin inhibitor
- Reduced P-gp efflux liability- best in class within tubulin binders
- Induces strong ICD
- IC_{50}: 0.3 - 4.2 nM



R = H (Exatecan)
R = Gly (Gly-Exatecan)

Exatecan/Gly-Exatecan

- Topo1 Inhibitor
- Close analog to Daiichi DXd-ADC
- More potent Topo1 inhibition and cell killing than CPT-11, SN-38, Topotecan
- Multiple novel linker payload for optimized TI's
- Not a P-gp substrate
- IC_{50}: 0.32 - 12nM/3.8-44 nM



PNU anthracycline class

- Topo1-inhibitor, multiple effects on DNA including intercalation and alkylation
- High potency, no need for high DAR
- Multiple stable linkers in evaluation
- Not a P-gp substrate
- strong ICD inducers, best in class activators of DCs
- IC_{50}: 0.01 - 0.05 nM

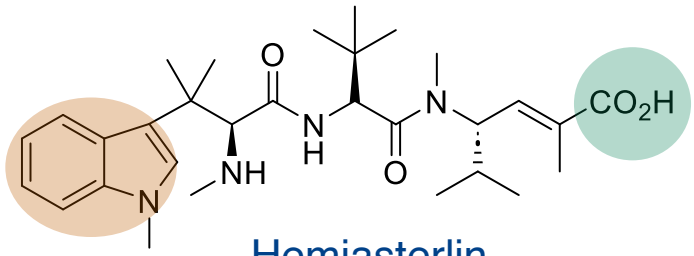
Hemiasterlins: From Natural Product to ADC Payload

hemiasterella minor

Isolation & characterization

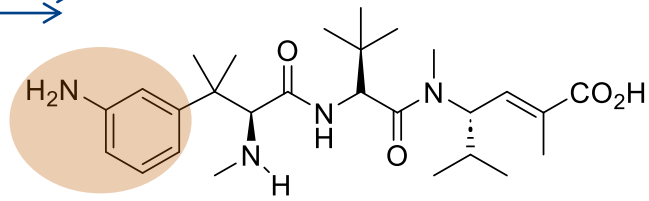
Tet Letters, 1994, 4453

Tetrahedron, 1995, 10653



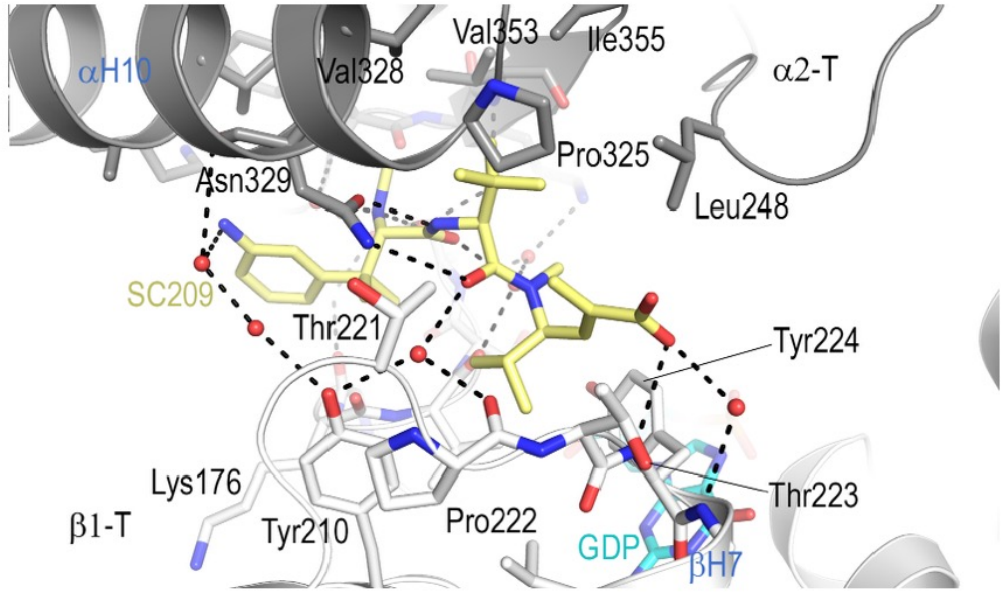
Hemiasterlin

SAR



SC209

- Co-crystallization of SC209 is binding to the vinca-site of two α,β-tubulin interdimer interface
- Sutro's tubulin inhibitor class payload for ADC programs
- Low to sub nM cell killing activity across various cancer cells
- Active against P-gp overexpressing cells
- Induced strong ICD, characterized by secretion of damage-associated molecular patterns (DAMPs)
- vc-SC209 (SC239) optimized LP utilized for two DAR4 ADC clinical programs



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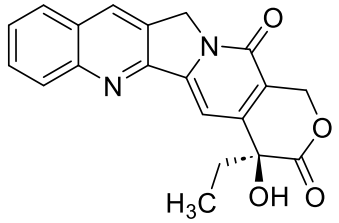
CPT Derivatives and DNA Targeting Cytotoxins Explored as ADCs

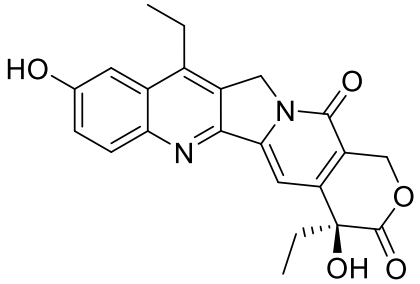
Camptothecins (DNA topoisomerase I inhibitor)

Camptotheca acuminata

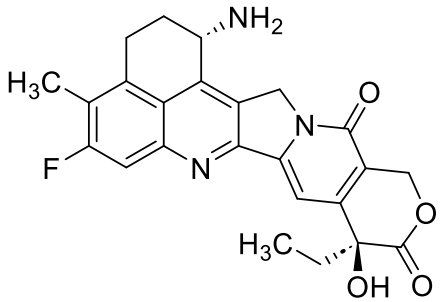


Camptothecin

Camptothecin (CPT) derivatives

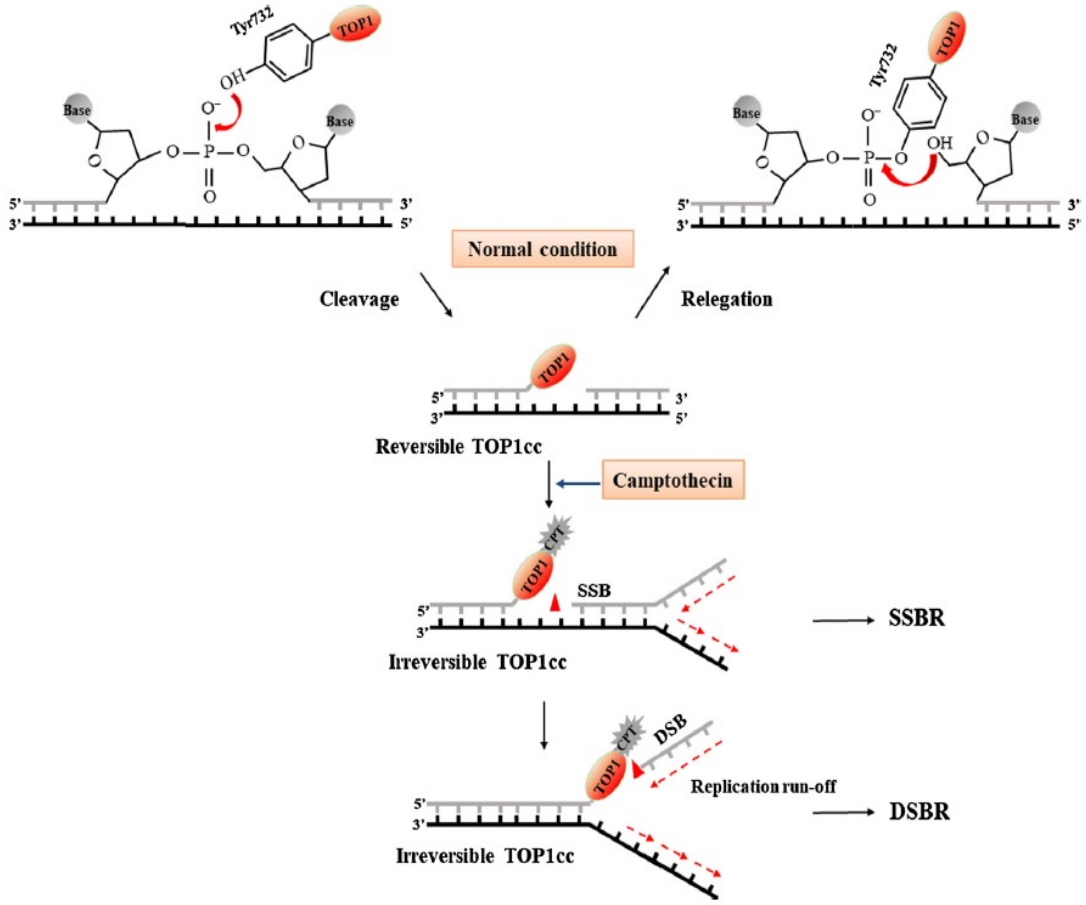


- **SN-38 (SC2609)**
- Poor solubility
- Less potent Topo-1 inhibition
- Catabolite to β-Glu SN-38 not potent



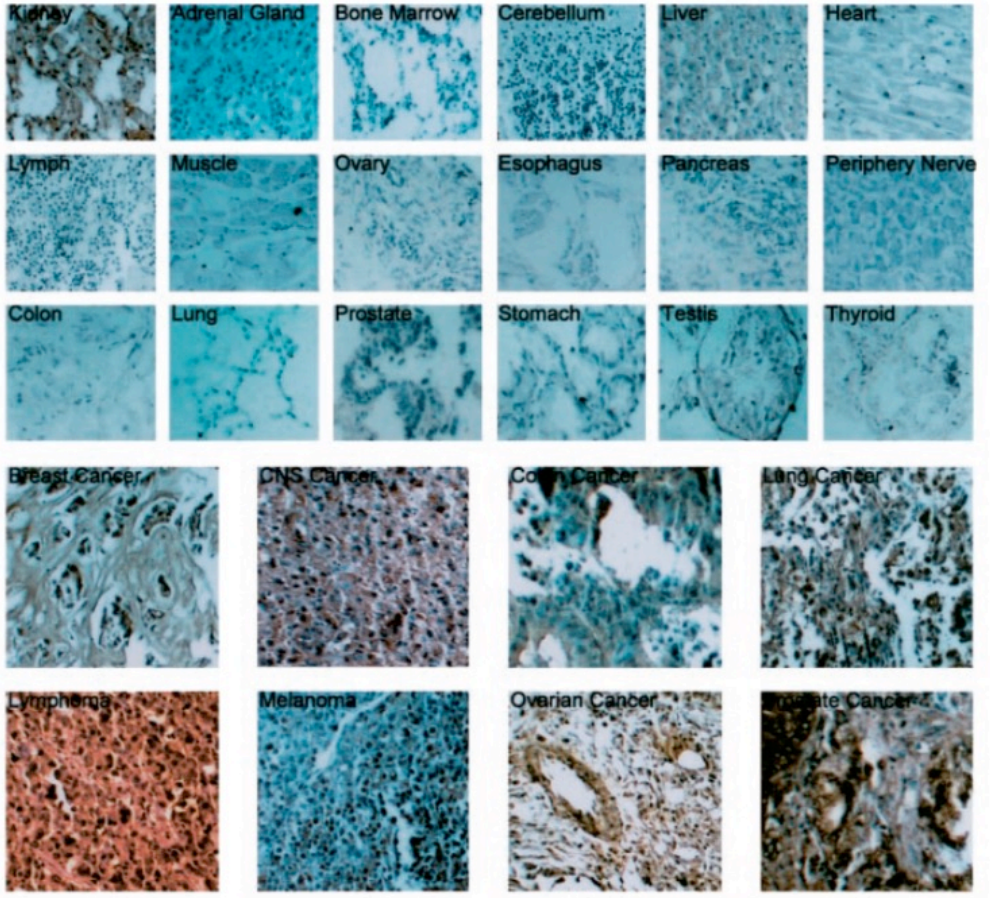
- **Exatecan (SC3386)**
- More stable lactone, not a P-gp substrate
- Water soluble CPT derivative

CPTs induce DNA damage through trapping of Topoisomerase 1



Biomedicine & Pharmacotherapy (2020) 109875

Legumain (LGMN) Expression in Human Tumors for ADC Linker



- Legumain expression in normal human tissues and tumors

- The Cys protease Legumain, also known as Asparaginyl endopeptidase, specifically cleaves Asn amide bonds at acidic pH
- Legumain is overexpressed in the majority of human solid tumors
- This protease is known to be upregulated in multiple cancer types, actively in cancer invasion and metastasis

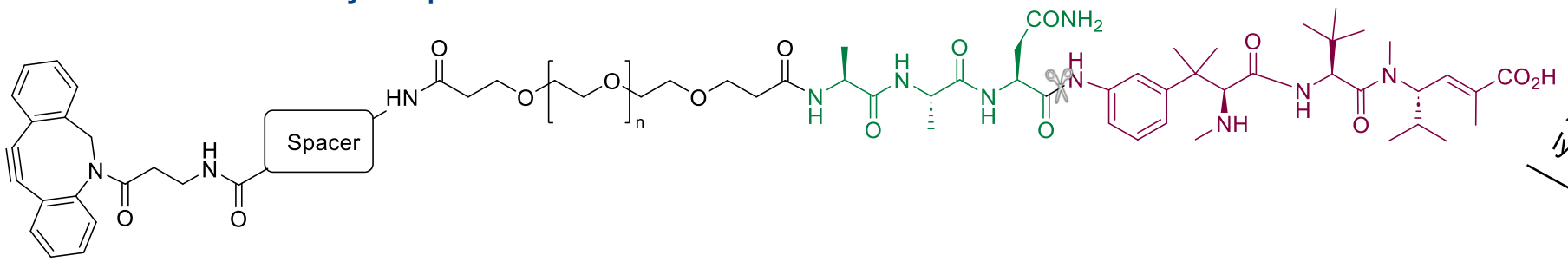
Table 1 *Legumain detection in human solid tumors*

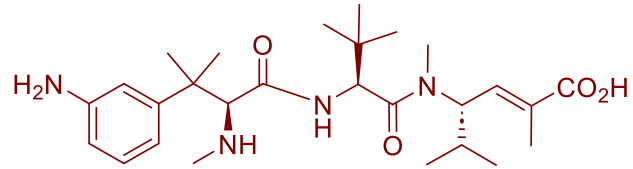
Carcinoma type	Number analyzed	Number positive	Percentage positive	Degree of positivity
Breast carcinoma	43	43	100%	+++
Colon carcinoma	34	32	95%	+++
Lung carcinoma	24	14	58%	+++
Prostate carcinoma	56	42	75%	++++
Ovarian carcinoma	23	17	73%	++
Central nervous system tumors	8	8	100%	++
Lymphoma	14	8	57%	+
Melanoma	12	5	41%	+

Cancer Res. 2003 Jun 1;63(11):2957-64

Legumain Protease Cleavable Hemiasterlin and Topo1i LP's for ADCs

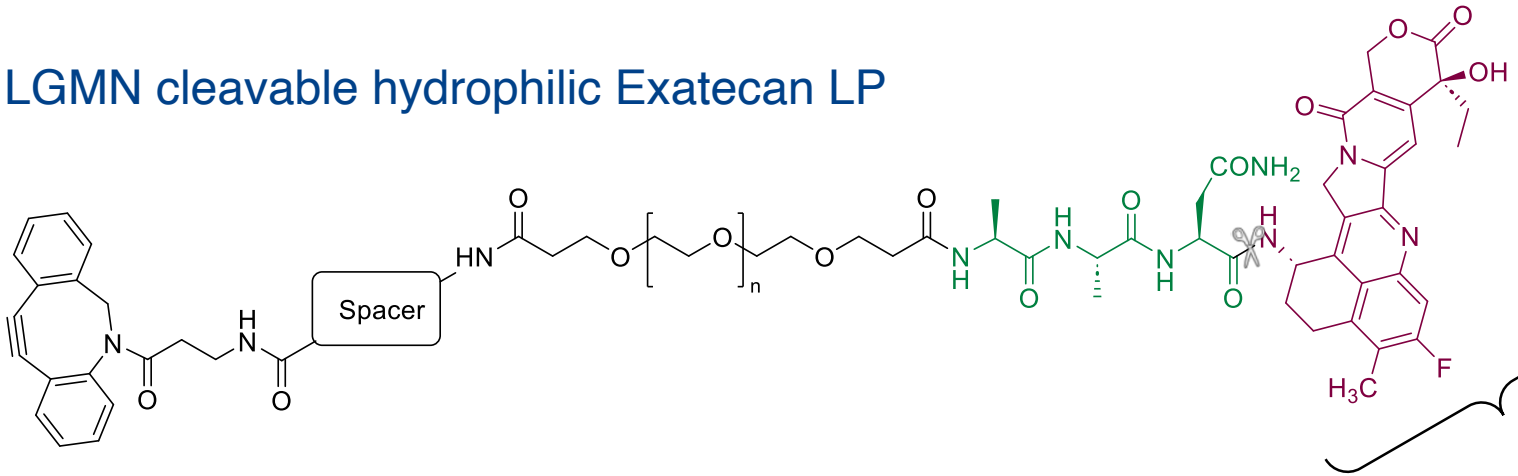
LGMN cleavable hydrophilic Hemiasterlin LP





Hemiasterlin Payload (SC209)

LGMN cleavable hydrophilic Exatecan LP



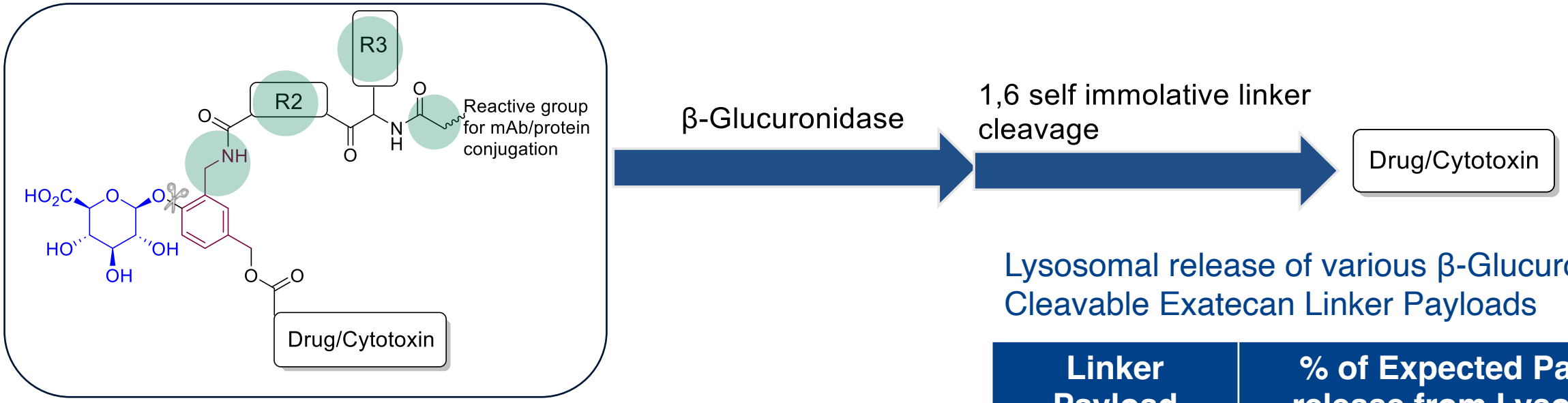
Exatecan Payload (SC3386)

- Selective to the LGMN
- Stable to hNE
- It is stable to serine proteases and or other cysteine proteases
- No pABC required, less hydrophobic and stable to Ces1C

β-Glucuronidase (β-Glu) Overview

- β-Glu is overexpressed in the majority of known solid and blood cancers
- β-Glu is only active and present in cancer cell lysosomes and tumor necrotic regions
- Active only at acidic pH
- Non peptide based, stable to hNE & or to other serine/cysteine proteases
- Minimal expression in normal cells, not active at physiological pH
- Intrinsic hydrophilicity due to the sugar linker
- Serum stable linker

Design of Novel Proprietary Tumor Specific β-Glucuronide Linker for ADCs



Several β-Glu cleavable linkers have been explored across diverse vectors to optimize the proprietary β-Glu linkers for ADCs

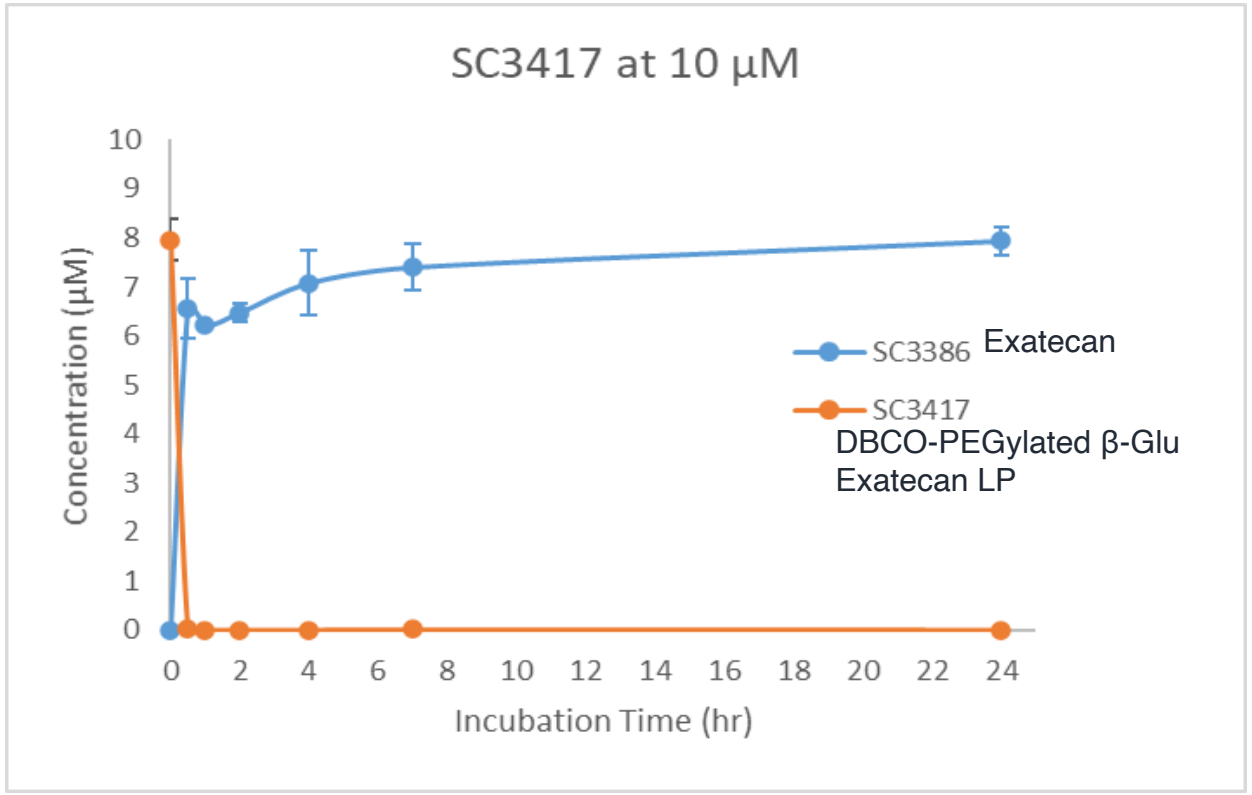
- Improved stability
- Improved hydrophilicity
- Improved Enzyme release kinetics
- Optimized hydrophilic linker enabling high DAR ADCs with improved PK and physicochemical properties

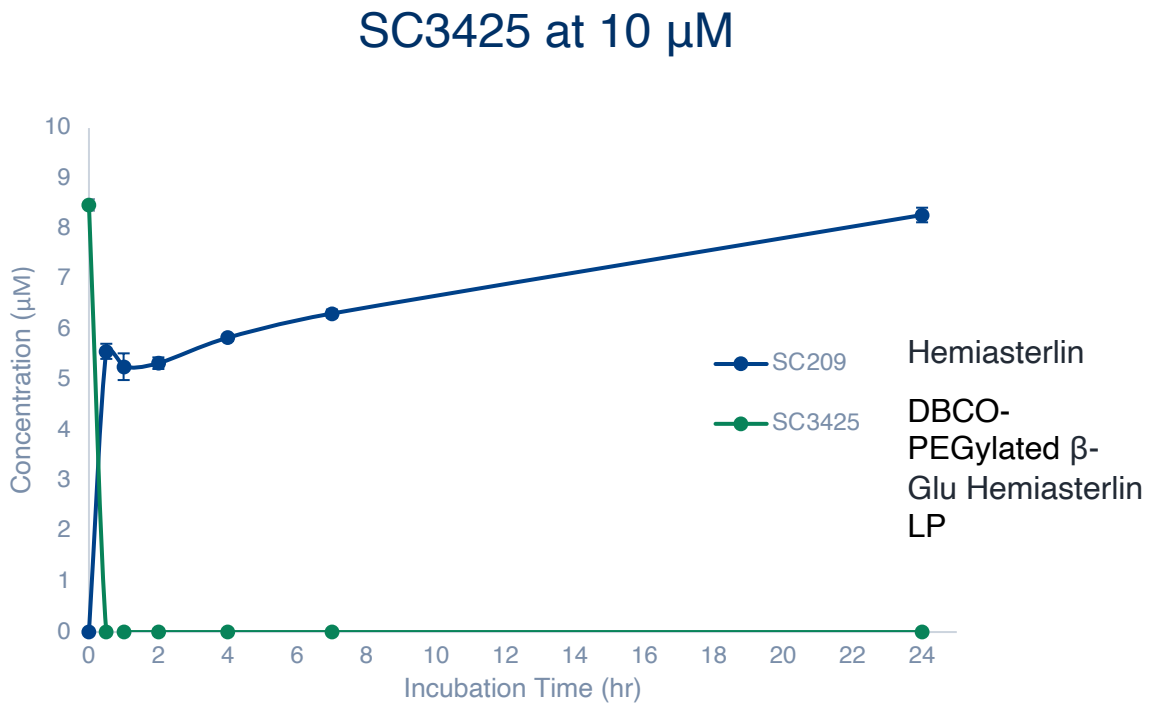
Lysosomal release of various β-Glucuronidase Cleavable Exatecan Linker Payloads

Linker Payload	% of Expected Payload release from Lysosomal Incubation @ 24h
SC3417	98.2%
SC3730	90.6%
SC3731	100%
SC3732	86.2%

β-Glucuronidase Enzyme Release of Optimized Hydrophilic β-Glu Linker Payloads

β-Glu enzyme cleaves novel hydrophilic β-Glu cleavable Exatecan (SC3417) and β-Glu cleavable Hemiasterlin (SC3425) LPs

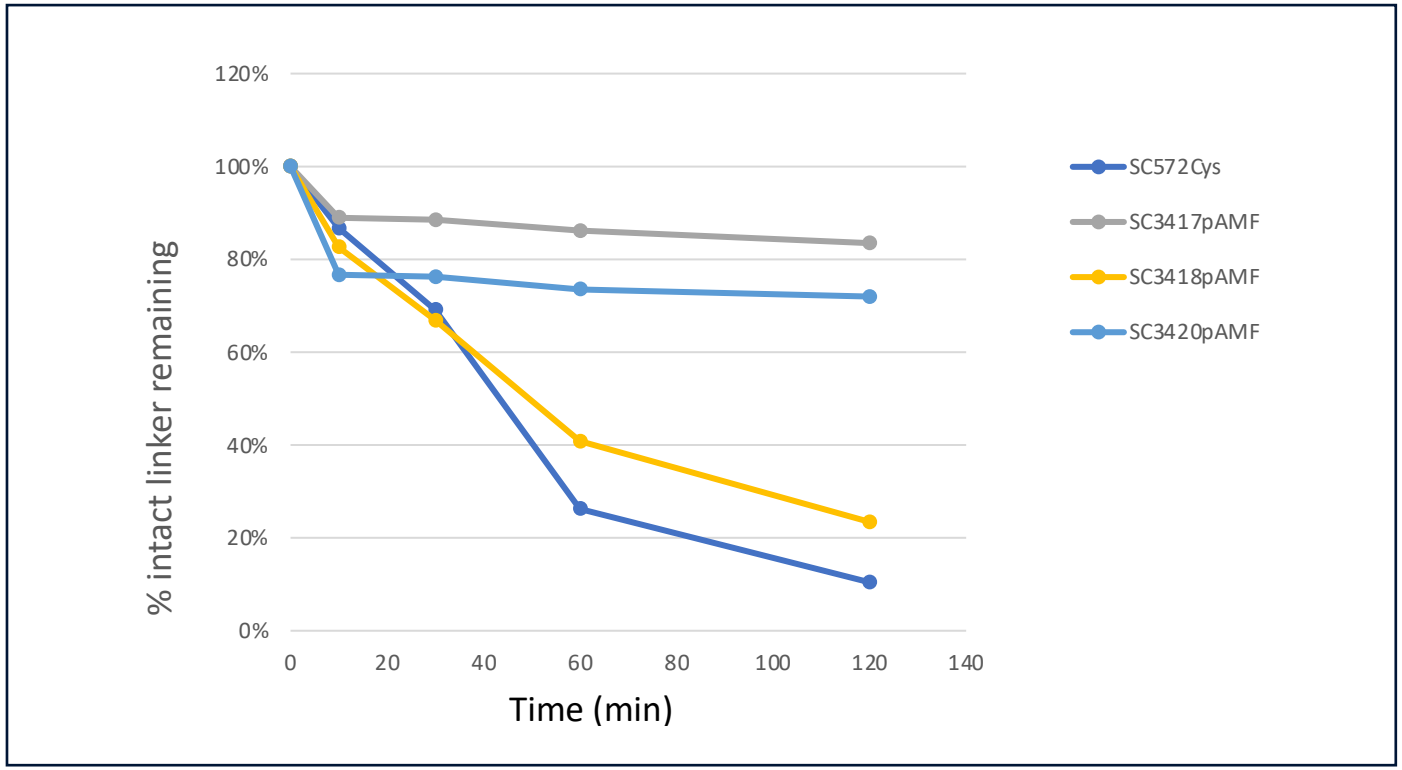




- The Optimized β-Glu Exatecan (SC3417) and β-Glu Hemiasterlin (SC3425) LPs exhibited comparable corresponding payload release concentrations of SC3386 and SC209

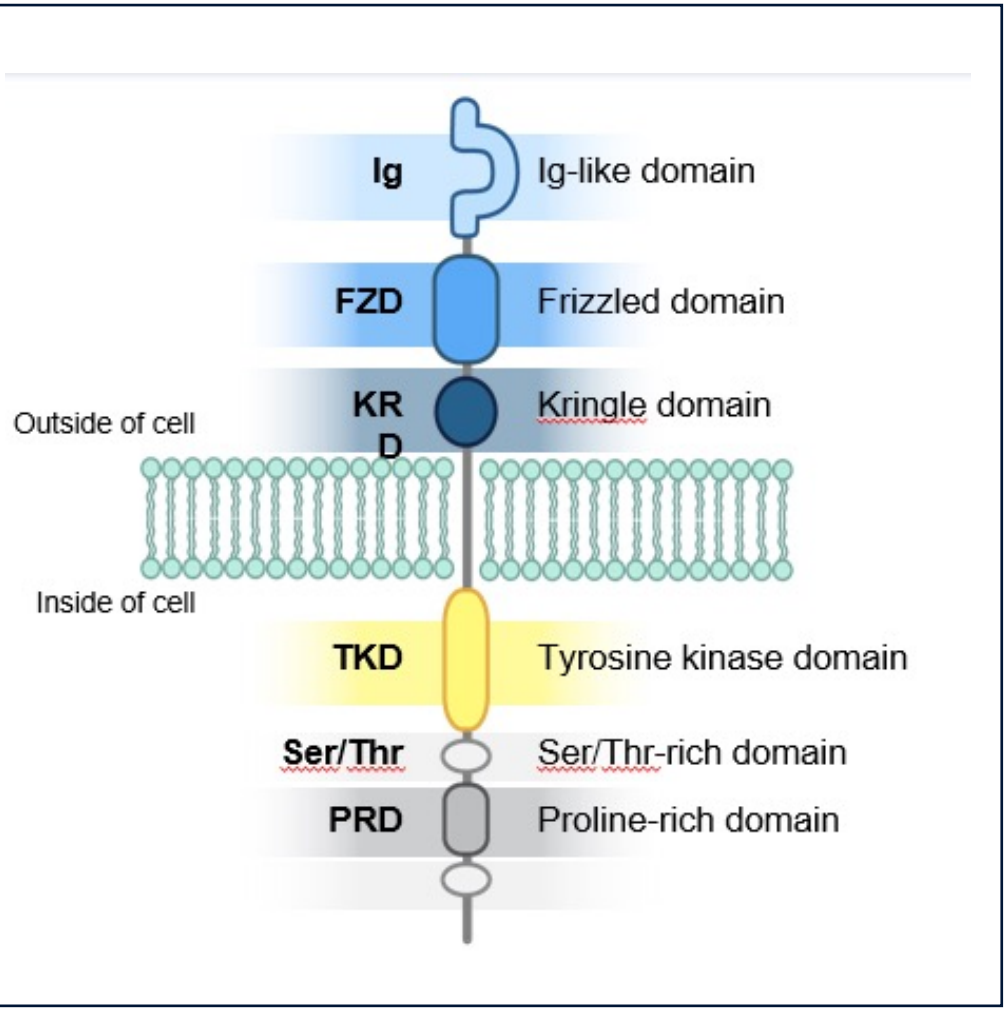
human Neutrophil Elastase (hNE) Stability of Various Linker Payloads

hNE Cleavage of Different pAMF/Cys Quenched Linker Payloads



- hNE cleavage was observed for SC572Cys (vcMMAE) ~ SC3418pAMF (cathepsin cleavable Exatecan LP)>> SC3420pAMF (LGMN cleavable Exatecan LP)
- No release is observed for SC3417pAMF (β-Glu cleavable Exatecan LP) and is stable in the hNE assay for 2h

ROR1 Background



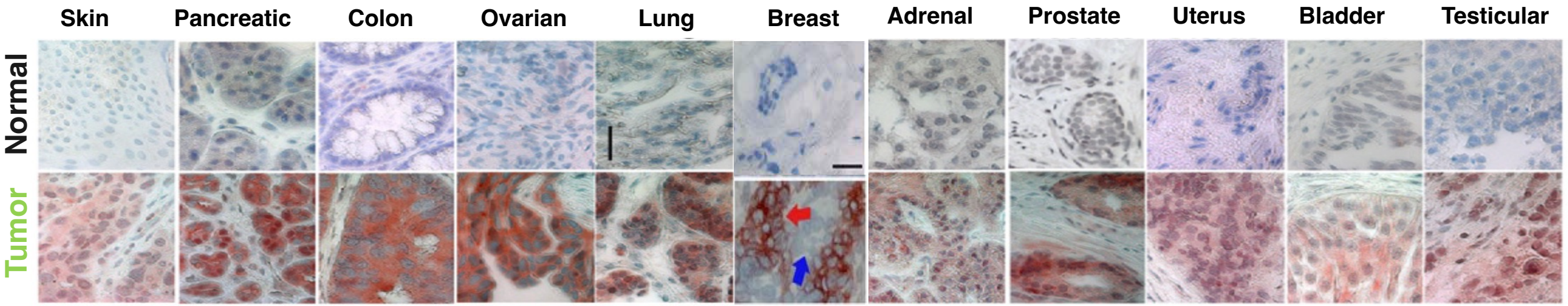
Schematic domain structure of ROR1

- Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is a cell-surface, onco-fetal protein whose expression is correlated with oncogenic properties such as enhanced proliferation, survival, and chemoresistance
- ROR1 expression is highly associated with epithelial-mesenchymal transition EMT genes and the silencing of ROR1 reduces the ability of MDA-MB-231 cells
- Tyrosine Kinase ROR1 as an attractive Target for Anti-Cancer Therapies

- *Zhao et al. Frontiers in oncology May 2021*
- *Nicholas et al. Protein Cell 2014, 5(7):496–502*

ROR1 is an Attractive ADC Therapeutic Target for Solid/Hematological Malignancies

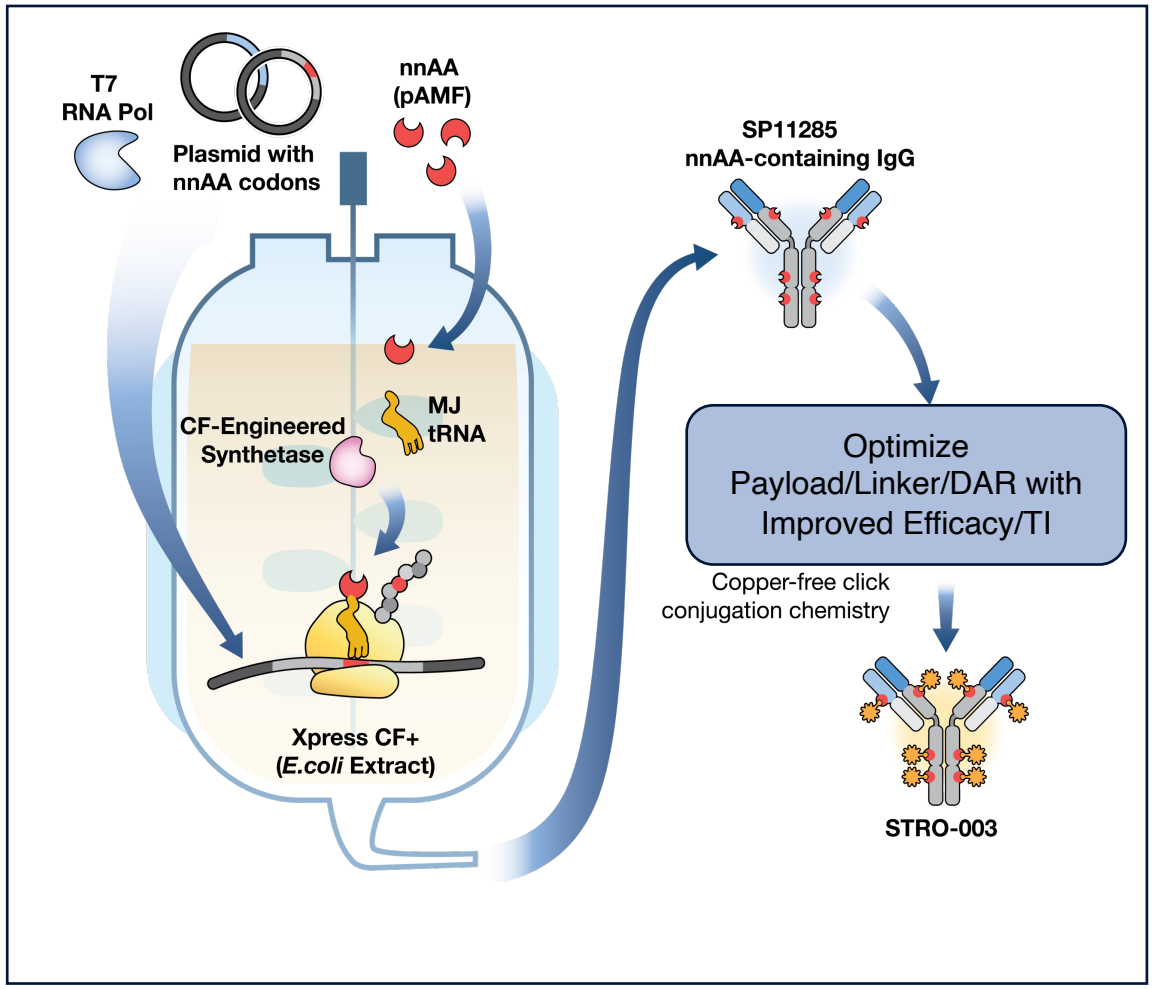
ROR1 Expression Across Various Solid Tumors



- ROR1 is a favorable target for an ADC due to its low expression in normal tissues
- As well as it's prevalence in solid tumors and B cell malignancies, including CLL, DLBCL, MCL, TNBC, NSCLC and ovarian cancer
- ROR1 expression is correlated with poor prognosis in different cancers, for e.g., TNBC and CRC

- *Zhang, et al. Am J Pathol. 2012; 1903-10*
- *Balakrishnan, et al. Clin Cancer Rese 2017, 3061-3071*
- *Zheng, et al Sci Rep. 2016 Nov 10;6:36447; Zhou et al, Oncotarget. 2017 May 16;8(20):32864-32872*

α-ROR1 nnAA pAMF Labeled Ab Discovered Using Sutro's CF Platform Technology



Schematic representation of cell-free α-ROR1 Ab synthesis for homogeneous ROR1 targeting ADC

- α-ROR1 STRO-003 Ab, high affinity binding to ROR1
- Fully human IgG1 aglycosylated Ab
- 8 pAMF nnAA incorporated at LC and HC
- Binding to ROR1 Ig domain
- Cross reactive to human/cyno/rodent
- No binding to ROR2

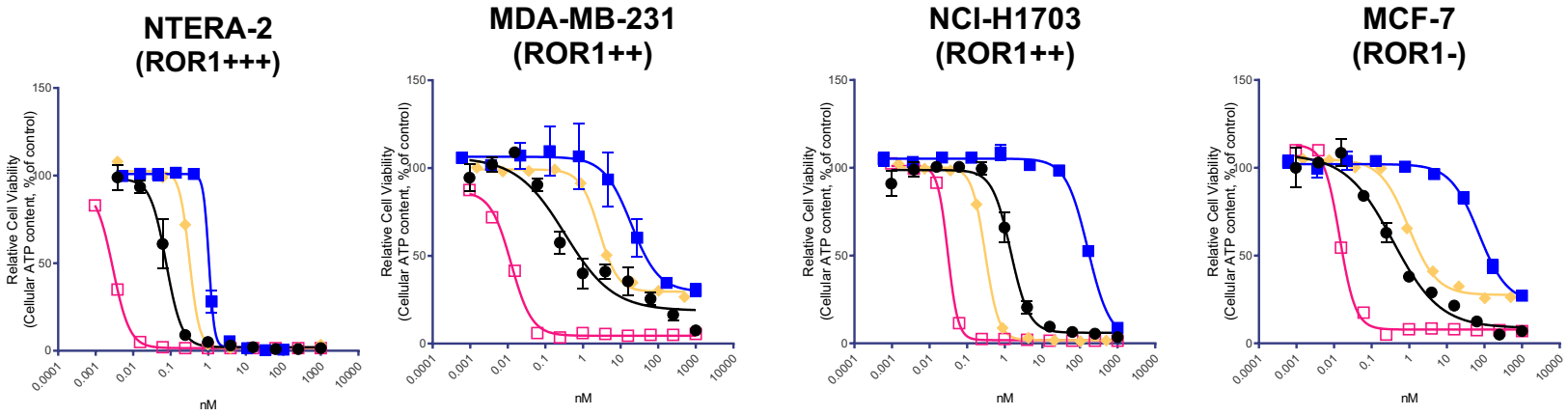
Samples	CHO-hROR1		HEK293-mROR1		HEK293-rROR1	
	Bmax	Kd (nM)	Bmax	Kd (nM)	Bmax	Kd (nM)
STRO-003 Ab	22295	0.24	17122	1.9	22176	1.5

Design/Optimization of Novel Linker Payload for α-ROR1 ADC

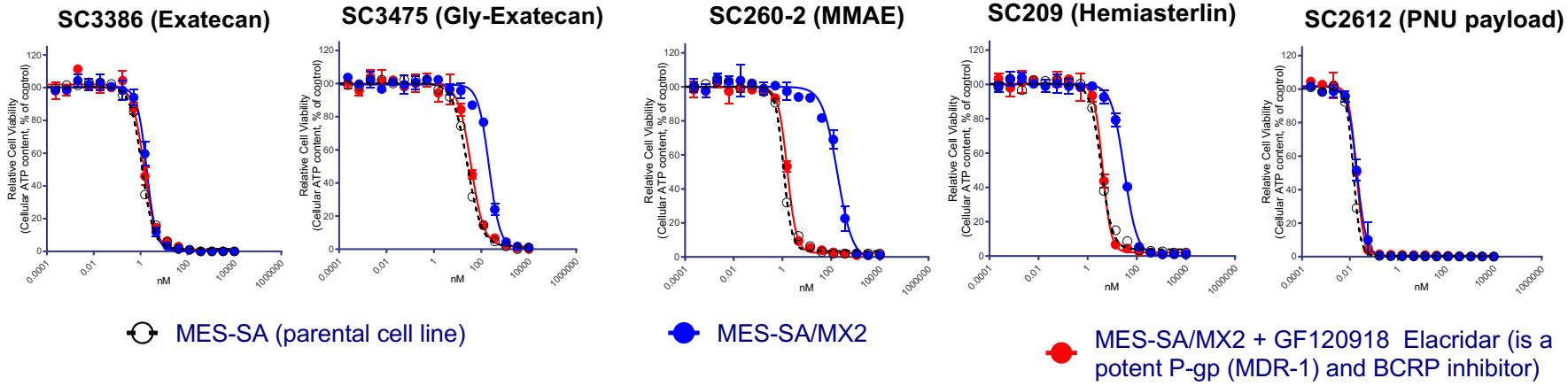
- ROR1 is expressed on multiple hematologic and solid tumors but not on normal tissues
- Heterogenous expression, low receptor copy number (ranging between 50,000-150,000)
- Payload selection Tubulin vs DNA targeting
- Low/not a substrate for drug efflux from MDPR/BCRP1 transporters
- Improved passive permeability (P_{app}) for better bystander affect
- Moderate potency payload; high drug loading ADCs (DAR8) to drive the efficacy and modulate the PK, safety
- Explored different drugs and tumor specific release mechanism-based linkers, to minimize Cmax the free payload in tissues and maximize target exposure to ADC
- Better physicochemical properties of ADC

Payload Selection for α-ROR1 ADC

In-vitro cell killing on ROR1 expressing tumor cells of different classes of payloads



In-vitro cell killing on P-gp overexpressing MES-SA/MX2 tumor cells of tubulin/DNA targeting payloads



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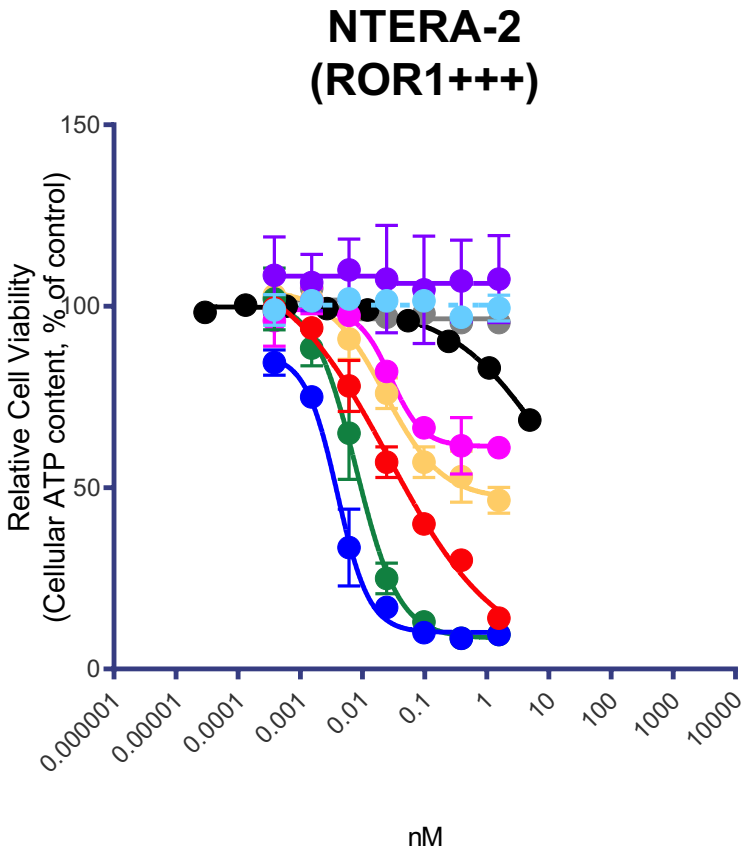
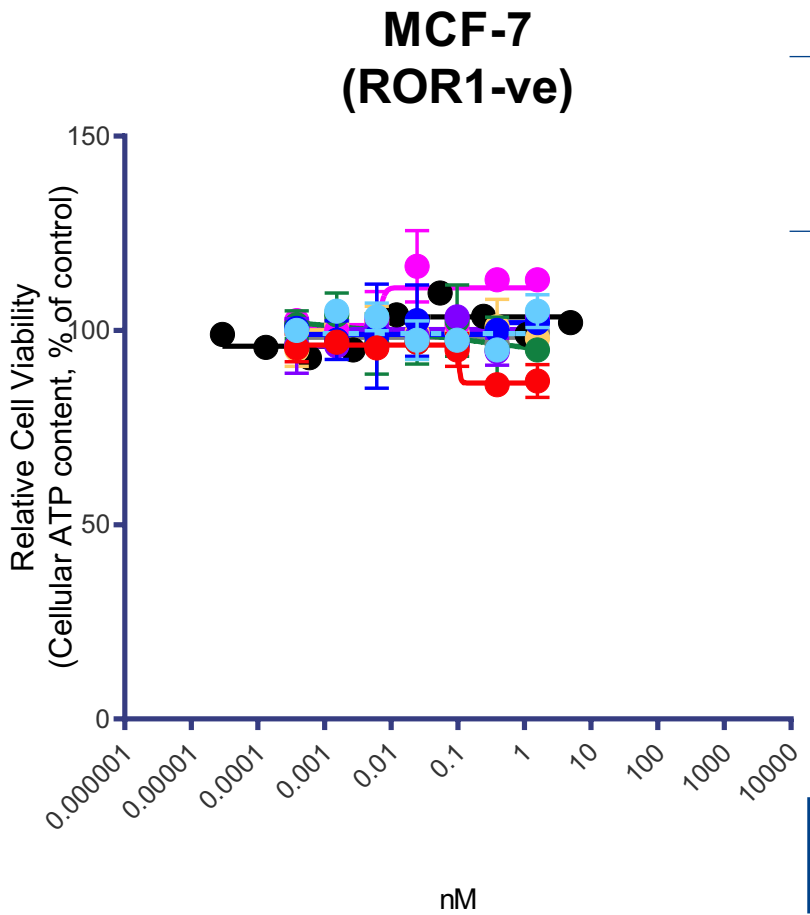
In vitro Potencies of α-ROR1 DAR8 ADCs with Different Linker/Payloads





SP10978 2188-D04 + SC3411 (charge-PEG-tripeptide cathepsin sensitive Exatecan) DAR8

SP10979 2188-D04 + SC3417 (PEGylated bGlu Exatecan) DAR8

SP10980 2188-D04 + SC3418(PEG-tripeptide cathepsin sensitive Exatecan) DAR8

SP10981 2188-D04 + SC3419 (PEG-dipeptide Exatecan) DAR8

SP10982 2188-D04 + SC3420 (PEG-tripeptide LGMN cleavable Exatecan) DAR8

SP10983 2188-D04 + SC3421 (PEG tripeptide Exatecan) DAR8

SP10873 2188-D04 + SC239 (hemiasterlin) DAR8

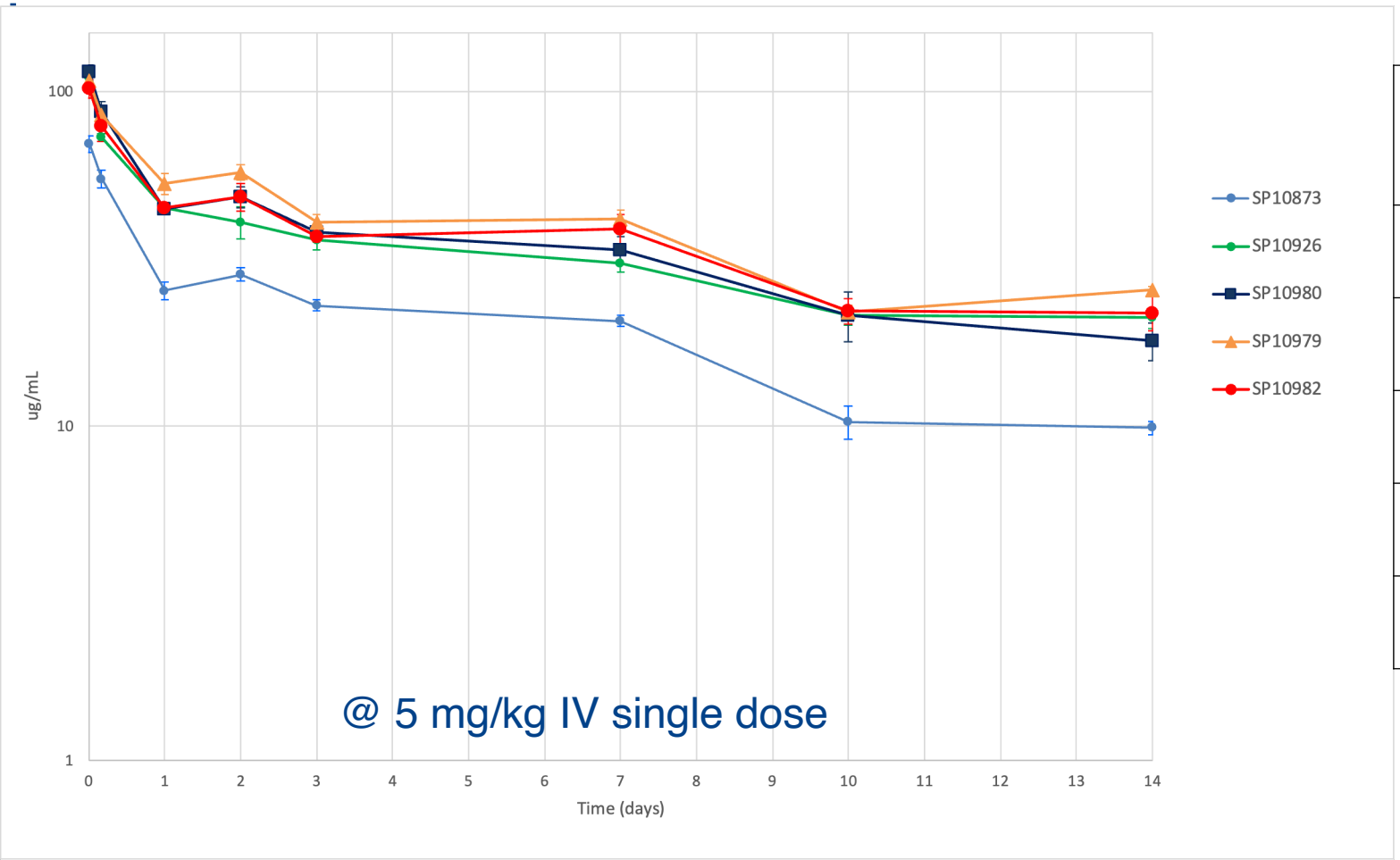
SP11068 UC961 + SC572 (DAR4)

SP10990 Velosbio Ab

- SC3418 (cathepsin), SC3417 (b-Glu), SC3420 (LGMN) ROR1 DAR8 ADCs demonstrated potent and ROR1 dependent cell killing.

Linker Payload	% of expected payload release from lysosomal incubation @ 24h
SC3411	Not released
SC3418	Payload released

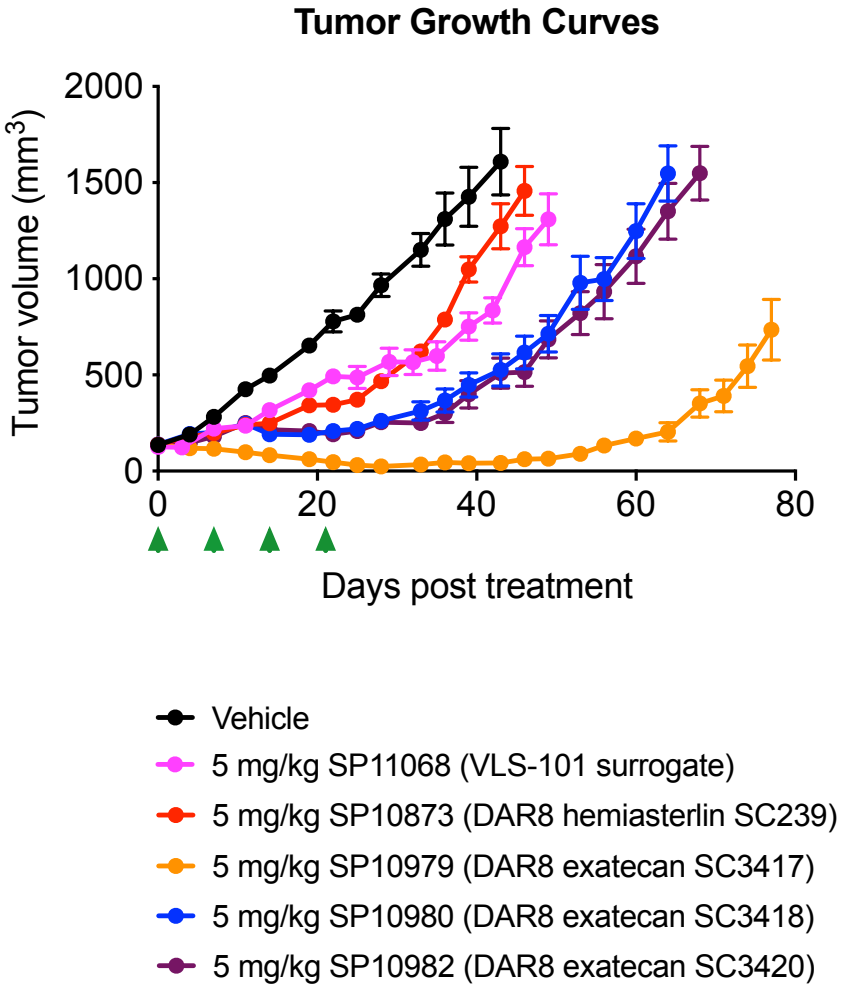
PK Summary of α-ROR1 DAR8 ADCs with Different Linker/Payloads in Non-Tumor Bearing Mice



Conjugate	Description	Terminal $t_{1/2}$ (day)
SP10873	ROR1 2188-D04-SC239 (DAR8)	7.56
SP10926	ROR1 2188-D04-SC3403 (DAR8)	12.0
SP10980	ROR1 2188-D04-SC3418 (DAR8)	8.67
SP10979	ROR1 2188-D04-SC3417 (DAR8)	10.2
SP10982	ROR1 2188-D04-SC3420 (DAR8)	10.7

- SC3417/SC3418/SC3420 with different cleavable linker based Exatecan DAR8 α-ROR1ADCs showed good PK properties when compared to the benchmark control LP (SC3403) as DAR8 ADC.

α-ROR1 DAR8 ADCs with Various Linker/Payloads in MDA-MB-231 Breast Cancer Model with Moderate ROR1 Expression

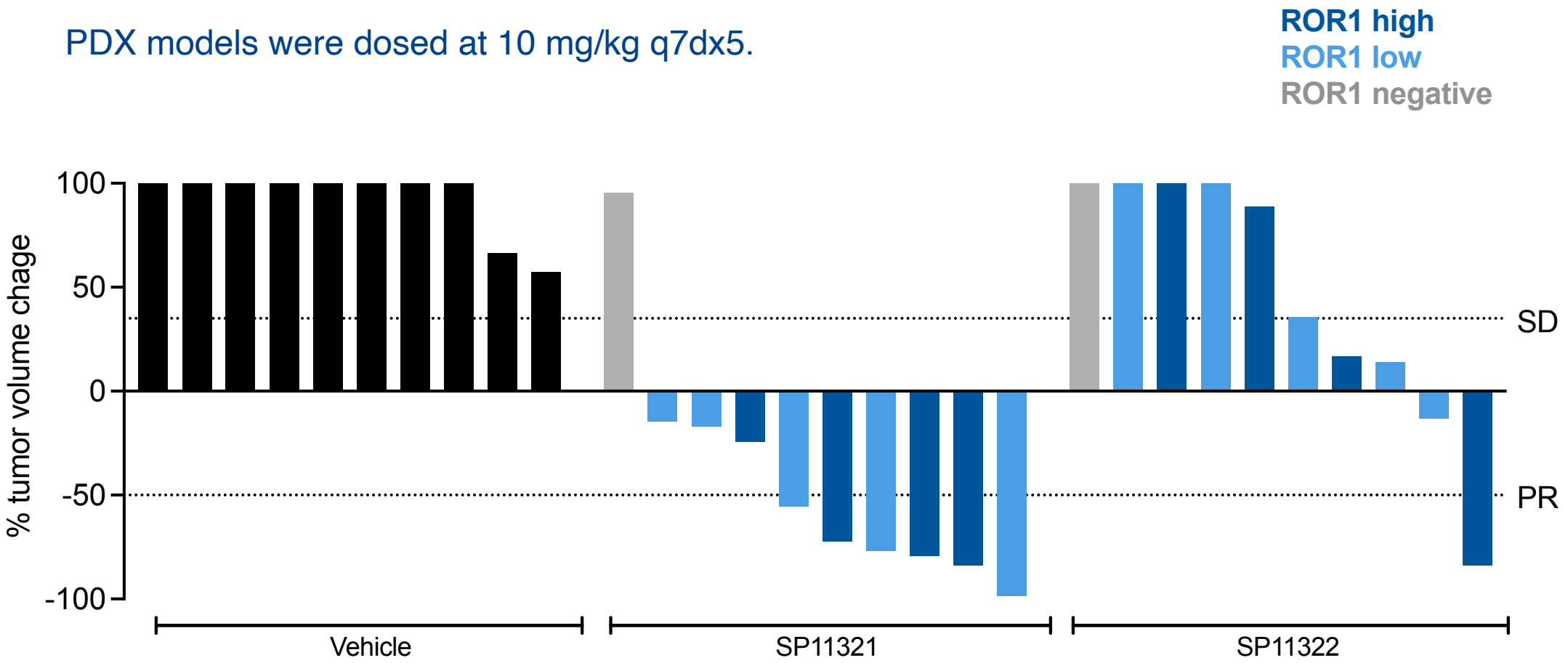


LP SC#	Description	Dose (qw x4)	% TGI (Day 42 or 43)
SC3417	DAR8 hydrophilic β-glu Exatecan Linker Payload	5 mg/kg	106%
SC3418	DAR8 CatB (tripeptide sequence) cleavable Exatecan Linker Payload	5 mg/kg	74%
SC3420	DAR8 LGMN (tripeptide sequence) Exatecan Linker Payload	5 mg/kg	75%
SC239	DAR8 CatB cleavable Hemiasterlin Linker Payload	5 mg/kg	23%
SC572	DAR4 CatB MMAE (VLS-101 Linker-Payload)	5 mg/kg	53%
	Vehicle		

- DAR8 β-Glu Exatecan conjugate outperformed compared to other α-ROR1 ADCs utilizing different Linker/Payloads

α-ROR1 DAR8 SP11321 (β-glu Exatecan) ADC Shows Greater Anti-Tumor Activity in High and Low ROR1 Expressing NSCLC PDX Models than SP11322 ADC

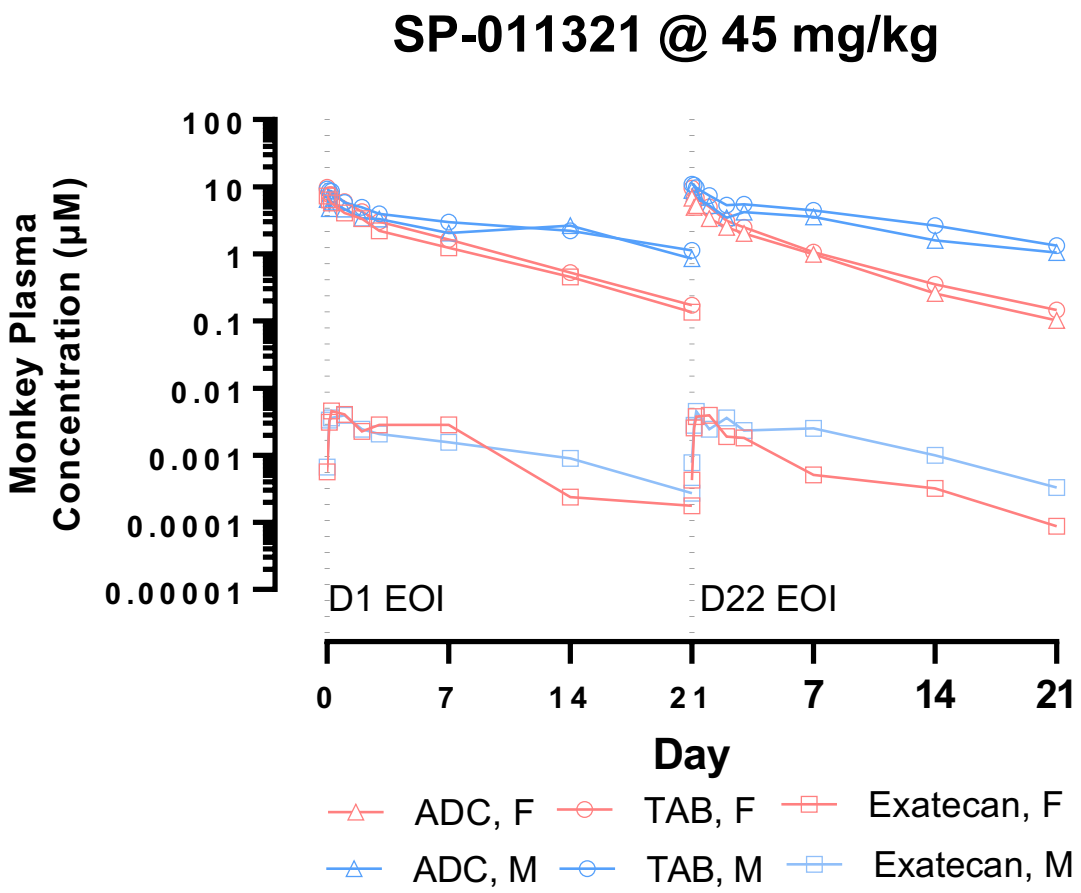
PDX models were dosed at 10 mg/kg q7dx5.



DAR8 hydrophilic β-glu Exatecan Linker Payload α-ROR1 ADC

DAR8 CatB (tripeptide sequence) cleavable Exatecan Linker Payload α-ROR1 ADC

α-ROR1 DAR8 SP11321 was Stable in Circulation and Well Tolerated in NHP up to 45 mg/kg

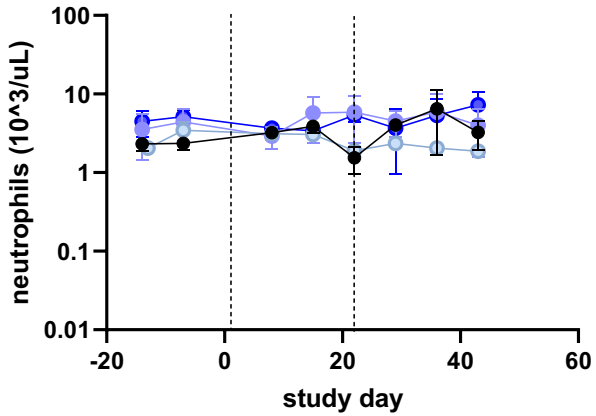


- Non-human primates were dosed with SP11321 every three weeks in a repeat dose toxicity (Q3wx2 at 10, 20, 45 mpk) and toxicokinetic study
- SP11321 was well-tolerated in a multi-dose non-GLP NHP up to 45 mg/kg, the highest dose tested
- SP11321 was stable in circulation with superimposable ADC and total antibody plasma concentrations
- Plasma concentrations of released Exatecan payload (SC3386) were in the sub to low nM range, and at least 100-fold lower than TAB or ADC

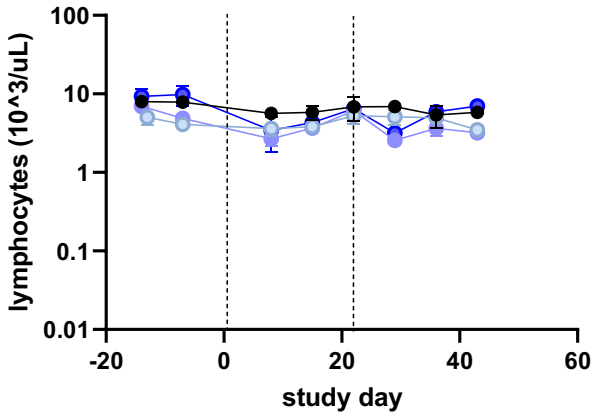
α-ROR1 DAR8 SP11321 Demonstrated a Wide Safety Window in Non-Human Primates

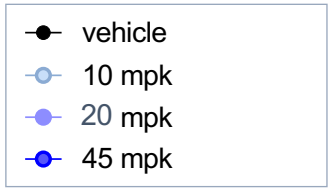
- α-ROR1 DAR8 SP11321 (β-glu Exatecan Linker Payload) was well tolerated in a multi-dose non-GLP NHP study up to 45 mg/kg
- No observed neutropenia or thrombocytopenia, no changes observed in WBCs

Neutrophils



Lymphocytes

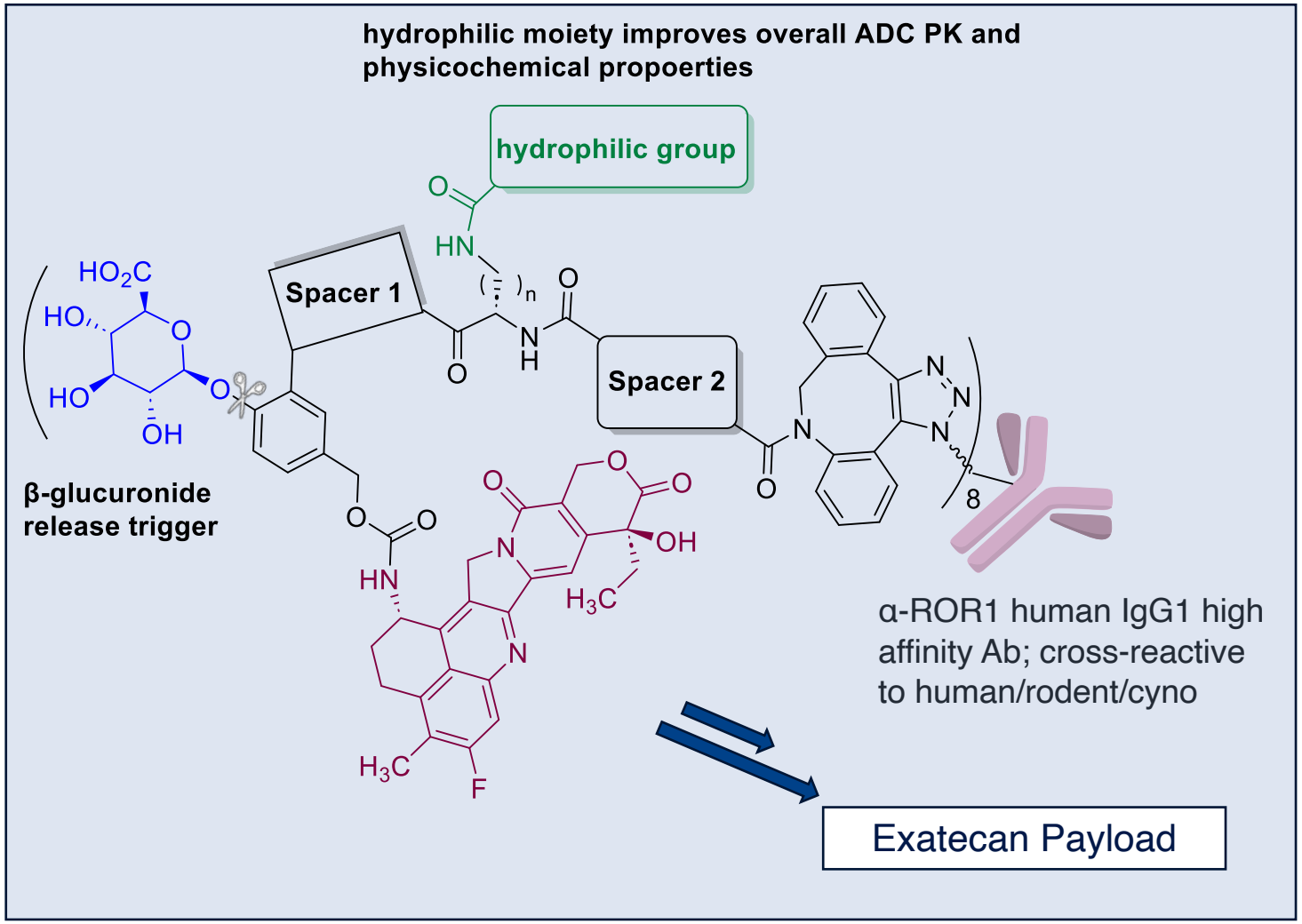




Additionally, no lung toxicities observed at 45 mg/kg SP11321 in NHPs

- No microscopic findings of toxicity were observed in the histopathology of animals dosed with SP11321
- In this NHP preclinical study, α-ROR1 DAR8 SP11322 (CatB cleavable Linker Exatecan SC3418) ADCs generated lung findings consistent with developing pneumonitis (and ILD) at 45 mg/kg

SP11321 α-ROR1 DAR8 ADC

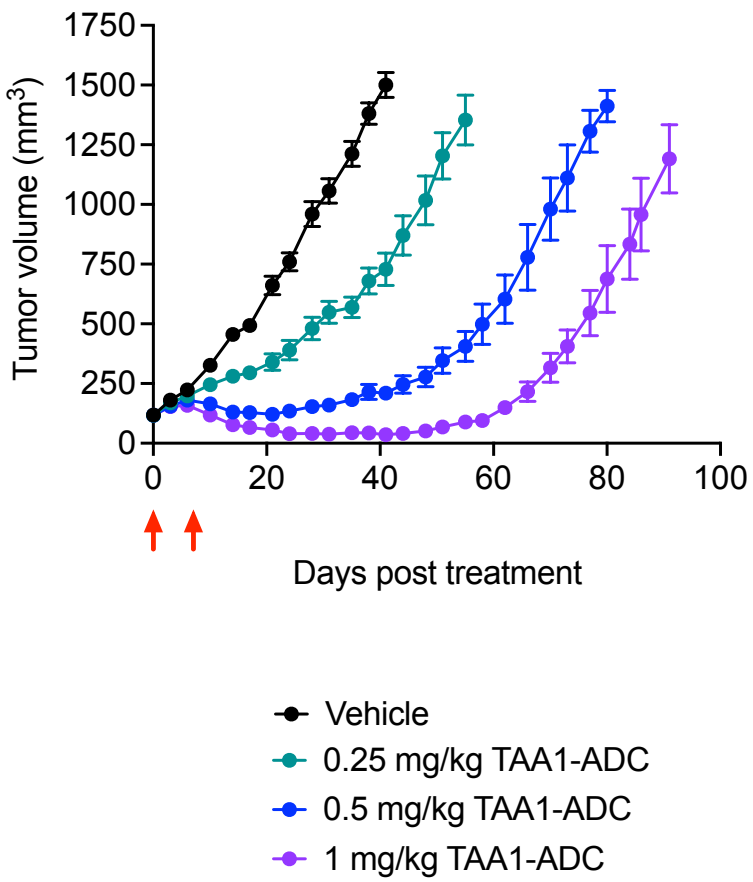


- High affinity ROR1 specific Ab; incorporating eight para-azidomethyl-phenyl alanine (pAMF) nnAA residues at optimal sites allowing for precise conjugation for enhanced efficacy and safety
- Optimized novel hydrophilic tumor selective and stable β-glucuronidase cleavable Exatecan linker payload (SC3417) for α-ROR1 and other TAA Sutro's ADCs
- Releases the high potency Exatecan Payload (SC3386). Payload (CPT class) have short systemic-half life
- High passive permeability payload, Exatecan ADCs showed greater bystander activity
- Efficacious in CDX, PDX models
- non GLP tox in NHPs was clinically well tolerated up to 45 mpk in repeated dose
- Overall SP11321 DAR8 ADC demonstrated improved efficacy/safety from preclinical studies; designed/optimized for **significantly superior clinical performance**

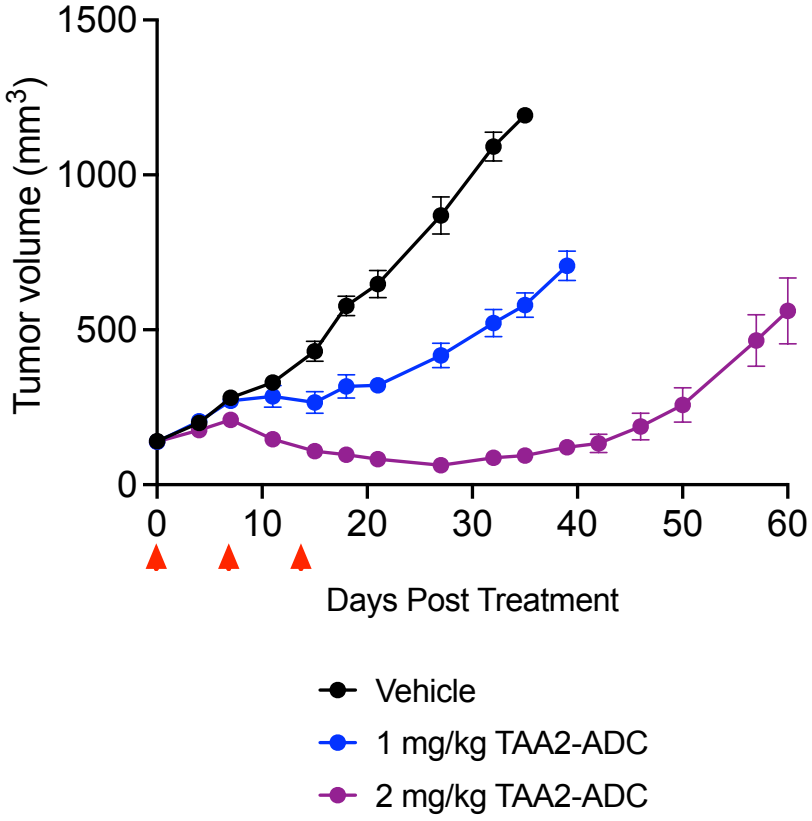
SP11321 is homogeneous α-ROR1 DAR8 ADC for the treatment of ROR1-expressing solid/hematological carcinomas

Sutro's Site-Specific TAA ADCs Utilizing the Novel Hydrophilic β-Glucuronidase Cleavable Exatecan Linker Payload (SC3417)

TAA1 β-glu Exatecan ADC in vivo data in breast cancer CDX model



TAA2 β-glu Exatecan ADC in vivo data in breast cancer CDX model



Acknowledgments

Many thanks to Sutro’s dedicated scientists across different cross functional teams as well as the managers who made valuable contributions!