



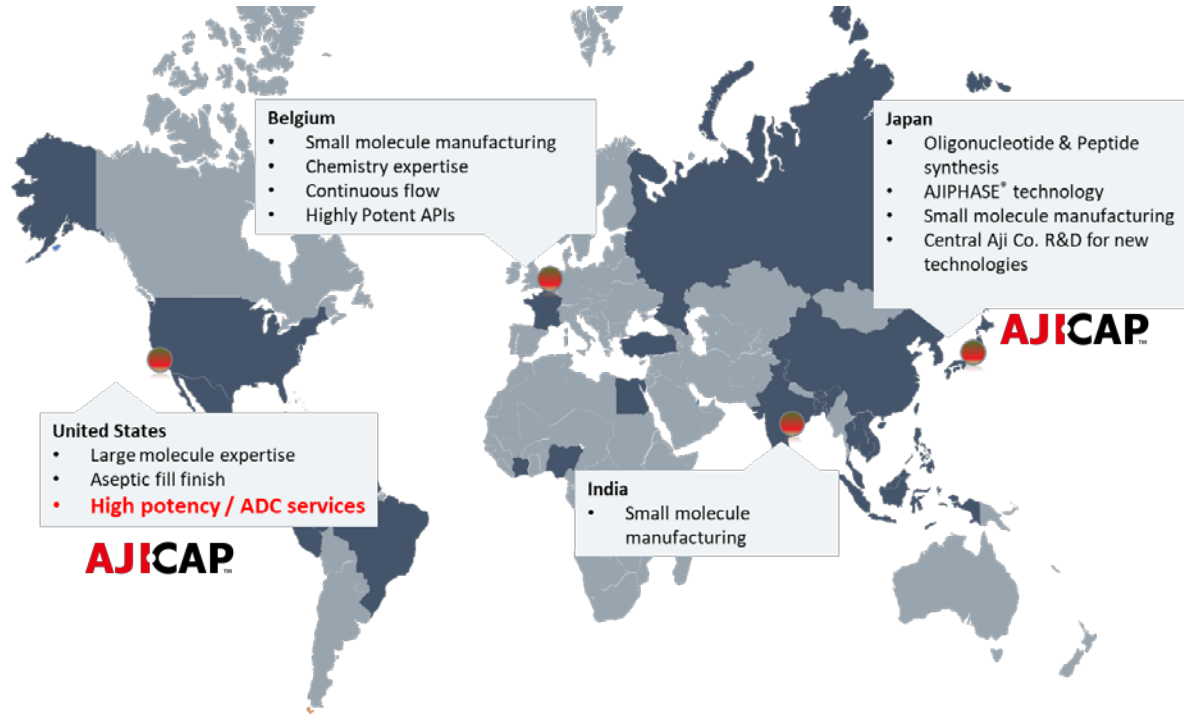
Technology of Site Specific Conjugation for Next Generation ADCs



BIO•PHARMA
S E R V I C E S

BRIAN MENDELSON
NOVEMBER 13, 2018
PRESENTATION FOR WORLD ADC SAN DIEGO 2018

Presentation Outline



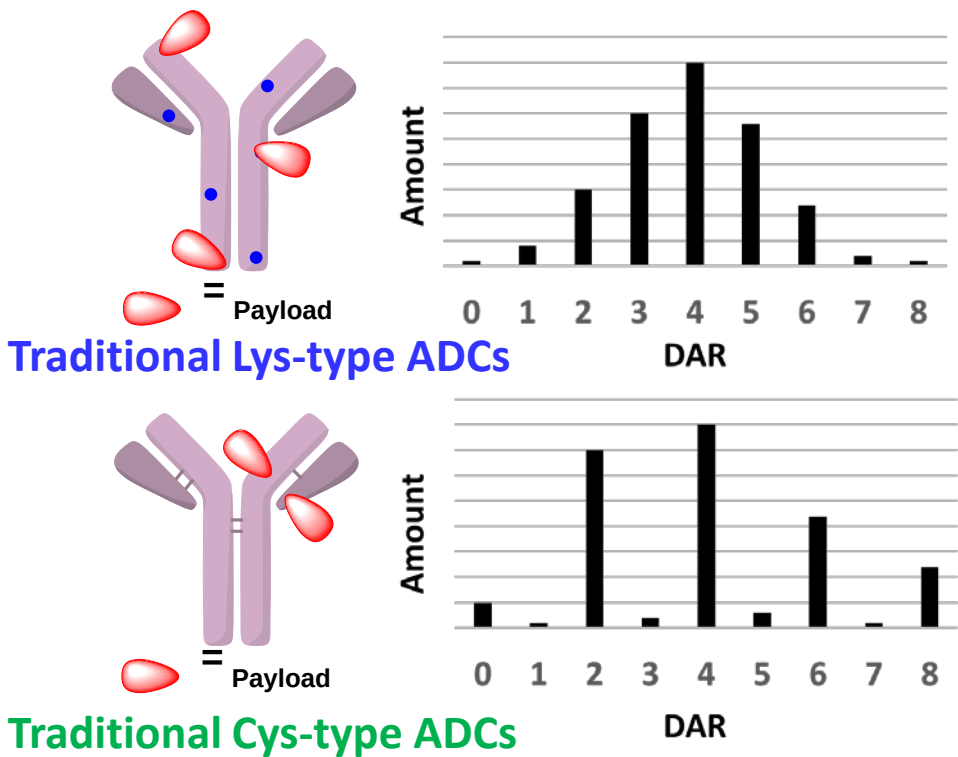
- 1 Background of AJICAP™ Technology
- 2 Capabilities of AJICAP™ Technology
- 3 Ajinomoto Bio-Pharma Services



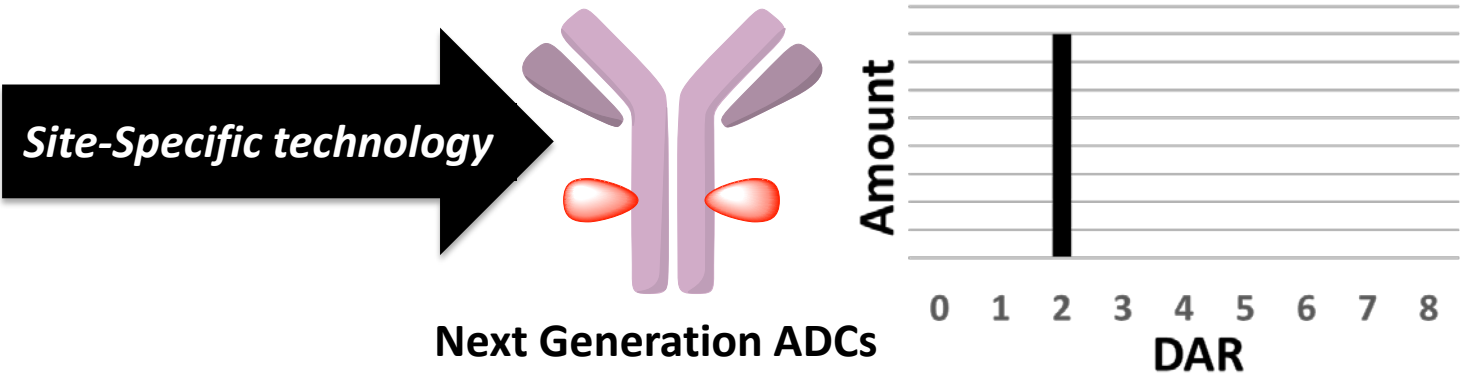
Chapter 1

Background of AJICAP™

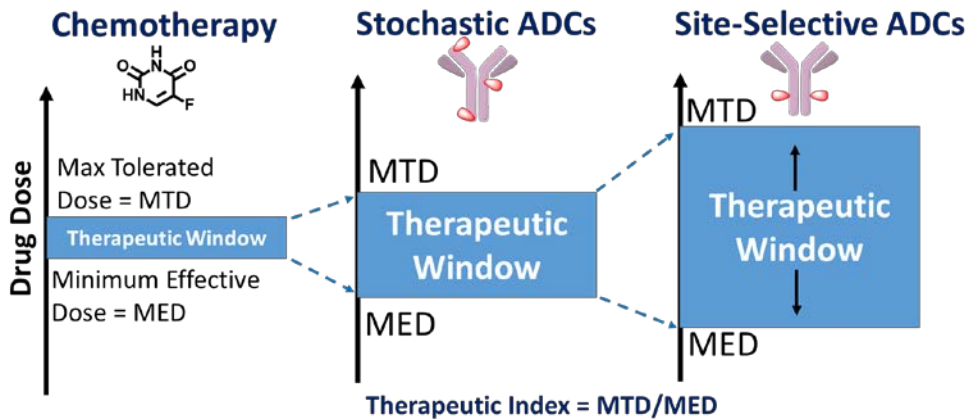
Introduction



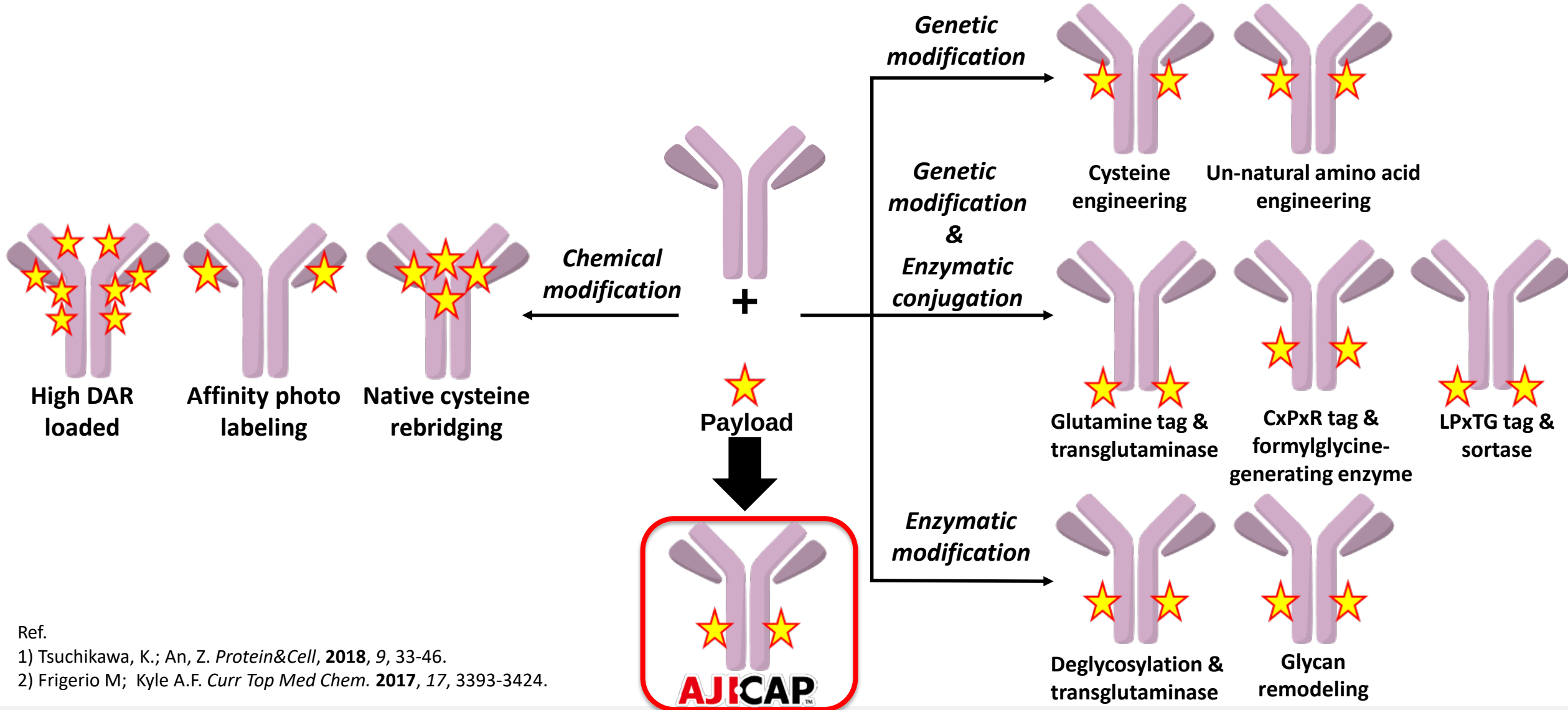
CMC Advantages
✓ Reproducible, simplified analysis



Site-specific technologies are being employed in many of the next generation ADCs due to enhanced biological properties:
Enhanced Potency, Reduced Toxicity → Increased TW



Prior Art



Ref.

1) Tsuchikawa, K.; An, Z. *Protein&Cell*, **2018**, 9, 33-46.

2) Frigerio M; Kyle A.F. *Curr Top Med Chem*. **2017**, 17, 3393-3424.

Summary of Advantage of AJICAP™



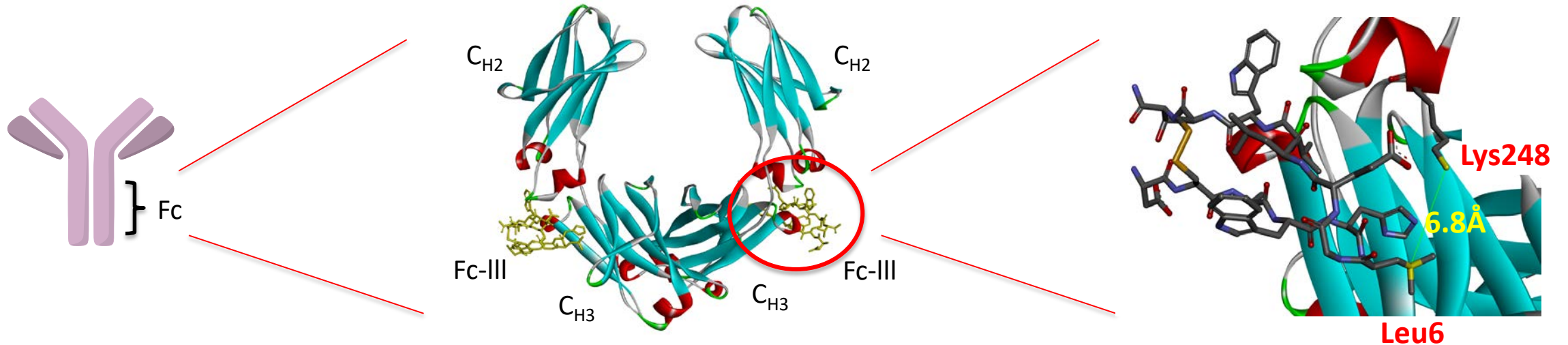
Ajinomoto Co. has developed an innovative direct chemical site-selective conjugation method for use with intact native antibodies.

Our novel technology provides significant advantages for next generation ADCs:

- 1. Straightforward CMC**
 - Direct modification of intact antibody
 - No genetic modification required
- 2. Enhancement of Therapeutic Window compared to stochastic ADCs**
 - Site specific, controlled DAR 2
- 3. Wide drug-linker compatibility: reactive group compatibility includes thiol, alkyne, azide, etc**
- 4. High yields, short conjugation reaction times, ease to manufacture**

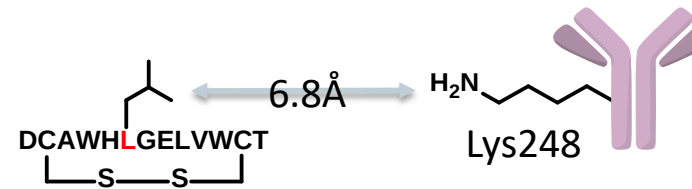
Background of Affinity Peptide and Reaction Concept of AJICAP™

Human IgG Fc and Fc-III peptide co-crystal structure¹⁾



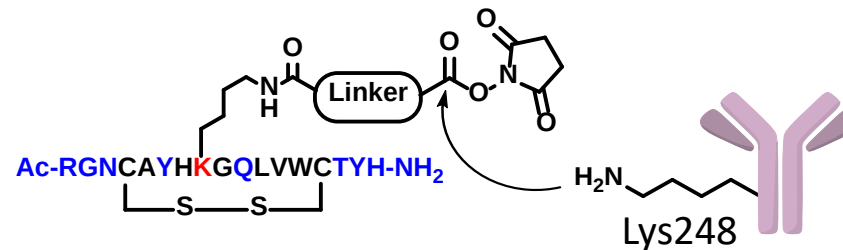
Fc-III

- ✓ K_D 20nM
- ✓ 13 amino acids



Peptide for AJICAP²⁾

- ✓ K_D 4 nM
- ✓ 17 amino acids
- ✓ Selected by phage display



Ref.

1) DeLano, W. L.; Ultsch, M. H.; de Vos, A. M.; Wells, J. A. *Science*, **2000**, 287, 1279-1283.

Aj
AJINOMOTO

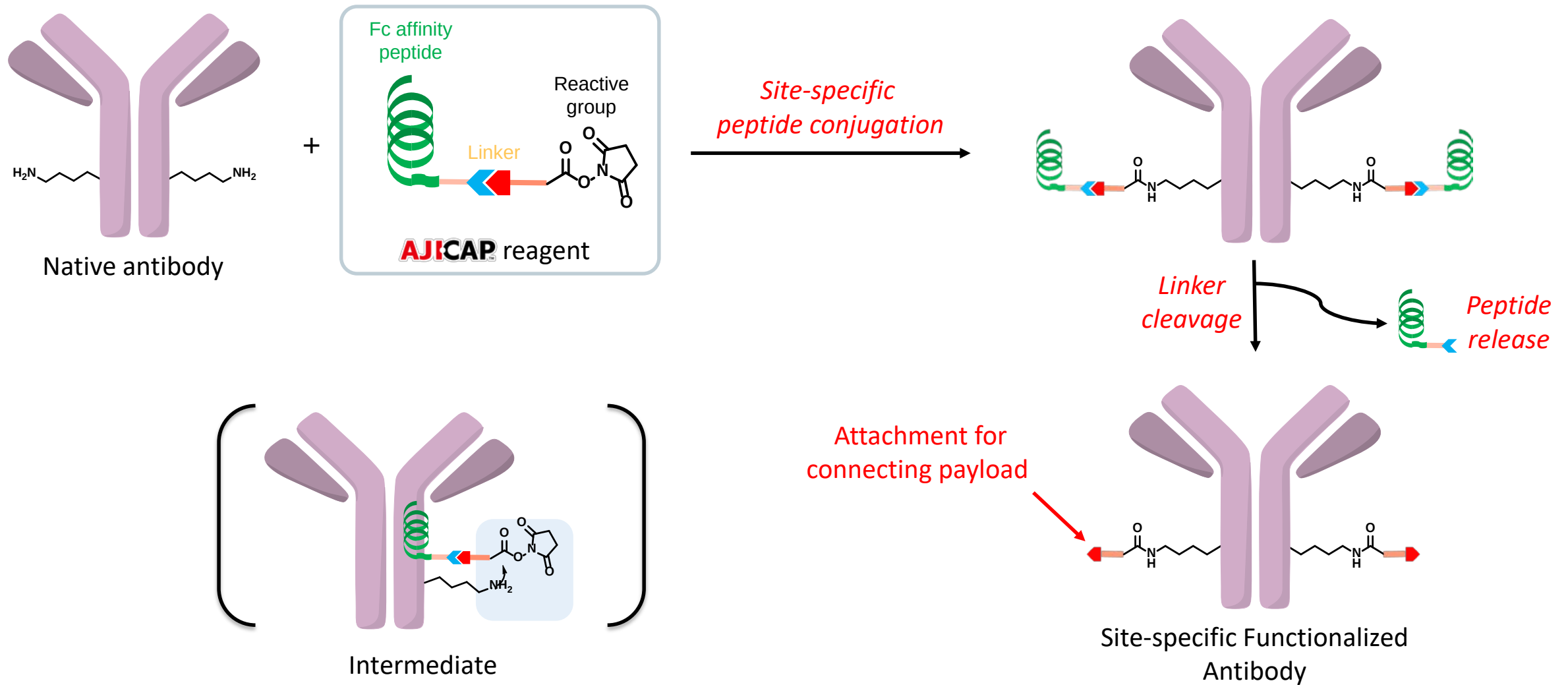
BIO-PHARMA
SERVICES

THE POWER TO MAKE™

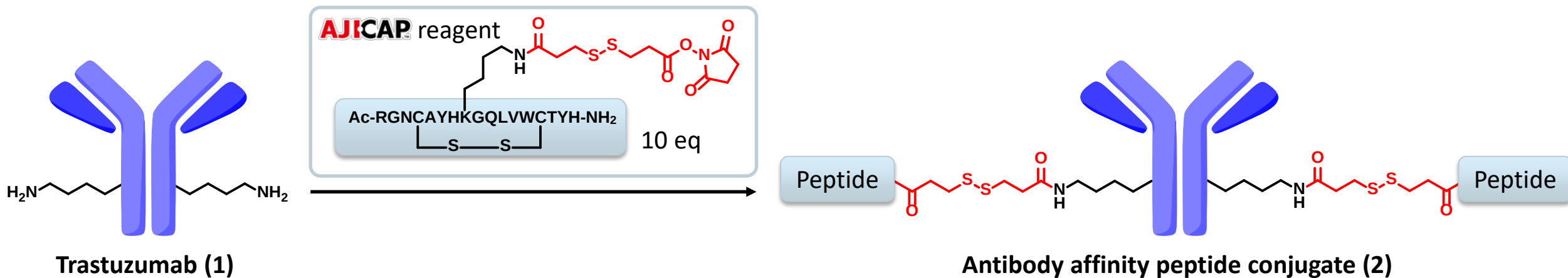
Ref.

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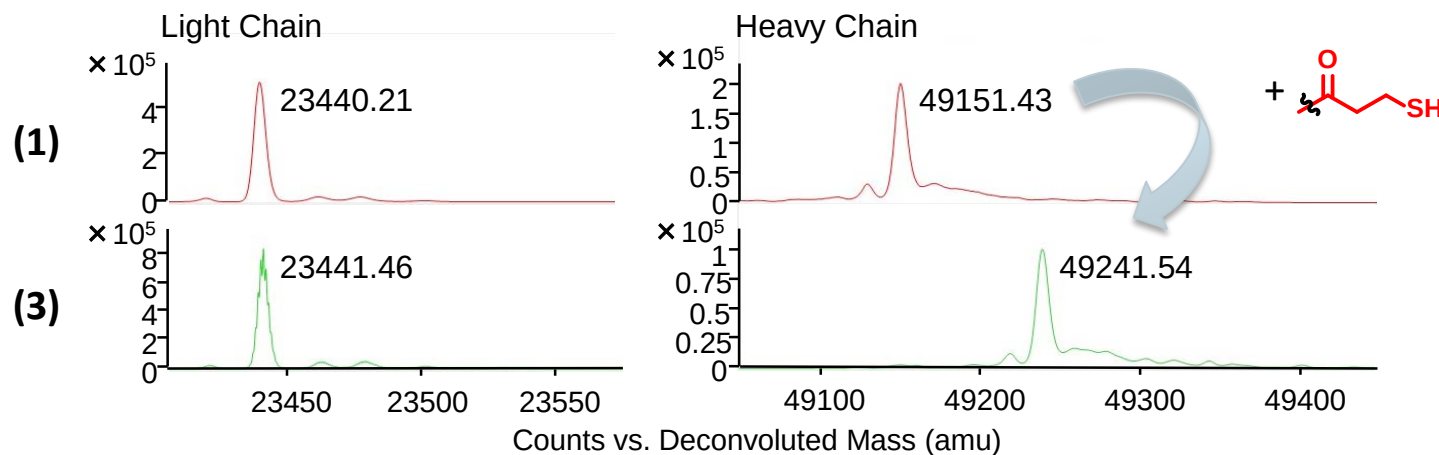
Strategy of AJICAP™



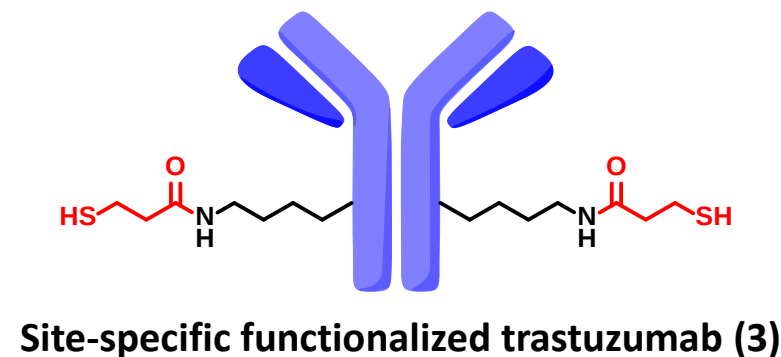
Site-Specific Functionalization of Trastuzumab at Lys248



ESI-TOFMS (Reduced condition)

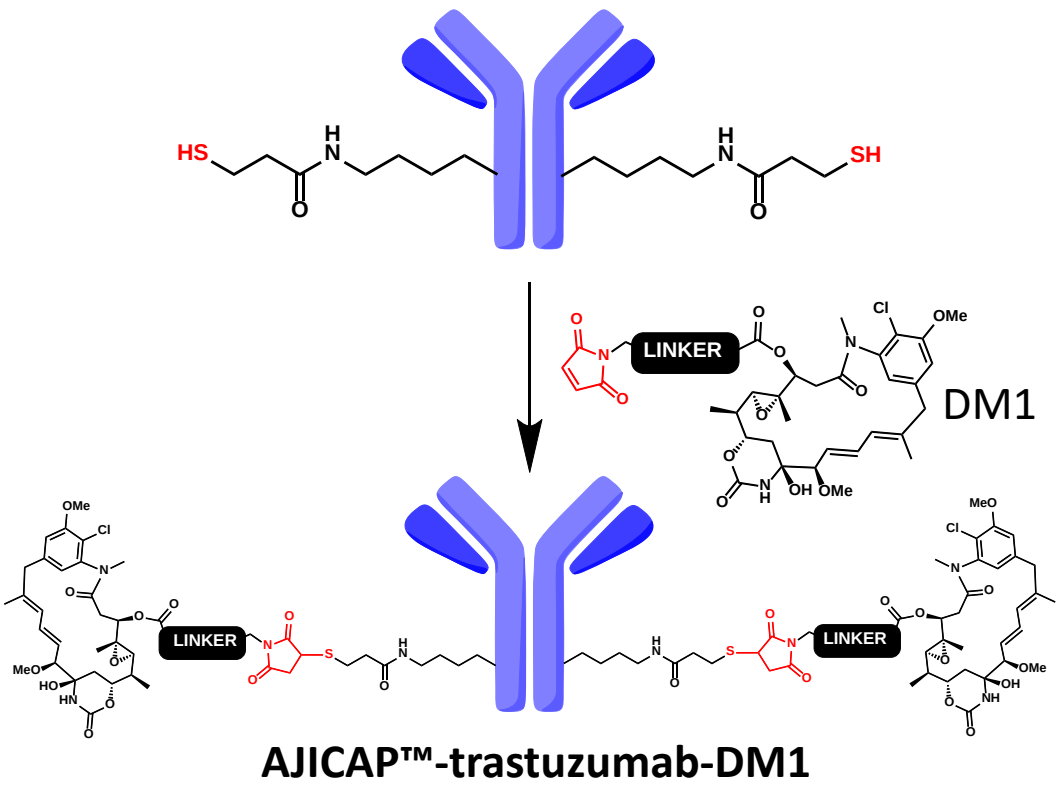


Redox cleavage



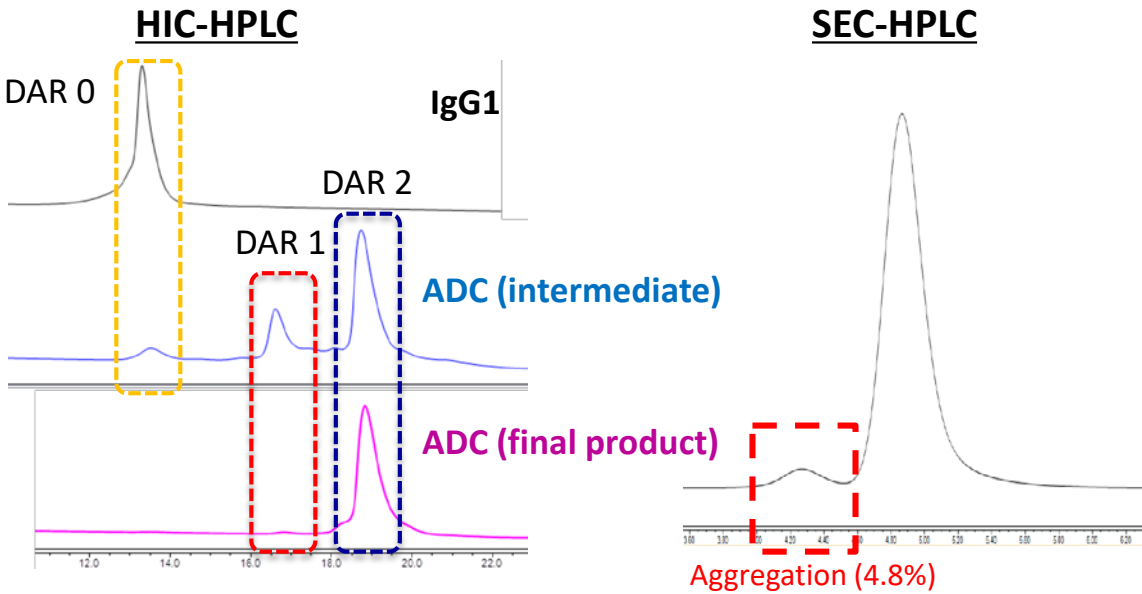
Analysis of AJICAP™-ADC

We began testing the AJICAP™ technology by using commercially available trastuzumab and a DM1-maleimide drug-linker

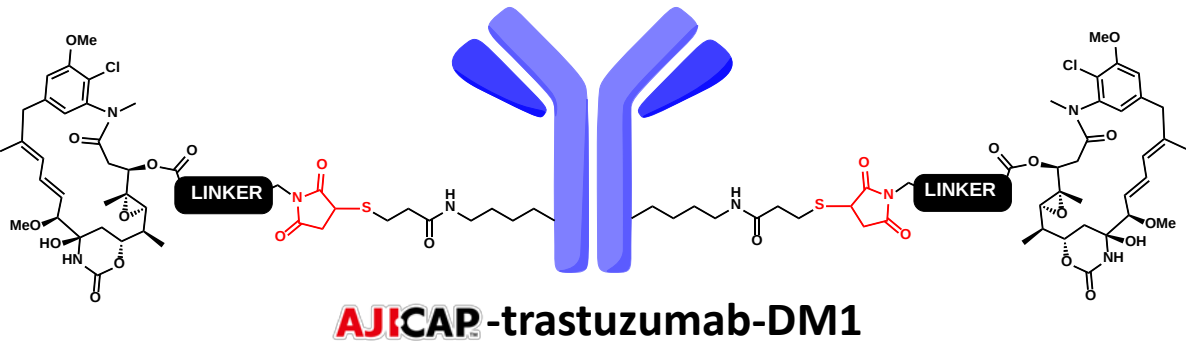


Target parameters

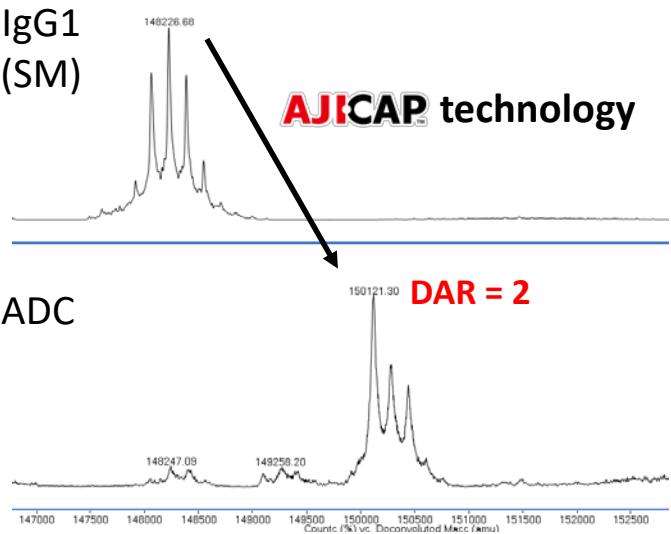
ADC	Trastuzumab-DM1
DAR	1.6~2.0 (Q-TOF, HIC)
Aggregation	< 10% (SEC)
Conjugated site	Confirmed by peptide mapping



Analysis of AJICAP™-ADC



Q-TOFMS



Peptide mapping

LC: 100% coverage

1	N-term	D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D V N T A V A W	35
36		Y Q Q K P G K A P K L L I Y S A S F L Y S G V P S R F S G S R S G T D F L T I	75
76		S S L Q P E D F A T Y Y C Q Q H Y T T P P T F G Q G T K V E I K R T V A A P S V	115
116		F I F P P S D E Q L K S G T A S V V C L L H N F Y P R E A K V Q W K V D N A L Q	155
156		S G N S Q E S V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E	195
196		V T H Q G L S S P V T K S F N R I G E C	214
	C-term		

HC: 93% coverage

1	N-term	E V Q L V E S G G G L V Q P G G S L R L S C A A S G F N I K D T Y I H	35
36		W V R Q A P G K I G L E W V A R I Y P T N G Y T R Y A D S V K I G R I F T I S A D T S	75
76		K I N T A Y L Q M N S L R A E D T A V Y Y C S R W G G D G F Y A M D Y W G Q G T L	115
116		V T V S S A S T K I G P S V F P L A P S S K S T S G G T A A L G C L V K I D Y F P E	155
156		P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S	195
196		L G T Q T Y I C N V N H K P S N T K V D K I K V E P K S C D K I T H T C P P C P A P	235
236		E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E	275
276		V K I F N W Y V D G V E V H N A K I T K P R I E E Q Y N S T Y R I V V S V L T V L H Q D	315
316		W L N G K I E Y K I C K I V S N K I A L P A P I E K T I S K I A K I G Q P R I E P Q V Y T L P	355
356		P S R E E M T K N Q V S L T C L V K I G F Y P S D I A V E W E S N G Q P E N N Y K I	395
396		T T P P V L D S D G S F F L Y S K I L T V D K S R I W Q Q G N V F S C S V M H E A L	435
436		H N H Y T Q K I S L S L S P G	449
	C-term		

THTCPPCPAPELLGGPSVFLFPPKP²⁴⁸KDTLMISR

Linker-DM1 at 4687.3 Da. This mass was found and indicates that **ONLY K248** was substituted.

Analytical summary of AJICAP-ADC

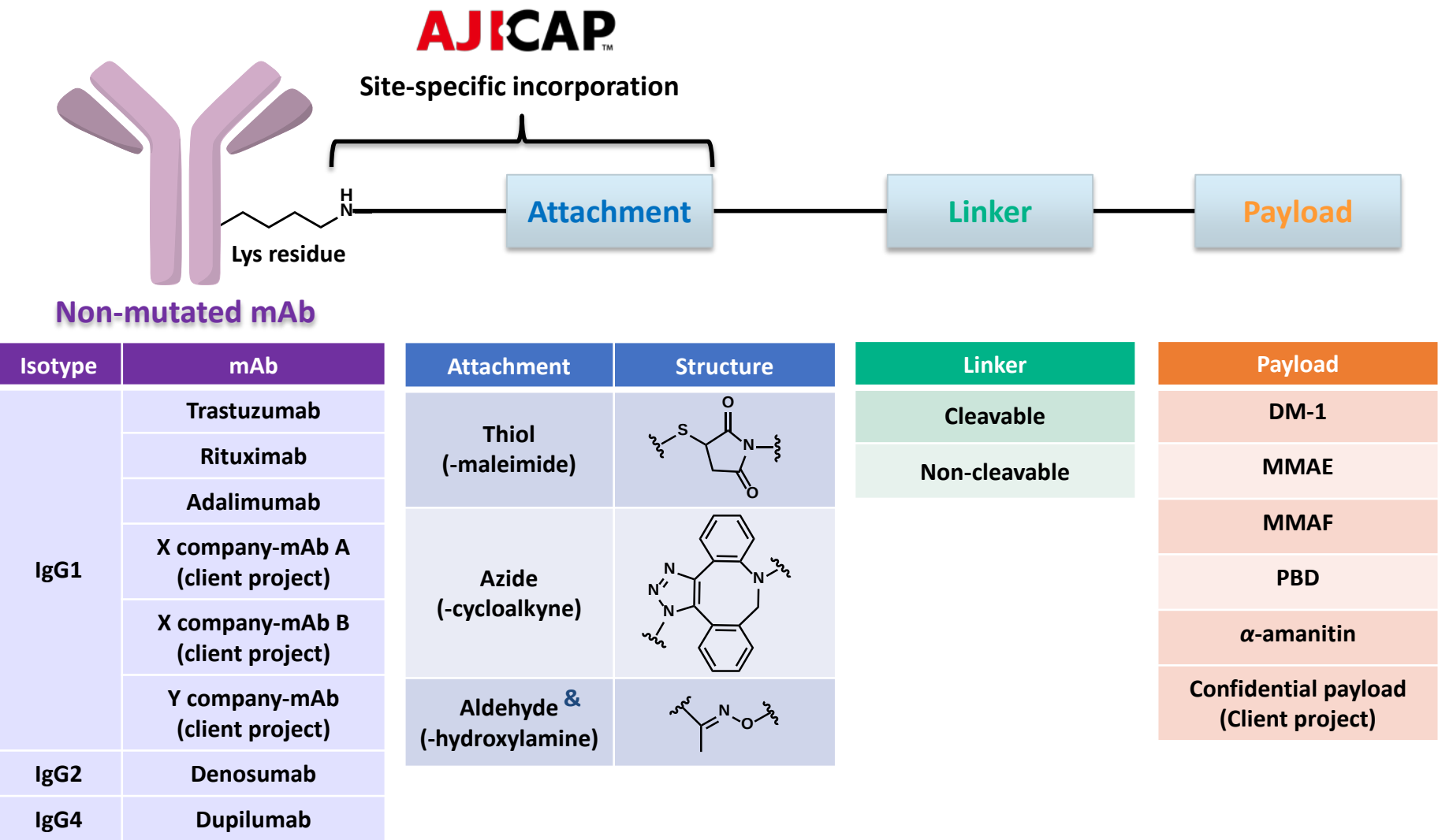
AJICAP™-trastuzumab-DM1	
DAR	1.9 (Q-TOF), 1.8 (HIC)
Aggregation	4.8% (SEC)
Conjugated site	K248 specific (peptide mapping)



Chapter 2

Capabilities of AJICAP™

Compatibility with Various mAbs, Payloads and Linkers

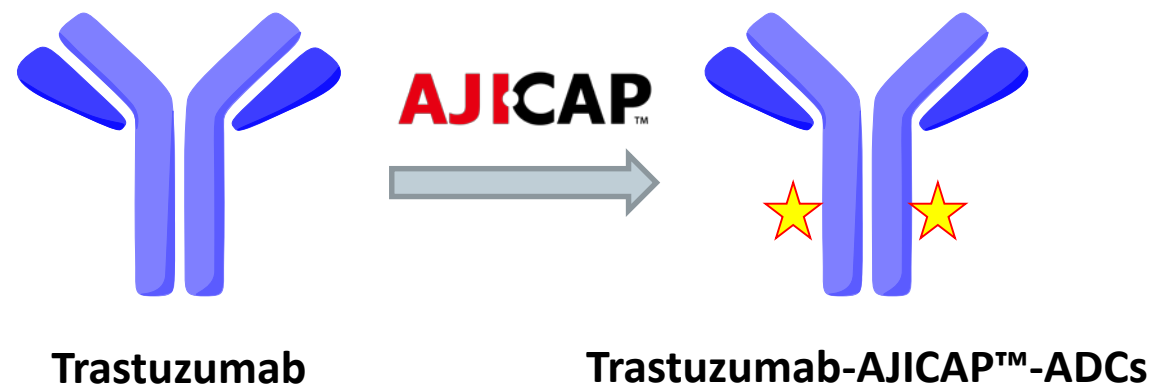


✓ AJICAP™-technology has great potential for mAb isotypes: IgG1, 2, 4

✓ Several client projects are ongoing

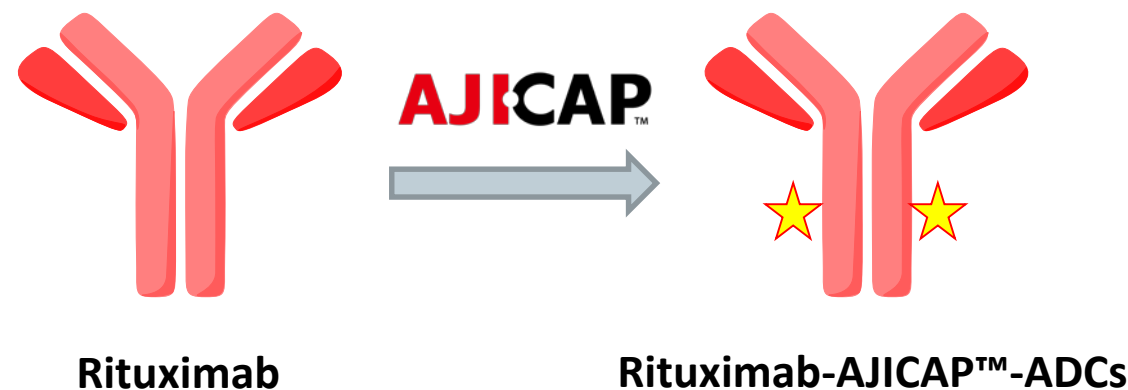
&Studies in progress

Materials for Proof of Concept Studies



AJICAP™-ADCs

Name of ADCs	mAb	Payload 
AJ1	Trastuzumab	DM1
AJ2		MMAE
AJ3		MMAF
AJ4		PBD



AJICAP™-ADCs

Name of ADCs	mAb	Payload 
AJ1R	Rituximab	DM1
AJ2R		MMAE
AJ3R		MMAF
AJ4R		PBD

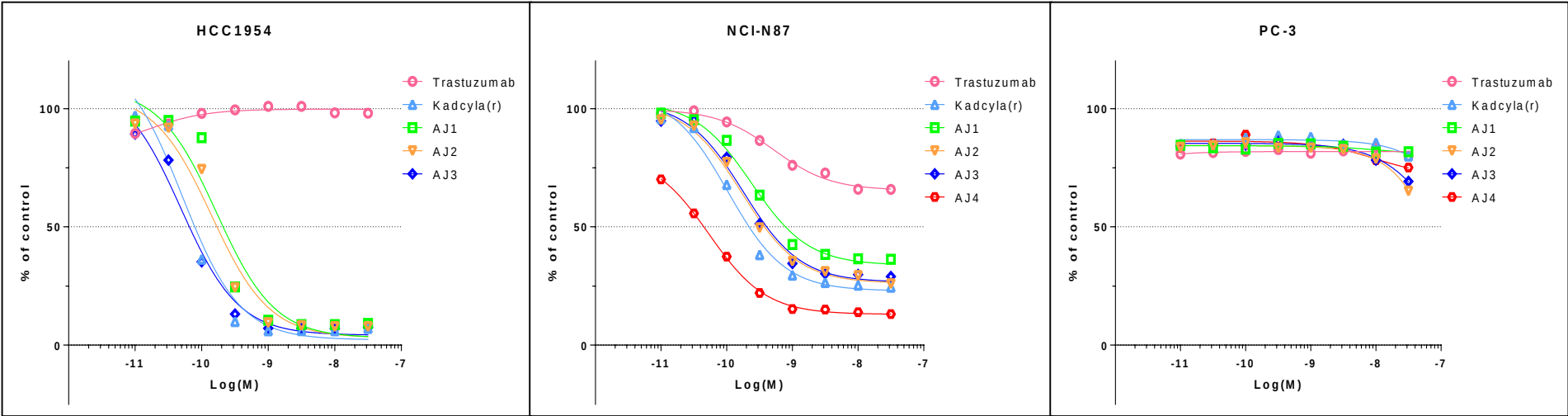
In Vitro Cell-Based Assay: Cytotoxicity Assessment

Cell lines

- HER2+
 - ✓ HCC1954
 - ✓ NCI-N87
- HER2-
 - ✓ PC3

Subject

- Trastuzumab ADCs



AJICAP™

			IC ₅₀ (nM)		
Name	mAb	Payload	HCC1954 (Ag+)	NCI-N87 (Ag+)	PC3 (Ag-)
AJ1	Trastu- zumab	DM1	0.17	0.24	no activity
AJ2		MMAE	0.14	0.17	no activity
AJ3		MMAF	0.050	0.18	no activity
AJ4		PBD	N.D.	0.054	no activity
Kadcyla®			0.050	0.10	no activity
Trastuzumab			no activity	slightly active	no activity

AJICAP™-ADCs showed *in vitro* cytotoxic activities reflective of DAR and payload potency.

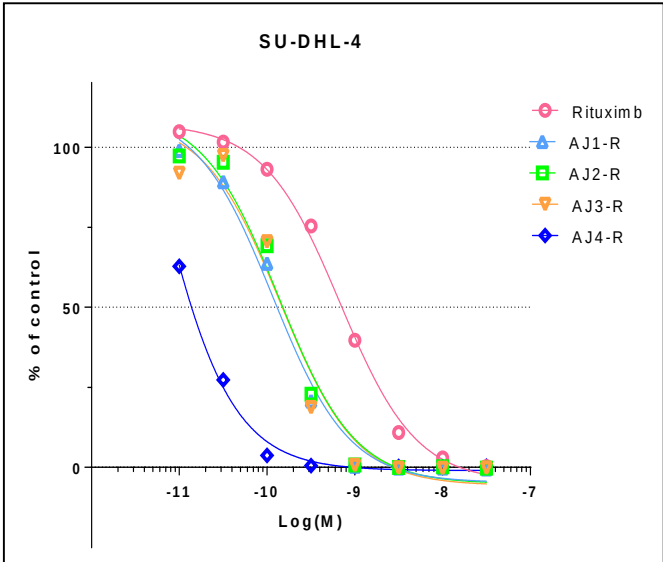
In Vitro Cell-Based Assay: Cytotoxicity Assessment

Cell lines

- CD20+
- ✓ SU-DHL-4

Subject

- Rituximab ADCs



AJICAP™

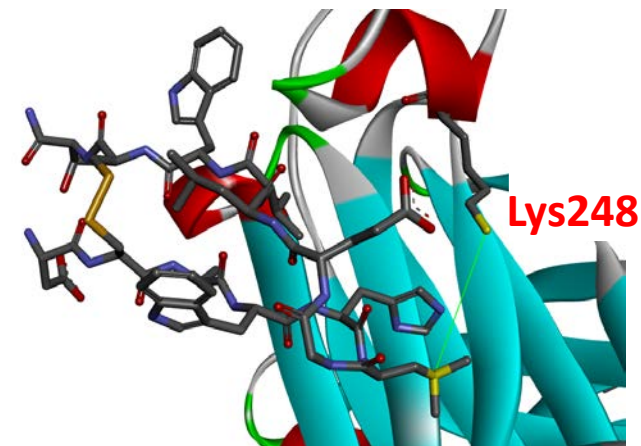
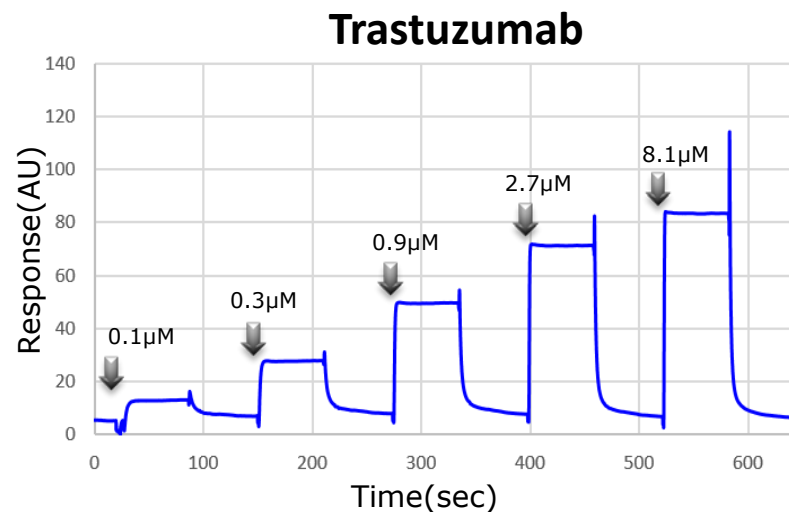
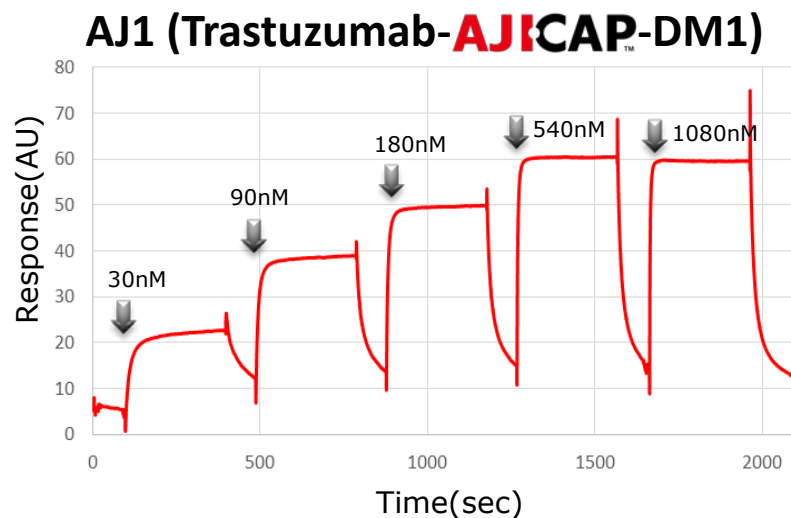
			IC ₅₀ (nM)
Name	mAb	Payload	SU-DHL-4 (Ag+)
AJ1R	Rituximab	DM1	0.12
AJ2R		MMAE	0.14
AJ3R		MMAF	0.14
AJ4R		PBD	0.0048
Rituximab			0.68

AJICAP™-Rituximab-ADCs showed *in vitro* cytotoxic activities against CD20+ cell line.

FcRn Binding: AJICAP™ ADC vs Parent mAb

- Objective: To confirm retained FcRn binding in AJICAP™-ADCs
- Material: AJ1 (Trastuzumab-**AJICAP**™-DM1), Trastuzumab
- Method: SPR analysis by Biacore™ instrument

Sensorgrams of each material at pH6.0



Binding affinity (K_D value)

	(nM)	
	pH6.0	pH7.4
AJICAP ™	54	No binding
Trastuzumab	400	No binding

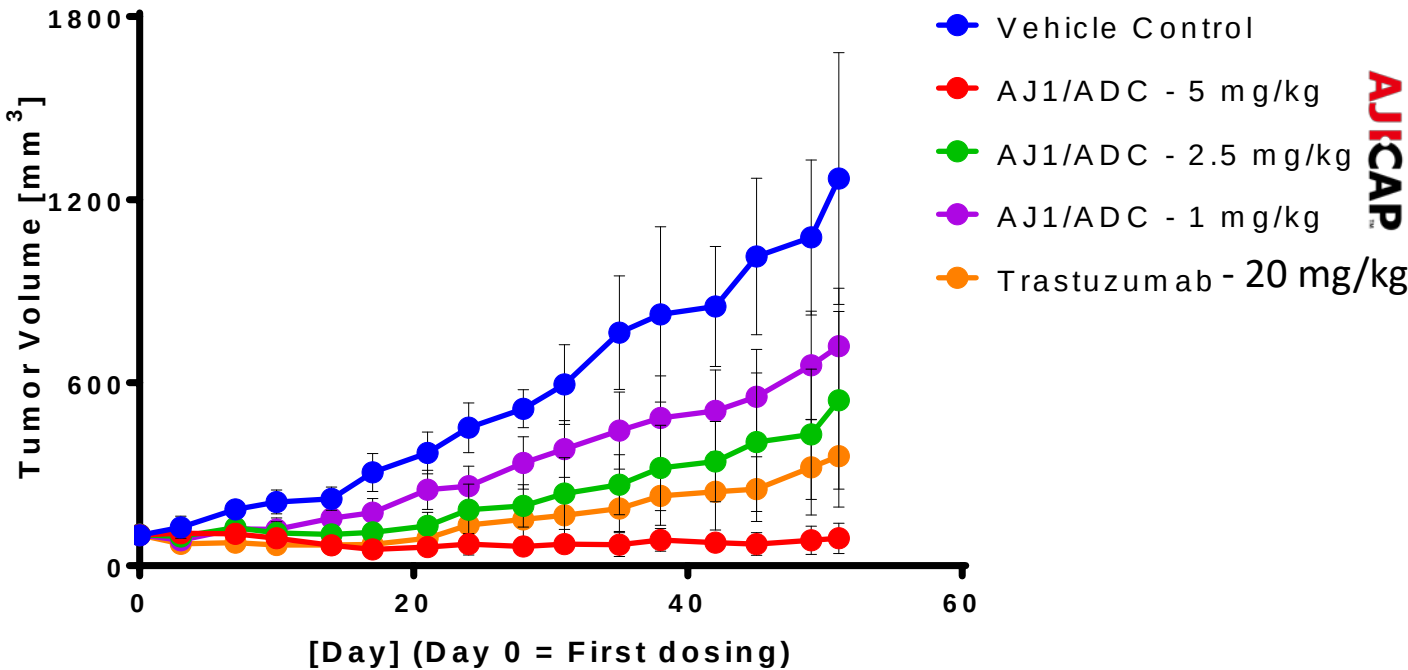
AJICAP™-ADCs retain the FcRn binding ability of the parent mAb despite Fc conjugation.

In Vivo Xenograft-NCI-N87 Mouse Model

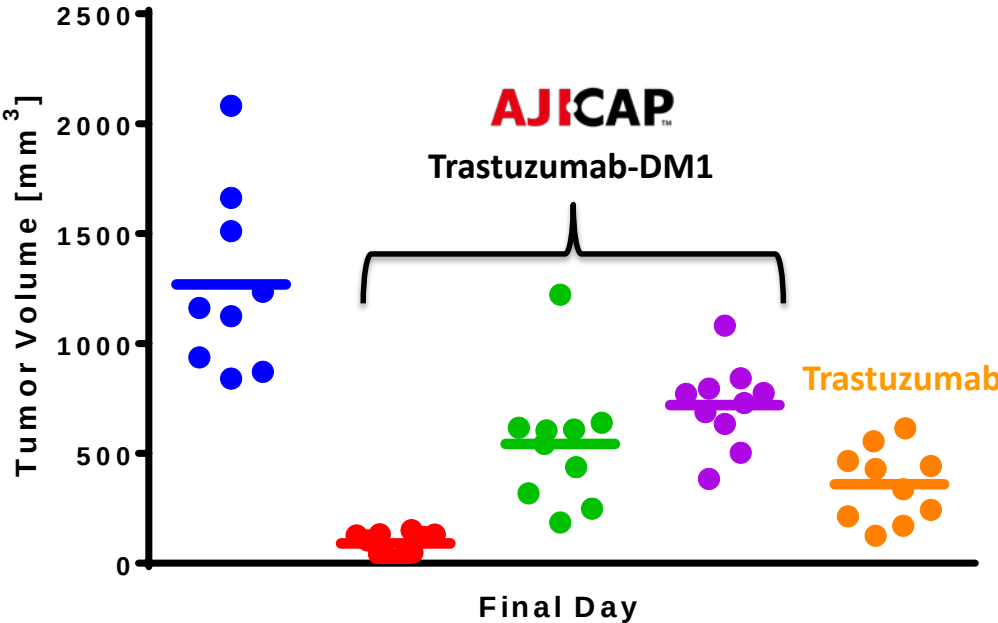
NCI-N87: HER2+, gastric cancer xenograft model
Multi-dose study
Objective: To measure the potency of AJICAP™-ADCs

AJ1: Trastuzumab-DM1

NCI-N87 Mouse Model



NCI-N87 Mouse Model



5 mg/kg AJICAP™ ADC shows sustained tumor inhibition

In Vivo Xenograft-NCI-N87 Mouse Model

NCI-N87: HER2+, gastric cancer xenograft model

Multi-dose study

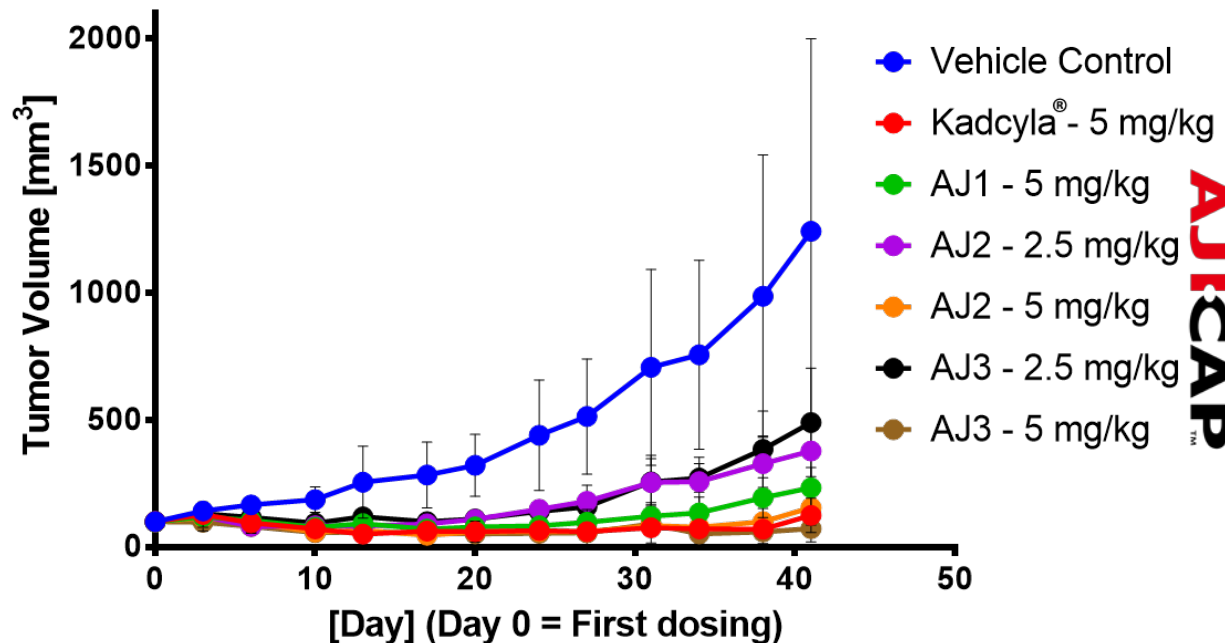
Objective: To measure the potency of AJICAP™-ADCs

AJ1: Trastuzumab-DM1

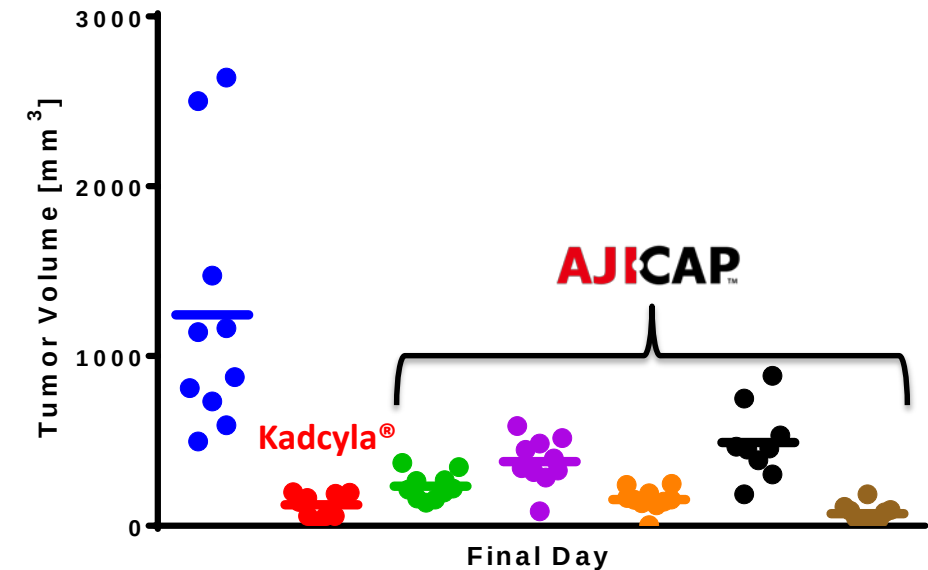
AJ2: Trastuzumab-MMAE

AJ3: Trastuzumab-MMAF

NCI-N87 Mouse Model



NCI-N87 Mouse Model



5 mg/kg AJICAP™ ADC shows tumor inhibition comparable to Kadcyła®

In Vivo Xenograft-JIMT-1 Mouse Model

JIMT-1: HER2+, ductal breast cancer xenograft model

Single dose study

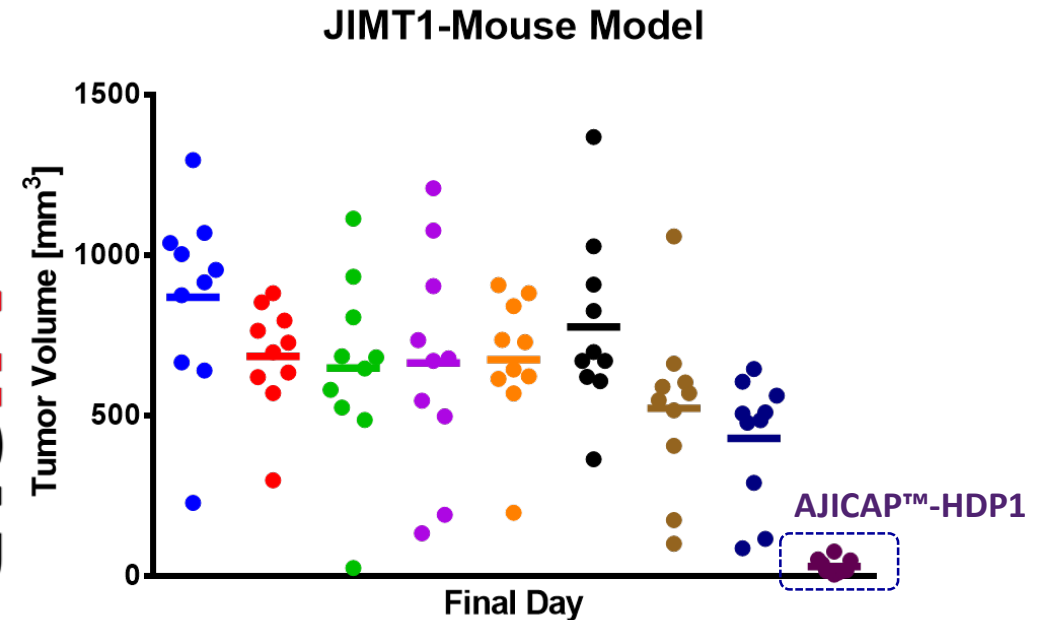
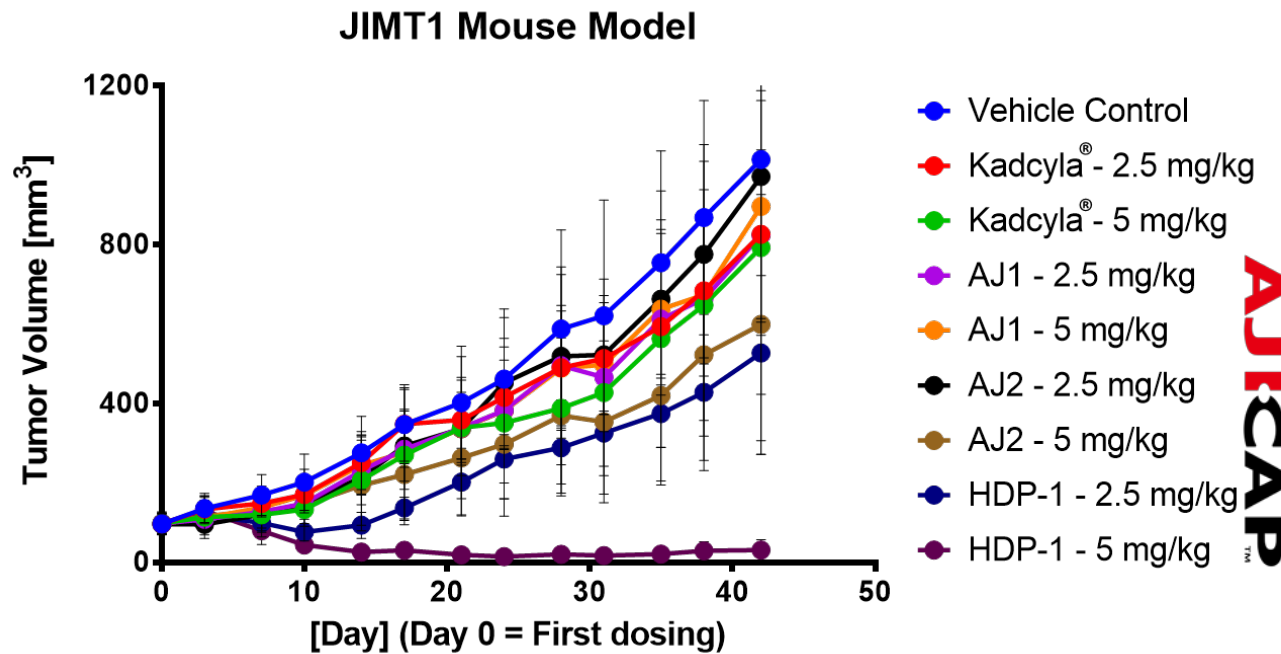
Objective: To measure the potency of AJICAP™-ADCs

AJ1: Trastuzumab-DM1

AJ2: Trastuzumab-MMAE

AJ3: Trastuzumab-MMAF

HDP-1: Trastuzumab- α -amanitin



5 mg/kg HDP-1 shows sustained tumor inhibition

Partner: 
Focused Cancer Therapies

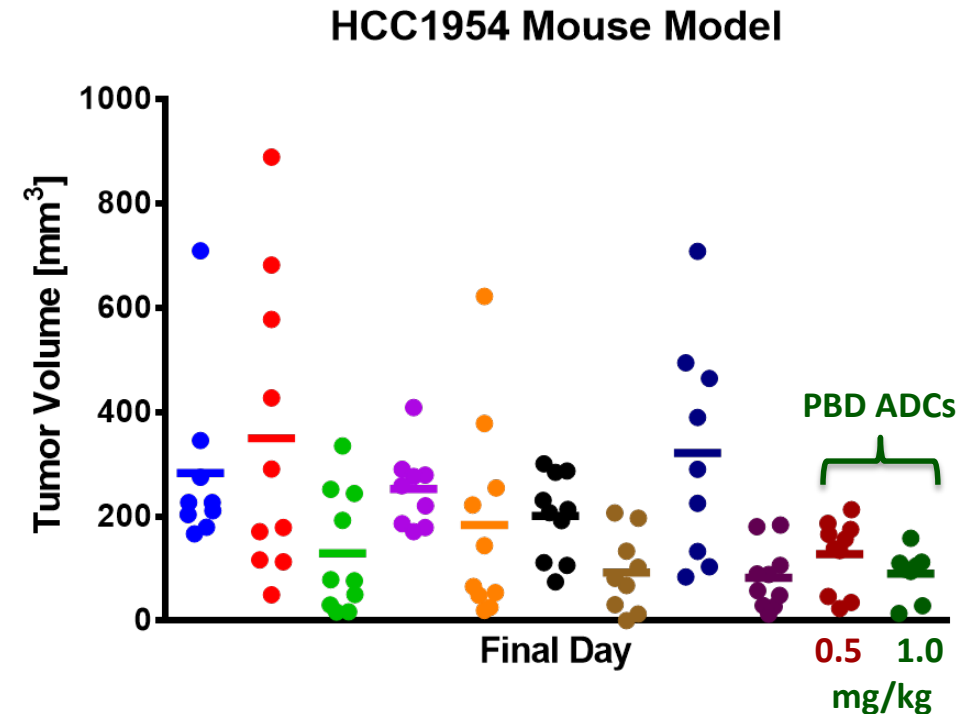
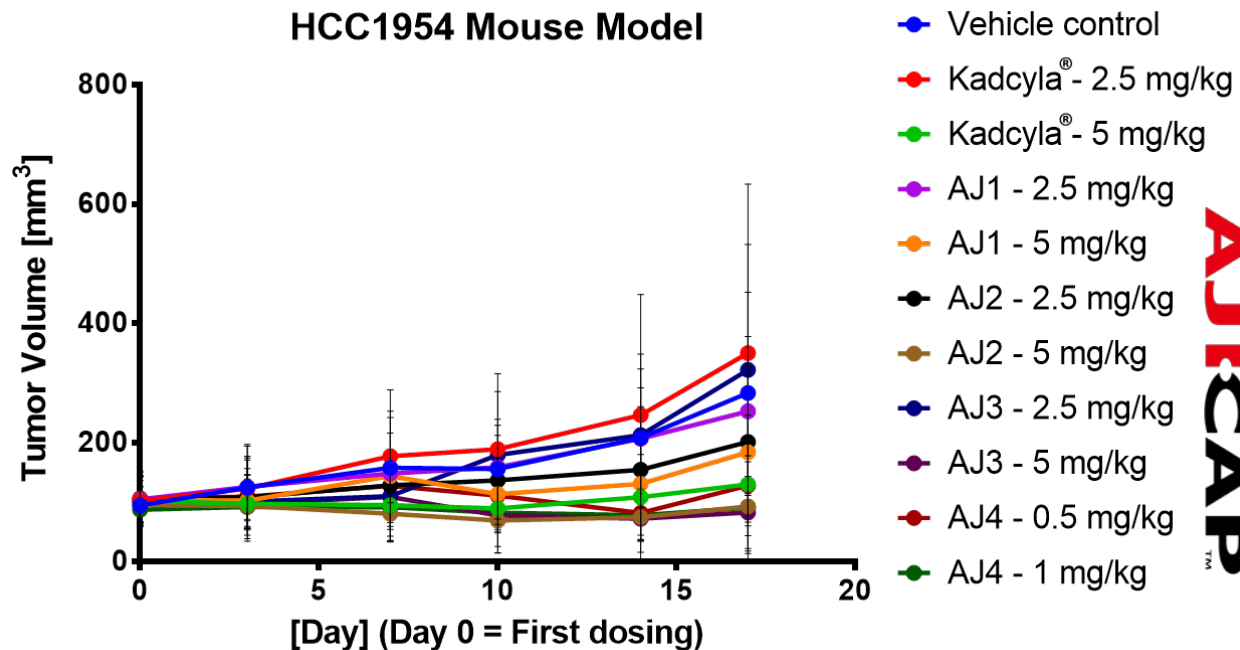
In Vivo Xenograft-HCC1954 Mouse Model

HCC-1954: HER2+, breast cancer model xenograft model – *in progress*

Single dose study

Objective: To measure the potency of AJICAP™-ADCs

AJ1: Trastuzumab-DM1
AJ2: Trastuzumab-MMAE
AJ3: Trastuzumab-MMAF
AJ4: Trastuzumab-PBD



Single-dose study comparing various payload activities: *study in progress*

Gram Scale Synthesis

**2.64 g, 10 mg/mL
Trastuzumab**

Step-1: peptide conjugation
Purification: TFF
Yield: 95%

2.59 g, 6.0 mg/mL

Step-2: linker cleavage
Filtration: 0.2 µm filter
Purification: TFF
Yield: 65% (*optimization in progress*)

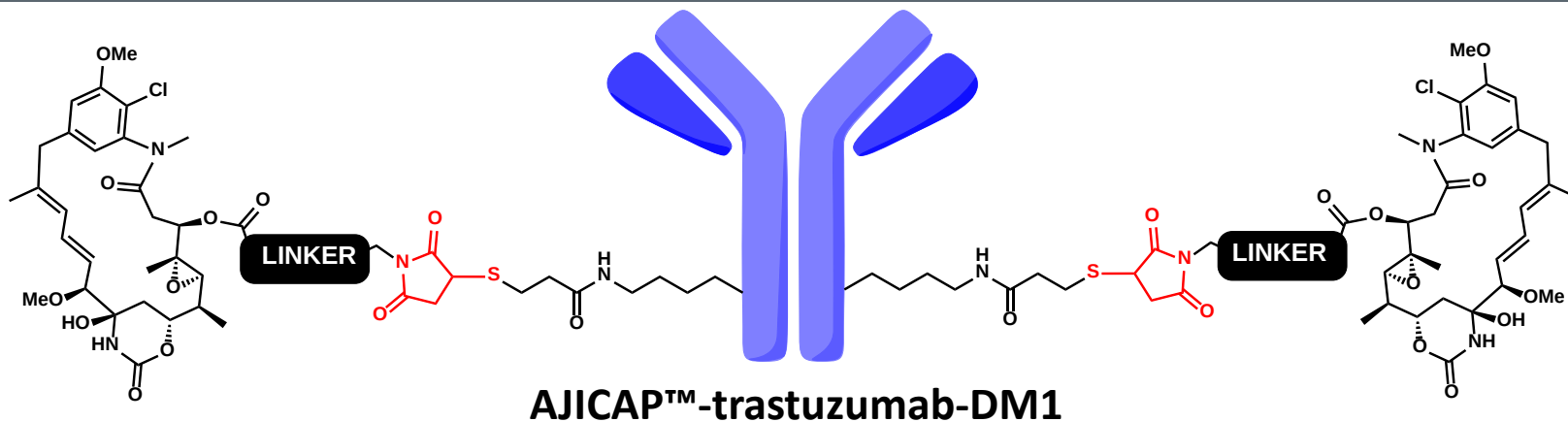
1.63 g, 6.0 mg/mL

Step-3: re-oxidation
Purification: TFF
Yield: 100%

1.63 g, 6.8 mg/mL

Step-4: Payload conjugation
Purification: TFF
Yield: 98%

1.67 g, 5.7 mg/mL
AJICAP™-ADC



AJICAP™-trastuzumab-DM1

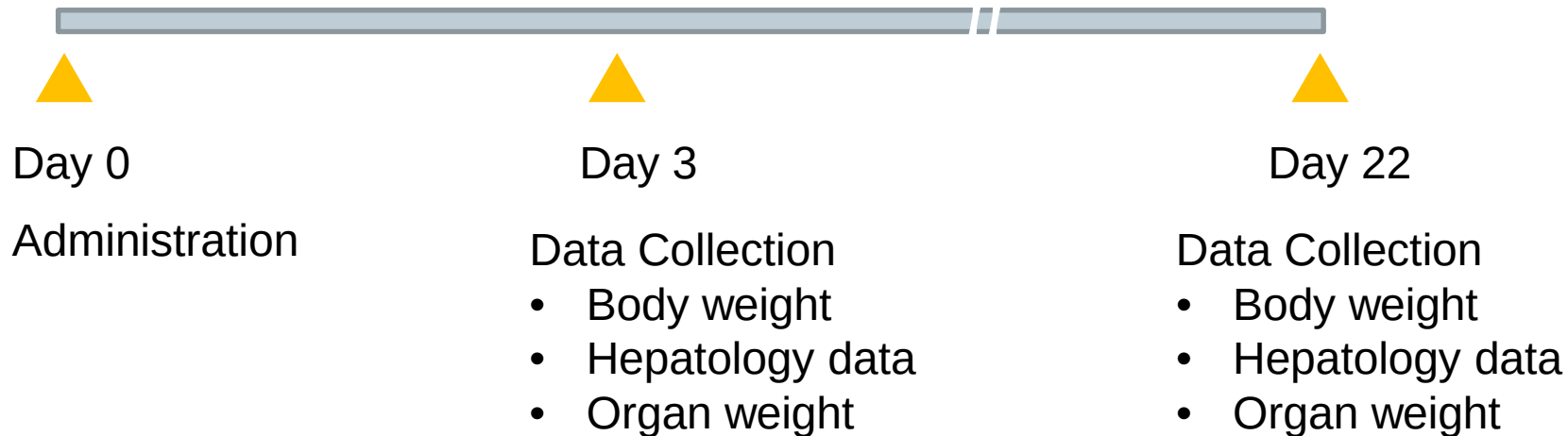
Analytical summary of AJICAP™-ADC

Amount	1.67 g
Overall yield	63% (4 steps overall)
DAR	1.9 (Q-TOF), 1.8 (HIC)
Aggregation	4.7% (SEC)
Percent free drug	Not detected by MS
Endotoxin	0.105 EU/mL

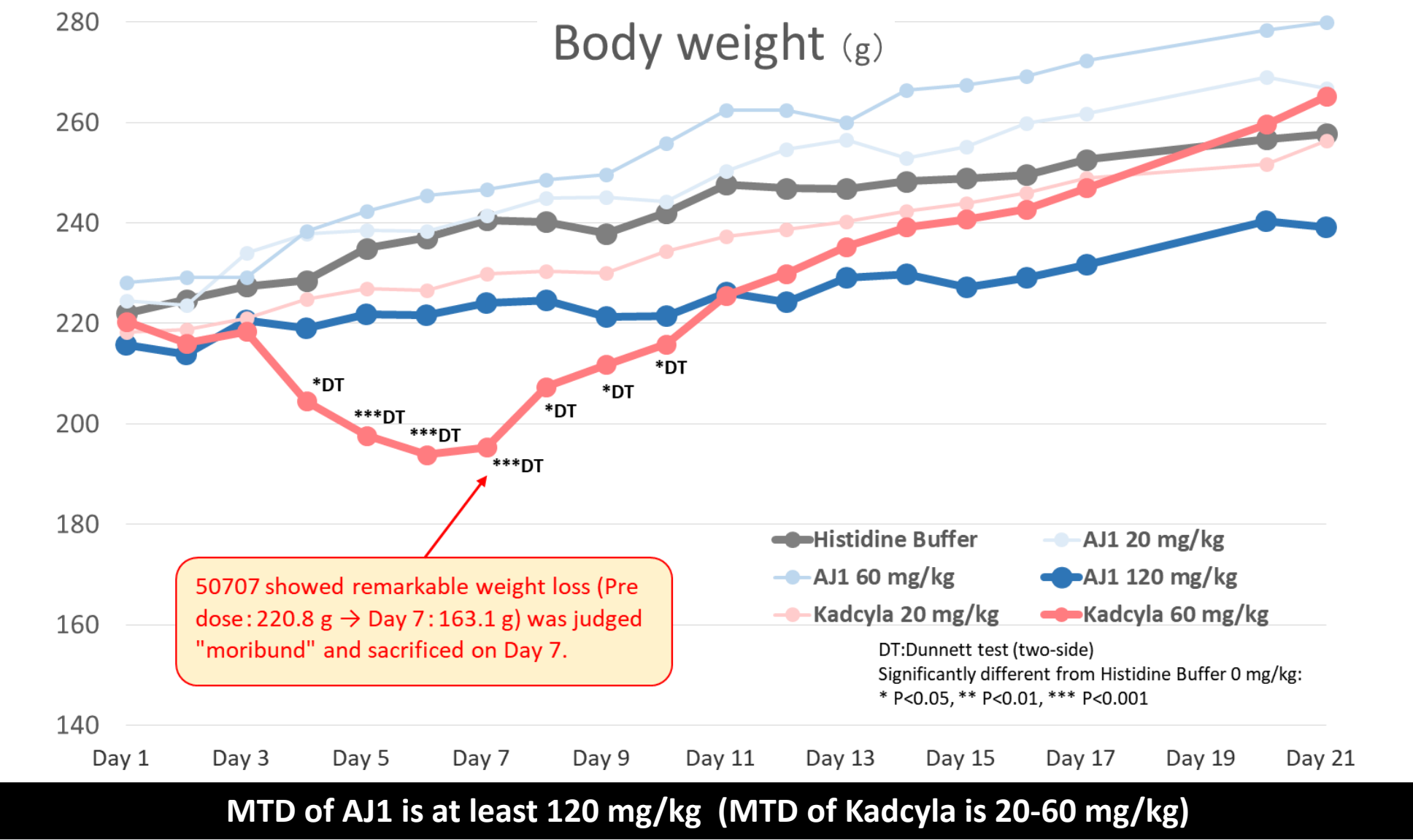
Max scale performed to date: 3.30 g mAb
Employed a scale-down manufacturing purification approach

In Vivo Safety Study: Design of Rat Toxicology Study

- **Objective:** Comparison of AJICAP™-ADC to a comparable stochastic ADC
- **Dose:** AJ1 (Trastuzumab-**AJICAP**™-DM1) – 20, 60, 120 mg/kg
Kadcyla® – 20, 60 mg/kg
- **Administration:** Single dose, intravenous administration
- **Animal:** 5 female SD rats / arm
- **Initial Age:** 9 weeks (approximate body weight 220 g)

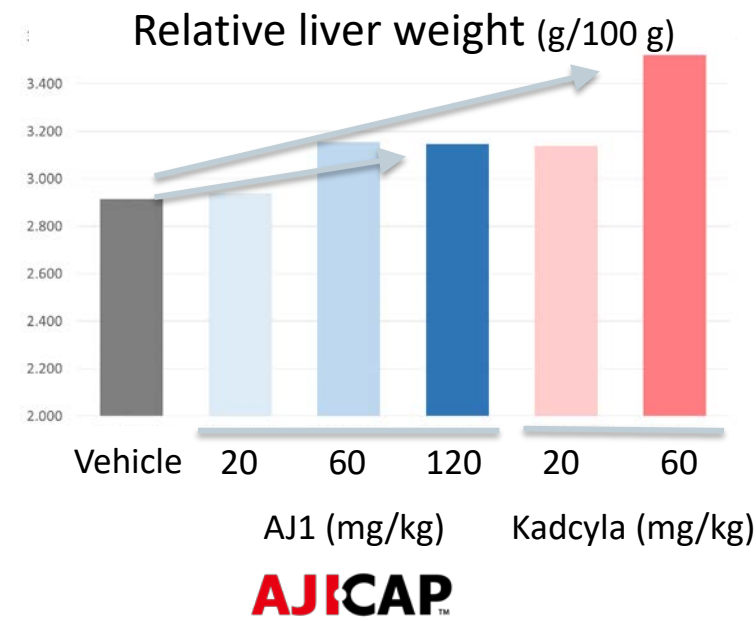
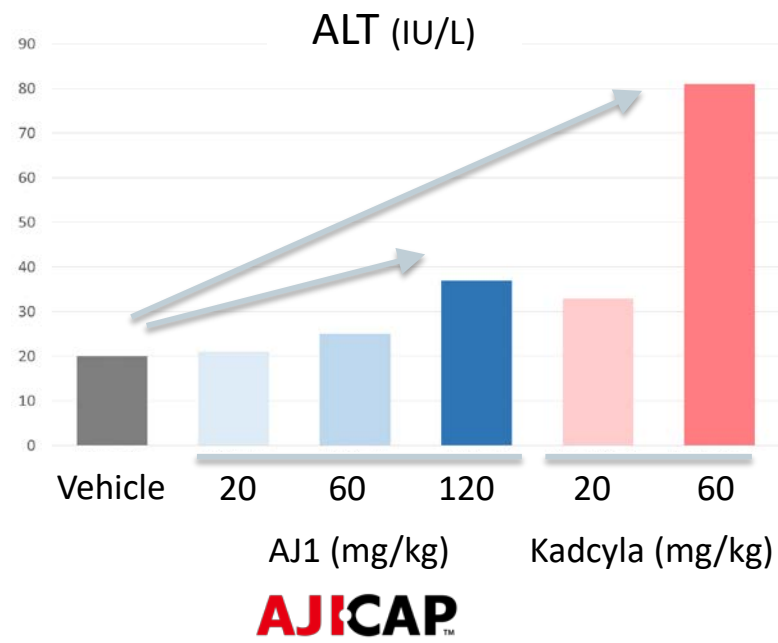
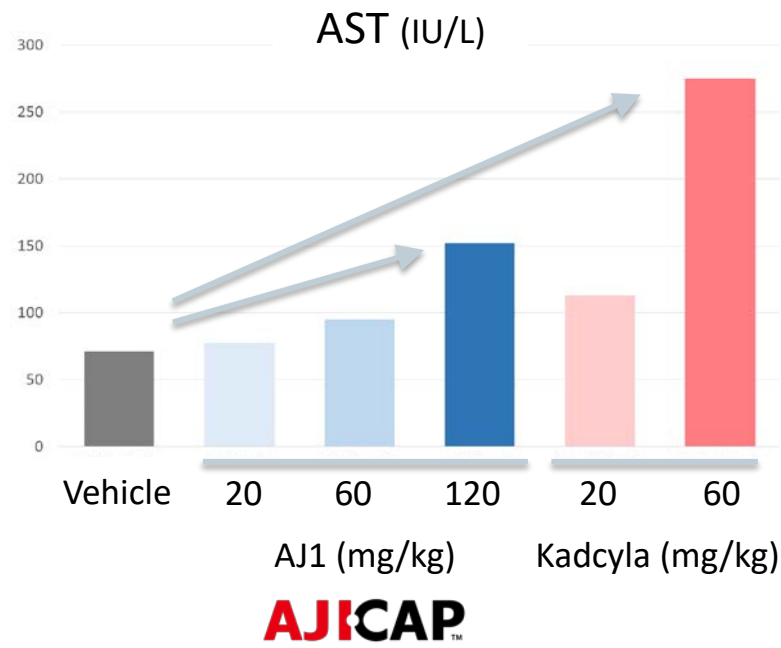


In Vivo Safety Study: Animal Body Weights



In Vivo Safety Study: Acute Clinical Chemistry

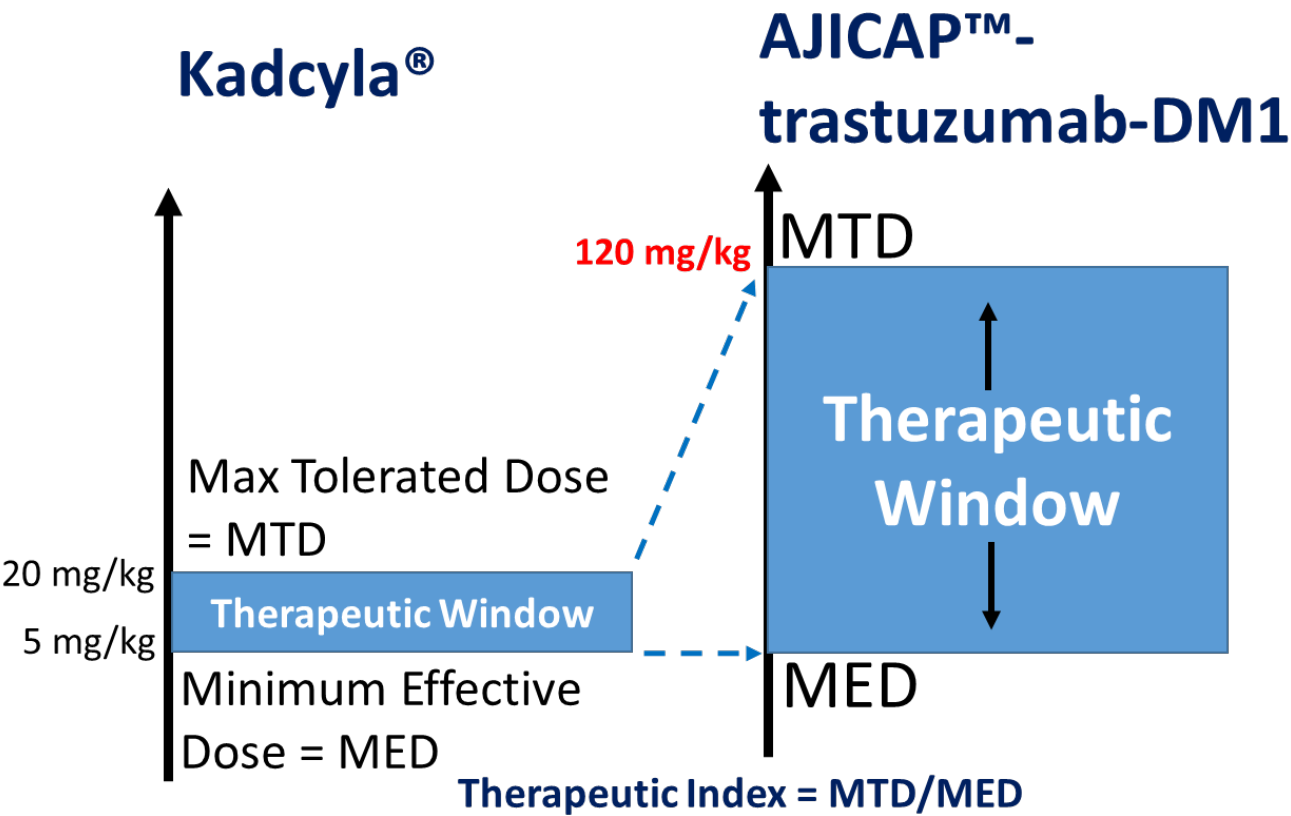
Data at Day 3



**Liver Parameters Support MTD Determinations:
MTD of AJ1 is at least 120 mg/kg (MTD of Kadcylla is 20-60 mg/kg)**

Summary of *In Vivo* Safety – Rat Toxicology Study

	AJICAP™ AJ1 120 mg/kg	Kadcyla® 60 mg/kg
Body weight	→	↓↓↓ (1/5 animal dead)
Relative liver weight	↑	↑↑↑
AST	↑	↑↑↑
ALT	↑	↑↑↑
Neutrophils	↑	↑↑↑
Reticulocytes	↓	↓↓↓
Lymphocytes	→	↓↓
Platelets	→	↓↓



AJICAP™-trastuzumab-DM1 has an **expanded Therapeutic Window**

Conclusion



Ajinomoto Co. has been developed an innovative direct chemical site-selective conjugation method used towards intact native antibodies.

Our novel technology provides significant advantages for next generation ADCs:

1. **Straightforward CMC**
 - Direct modification of intact antibody
 - No genetic modification required
2. **Enhancement of Therapeutic Window compared to stochastic ADCs**
 - Site specific, controlled DAR 2
3. **Wide drug-linker compatibility: reactive group compatibility includes thiol, alkyne, azide, etc**
4. **High yields, short conjugation reaction times, ease to manufacture**

Contacts:

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BIO•PHARMA
S E R V I C E S

ADC CDMO Services

Well Suited for Highly Potent / ADC Manufacturing

DRUG SUBSTANCE MANUFACTURING

- cGMP conjugation and purification for clinical and commercial applications
- Preparation of GLP tox material
- Isolator for potent compound handling
- Reactor sizes range from 1 -100 L
- TFF and chromatography system

PROCESS DEVELOPMENT

- Technology transfer / Optimization
- Process verification
- Conjugation process development milligram to gram scale
- Formulation development
- Material generation for pre-clinical studies

FOCUS ON SAFETY

- Facility designed to and tested to handle compounds with OEL down to 1 ng/m³
- Dedicated SHE personnel
- Barrier containment isolators and hoods
- Single use technology employed to minimize personnel risk

FILL/FINISH & LYOPHILIZATION

- Fully contained & integrated
- 120 vials/min for 2 mL vials
- Fill line batch sizes up to 70,000 vials
- Lyophilizer batch capacity up to 20,000
- Vial sizes: 2 mL – 50 mL
- Isolator technology

AJICAP™ TECHNOLOGY

- Site-specific conjugation technology
- Chemical mediated site specific modification to a native IgG molecule or fragment
- No mAb engineering required
- No observation of compatibility limitations

ANALYTICAL SERVICES

- On-site, dedicated analytical labs
- Analytical methods development
- Raw materials testing
- QC release / stability
- Product characterization
- Methods validation

High Potency and ADC Services

A single US site for high containment & ADC bioconjugation & fill finish



Bioconjugation and Fill Finish of ADCs



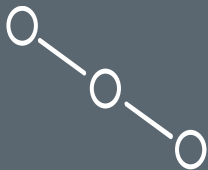
ADC Process Development and Purification



Fill Finish and Analytics of Highly Potent Oncology Drug Products



Full Analytical Suite to Support DS and DP release, Stability, Validation of ADC processes



A SIMPLIFIED SUPPLY CHAIN



Why Ajinomoto Bio-Pharma Services?

**LET'S
MAKE**

A NEW FUTURE



Responsive, Trusted, Innovative
and Reliable Partner

Global Regulatory Excellence

Clinical Studies Through
Commercial Success

Embrace your every manufacturing
challenge

**WHAT DO YOU
WANT TO
MAKE?**

