Development of a Novel Chemical Site-Specific Conjugation Platform for ADCs - AJICAP™ Technology -

Kei Yamada[1,2], Tomohiro Fujii[1], Takuya Seki[1], Yuri Ooba[1], Takahiro Narita[1], Akira Nakayama[1], Yoshiro Kitahara[1], Natsuki Shikida[1], Kazutaka Shimbo[1], Kunio Nakata[1]
Tatsuya Okuzumi[1*], Yuji Ito[2], Yutaka Matsuda[3], Zahra Khedri[3] and Brian A. Mendelsohn[3*]

[1]Ajinomoto Co., Inc., [2]Kagoshima University, [3]Ajinomoto Bio-Pharma Services

PepTalk THE PROTEIN SCIENCE WEEK
January 14-18, 2019 | San Diego Hilton Bayfront | San Diego, CA

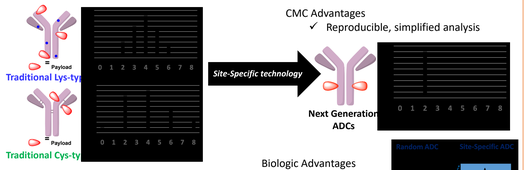
Introduction

1. About Antibody-Drug Conjugates (ADCs)

Protein modification has long inspired chemists and biologists. For instance, antibody–drug conjugates (ADCs) continue to fascinate because they offer the ability to target drug delivery to specific cells, based on the cell-specific recognition and binding of an antigen by a monoclonal antibody (mAb). Across various oncology indications, by delivering a cytotoxic payload to cancer cells, ADCs can be expected to provide treatment breakthroughs by offering increased efficacy and decreased toxicity compared to traditional chemotherapeutic cancer treatments.

2. About Site-Specific Conjugation

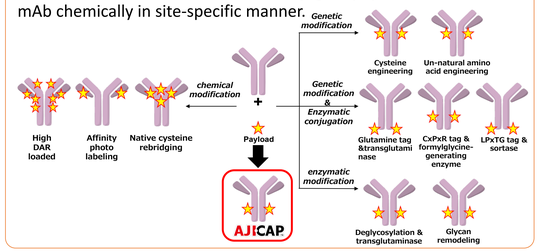
In order to prevent interference with antigen recognition and therefore ensure that the ADC is effective, the drug moieties can be connected to consistent fragment (Fc region) or conserved area away from the antigen-binding region of the mAb. In the recent scientific literature, various groups have reported that changing the number and position of drug payloads in ADCs affects the pharmacokinetics, drug release rate and biological activity. Using conventional stochastic conjugation methods for ADC preparation results in an ADC population with the drug/antibody ratio (DAR) and payload locations being heterogeneous. Site-specific conjugation technology can result in homogeneous (or low heterogeneity) ADCs and benefits to biologic activity as well as manufacturing.



Site-specific technologies are great demand for next generation ADCs

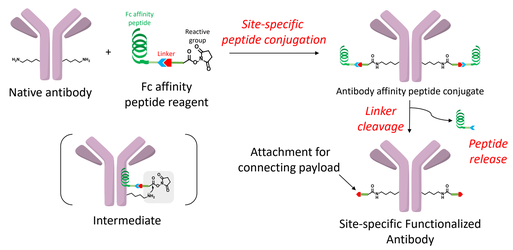
3. Site-Specific Conjugation Technologies

Most site-specific conjugation techniques developed for mAbs are based on genetic modification of the mAb, which aim to introduce unnatural amino acids to incorporate a biorthogonal functional group or free cysteine residue into an antibody as the conjugation handle. Enzymatic conjugation, such as transglutaminase-driven asparagine modification and glycan re-modelling, have also been tested.[1] AJICAP can enable us to conjugate payloads with a native mAb chemically in site-specific manner.

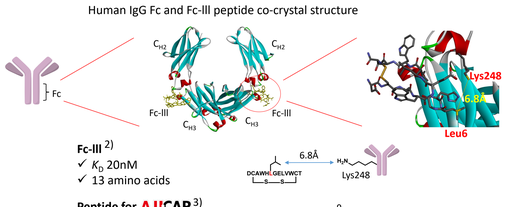


Methods

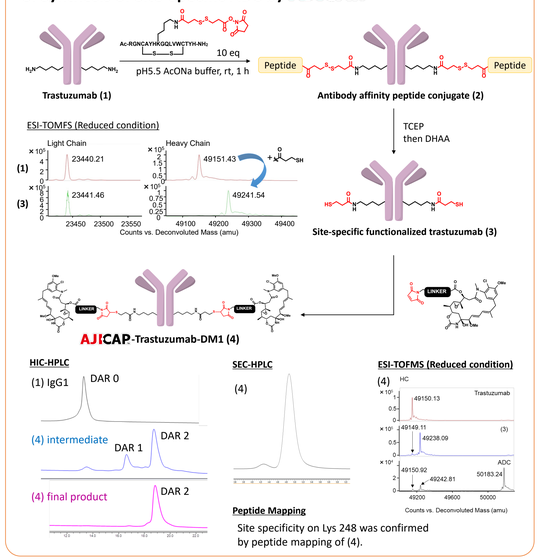
1. Concept of AJICAP



2. Determine Target Lysine Residue

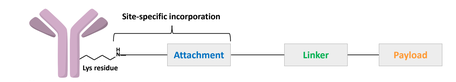


3. Synthesis of Site-Specific ADC by AJICAP



Results

1. Capability of AJICAP



Isotype	mAb
IgG1	Trastuzumab
	Rituximab
	Adalimumab
	X company-mAb A (client project)
	X company-mAb B (client project)
	Y company-mAb (client project)
IgG2	Denosumab
IgG4	Dupilumab

Attachment	Structure
Thiol (-maleimide)	
Azide (-cycloalkyne)	
Aldehyde & (-hydroxylamine)	

Linker
Cleavable
Non-cleavable

Payload
DM-1
MMAE
MMAF
PBD
α-amanitin
Confidential payload (Client project)

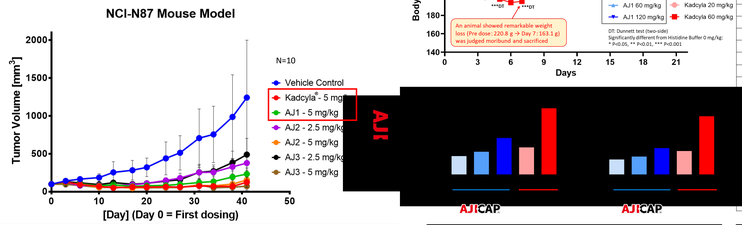
AJICAP technology is compatible with a wide variety of mAbs, attachments, linkers, and payloads.

4. FcRn Binding with SPR Assay

	K_D value (nM) pH6.0	pH7.4
AJ1	54	No binding
Trastuzumab	400	No binding

Binding dependency with pH was equally observed between AJ1 and Trastuzumab.

5. Mouse Xenograft Model

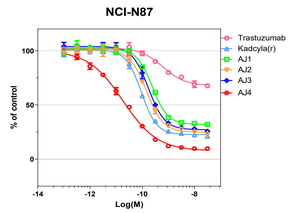


- Both AJ1 and Kadcyla are effective at approximately the same dose
- Effective dose: 5 mg/kg

2. Materials

Definition of ADCs	mAb	Conjugation	Attachment	Payload
AJ1	Trastuzumab	AJICAP	Thiol-Maleimide	DM1
AJ2				MMAE
AJ3				MMAF
AJ4				PBD
Kadcyla		Stochastic		DM1

3. *In Vitro* Cytotoxicity



- AJICAP ADCs have *in vitro* cytotoxic activity reflecting payload cytotoxicity.

6. Safety Study in Rats

- The MTD (maximum tolerance dose) for AJ1 is >120 mg/kg while MTD for Kadcyla is 20 mg/kg.

7. Therapeutic Index

Dose (mg/kg)	Kadcyla (DAR 3.6)	AJ1 (DAR 2.0)
>120		MTD
115		
110		
105		
100		
95		
90		
85		
80		
75		
70		
65		
60	Death	> X 24
55		
50		
45		
40		
35		
30		
25		
20	MTD	
15	X 4	
10		
5	ED	ED
0		

- TI (Therapeutics Index) for AJ1 is > 24-fold while TI for Kadcyla is 4-fold.

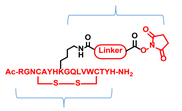
Conclusion

1. Features of AJICAP[4]

Ajinomoto Co. has 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 productivity, short conjugation reaction times, ease to manufacture**

2. A glance at second generation AJICAP technology



- ✓ Shorter reaction process
- ✓ No red/ox chemistry
- ✓ Lower aggregation propensity
- ✓ Introduction of azide group to mAb
- ✓ Expansion of conjugation sites (LysXXX, LysYYY, etc.) through peptide SAR
- ✓ Stability enhancement of peptide reagent

References

1) a) Tsuchikawa, K.; An, Z. *Protein&Cell*, **2018**, *9*, 33-46.
   b) Frigerio M; Kyle A.F. *Curr Top Med Chem.* **2017**, *17*, 3393-3424.
2) DeLano, W. L.; Ultsch, M. H.; de Vos, A. M.; Wells, J. A. *Science*, **2000**, *287*, 1279-1283.
3) WO2016/186206
4) WO2018/199337

Corresponding author

Ajinomoto Co., Inc. 1-1, Suzuki-cho, Kawasaki-ku, Kawasaki-shi 210-8681, JAPAN
Phone: +81-44-244-7163 E-mail: tatsuya_okuzumi@ajinomoto.com
Ajinomoto Bio-Pharma Services, 11040 Roselle St., San Diego, CA 92121, USA
Phone: +1-858-281-7470 E-mail: Brian.Mendelsohn@US.AjiBio-Pharma.com