

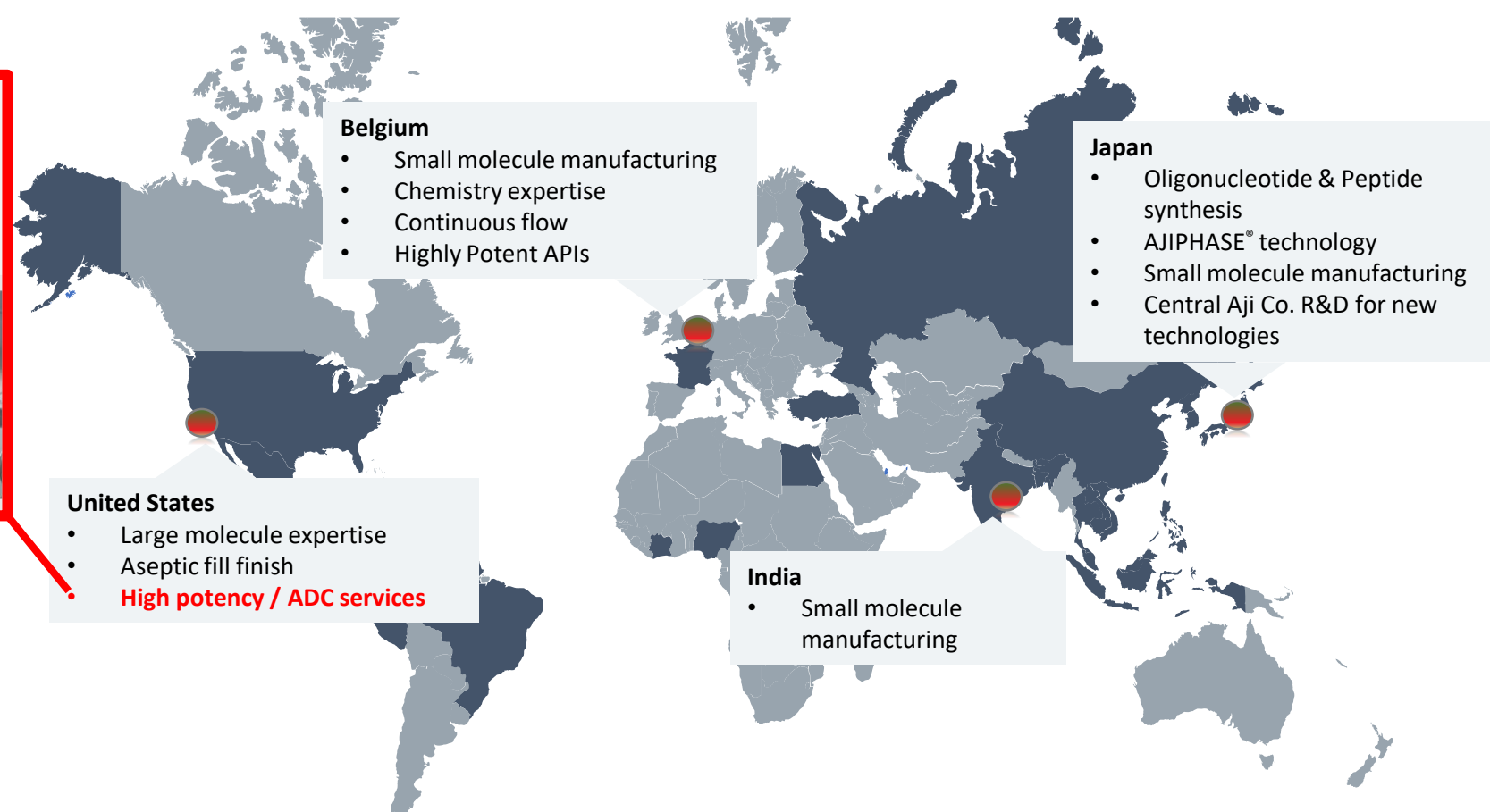
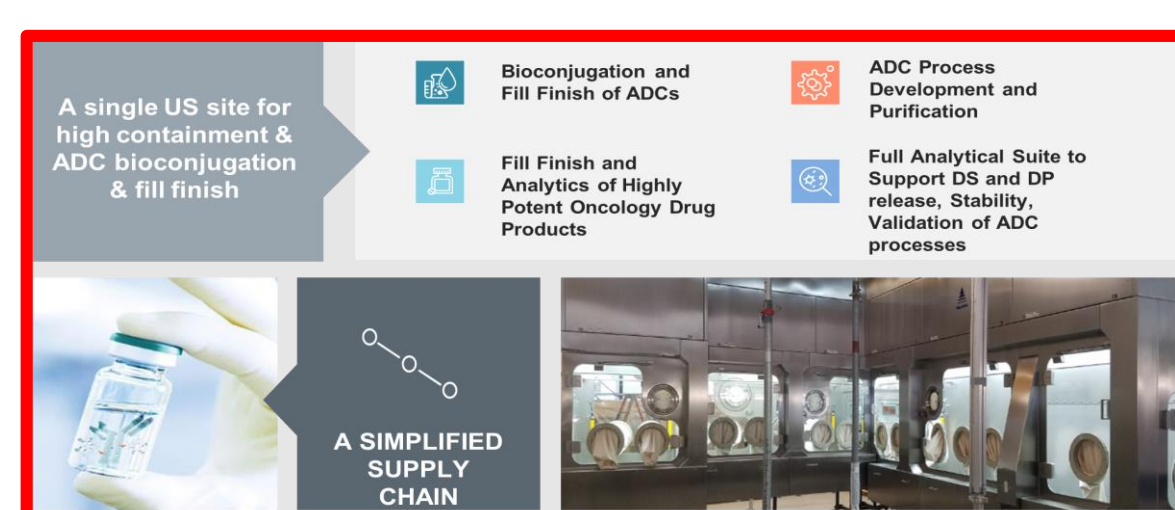
Good Manufacturing Practices Strategy Applied to ADC Synthesis

Produced by Site-Specific Chemical Conjugation: **AJICAP™** Technology

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Ajinomoto Bio-Pharma Services



Introduction: GMP Strategy¹

Ajinomoto Bio-Pharma Services has developed a phase appropriate cGMP process for the production of materials suitable for use in early development and GLP pre-clinical studies. Producing these GLP-ready materials with solid documentation packages will enable the filing of investigational new drug applications (INDs) and eventually new drug applications (NDA). This process was developed using guidance from multiple regulatory agencies to adapt traditional GMP production for site-specific ADC synthesis. Using this strategy, site-specific ADCs were produced on gram-scale and synthesized utilizing AJICAP™ technology.

Table 1. Comparison of R&D, GMP, and our approach to producing material suitable for GLP pre-clinical studies.

Topic	Research & Development	GMP	Our Approach
Facility	Areas are not product dedicated	Separate or defined areas to prevent cross-contamination	Use of segregated area in a GMP manufacturing facility to ensure no cross-contamination
Equipment	Equipment qualification not completed No defined maintenance schedules	Equipment installation, operational and performance qualifications (IOPQ) completed Established maintenance and calibration schedules	Equipment used for analytical testing is classified as GMP and has full IOPQ execution Routine maintenance, calibration and requalification
Manufacturing controls	Experiments and studies are documented Materials are off the shelf	All manufacturing operations are documented at the time of execution Second person verification is required RM control strategy in place	All process steps are documented at the time of execution with a second verifier, in a part 11 compliant ELN system RMs are qualified and released prior to use
Laboratory controls	Analytical methods are demonstrated to be suitable for their intended use but are not qualified	Appropriate qualification, validation and documentation of test methods in place	Analytical methods are documented prior to or at the time of completion depending on the maturity of the product lifecycle

Gram-Scale **AJICAP™** ADC Synthesis

Step-1: Peptide conjugation

Trastuzumab: **1900 mg**
Concentration: 10 mg/mL
Buffer: AcONa buffer (pH 5.5)
Peptide Reagent: **10 eq**

1 h, rt
Purification with TFF system
SoloVPE, free-peptide measurement by Q-TOF

Amount: 1.92 g, Yield: 98%

Step-2: Linker cleavage

Step-1 product: **1920 mg**
Concentration: 6.9 mg/mL
Buffer: reduction buffer
TCEP: **20 eq**

1 h, 37 °C
Purification with TFF system
SoloVPE, free-peptide measurement by Q-TOF

Amount: 1.79 g, Yield: 96%

Step-3: Re-oxidation

Step-2 product: **1790 mg**
Concentration: 6.6 mg/mL
Buffer: conjugation buffer
DHAA: **40 eq**

3 h, rt
Purification with TFF system
SoloVPE, Q-TOF, SEC

Amount: 1.78 g, Yield: 100%

Step-4: Payload conjugation

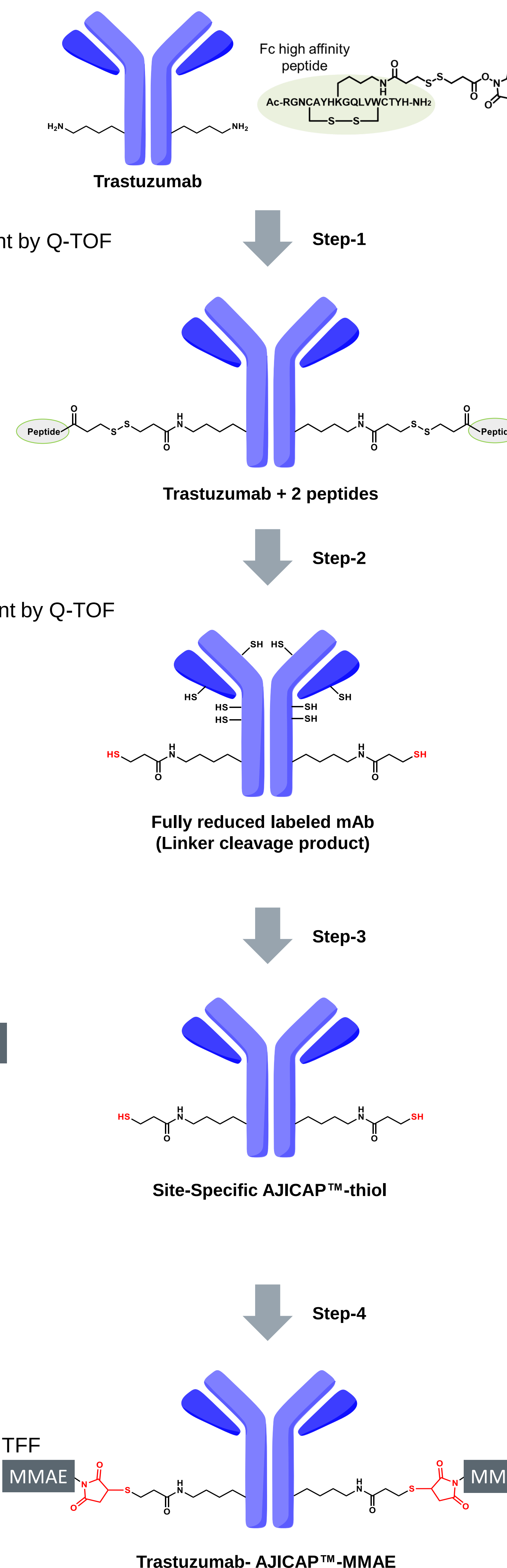
Step-3 product: **1780 mg**
Concentration: 6.8 mg/mL
Buffer: conjugation buffer
MC-VC-MMAE: **10 eq**

1 h, 20 °C
N-acetyl cysteine: **25 eq**
15 min, 25 °C

Filtration
Buffer exchange and purification by TFF
SoloVPE, Q-TOF, HIC, SEC

Amount: 1.73 g, Yield: 95%

Overall Yield: 90% (4 steps)



Analytical Results³

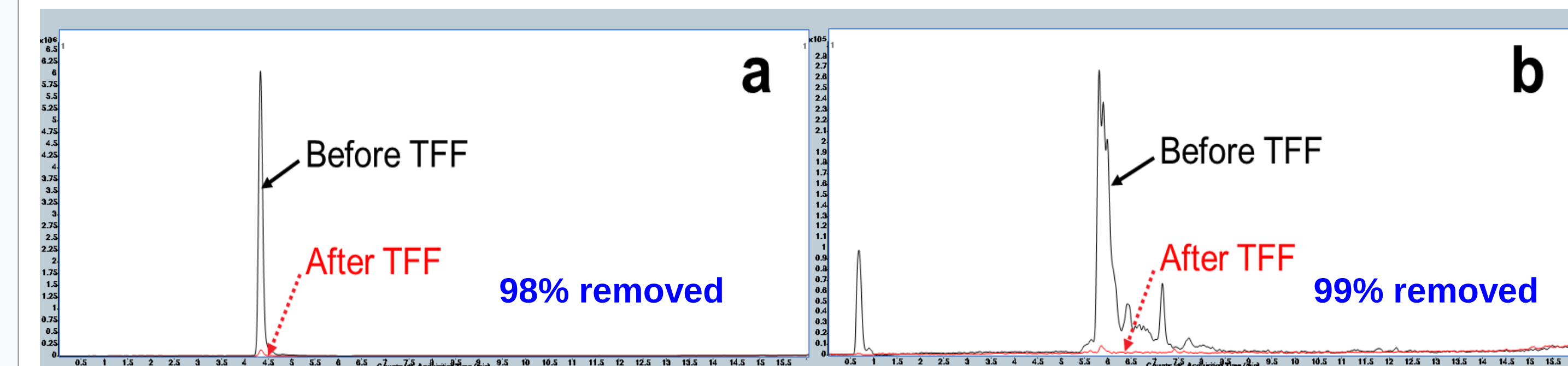


Figure 1. EICs showing a comparison of before (black) and after (red) TFF. a) EIC of hydrolyzed affinity peptide 1 with m/z ratio of 1134.97 ($z = 2$, $[M+2]^{2+}$) and b) EIC of NAC-quenched MC-vc-MMAE, with m/z ratio of 1479.68 (M^+).

Biological Assessment²

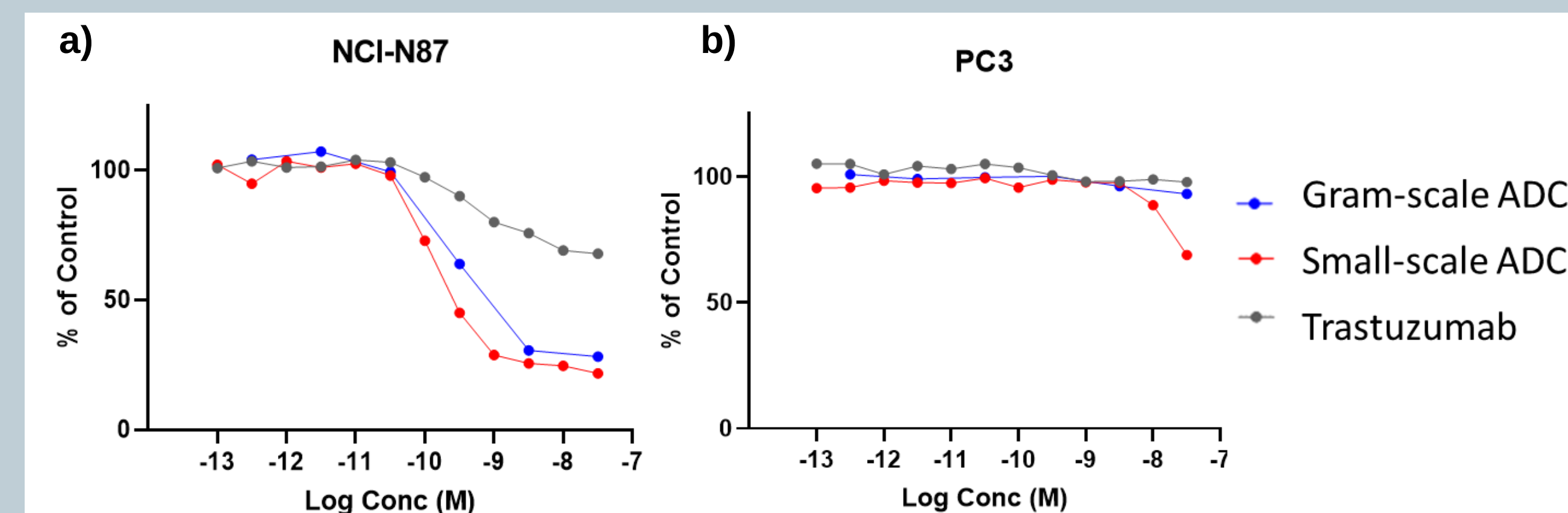


Figure 2. In vitro assessment in a HER2 antigen overexpressing cell line a) NCI-N87 gastric cancer cell and a low HER2 antigen expressing cell line b) PC-3, human prostate cancer cell. Both gram-scale and small-scale AJICAP™-ADCs show strong anti-tumor activity against the HER2 antigen overexpressing cells. In contrast, no cytotoxicity against the low HER2 expressing PC-3.

Table 2. IC50 Value of **AJICAP™** ADC

	NCI-N87	PC-3
Gram-scale	0.28 nM	>30 nM
Small-scale	0.16 nM	>30 nM
Trastuzumab	>30 nM	>30 nM

Conclusion

- A GMP strategy was established for use in GLP pre-clinical supply
- Successful gram-scale **AJICAP™** conjugation was conducted (amount: **1.73 g**, overall yield: **90%**)
- Both gram-scale and small-scale AJICAP™-ADCs show considerable potency and target selectivity toward cancer cells

References

- Matsuda, Y.; Clancy, C.; Tawfiq, Z.; Robles, V.; Mendelsohn, B. A. *in review*.
- Matsuda, Y.; Yamada, K.; Okuzumi, T.; Mendelsohn, B. A. *in review*.

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