# Design, Synthesis and Evaluation of a Novel Antibody-Drug Conjugate based on Cysteine Conjugation and a Polymer Linker

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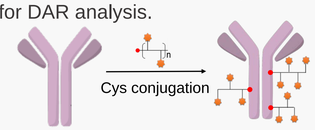
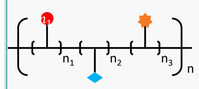
## Summary

NC18 ADC utilizes a polymer-based linker to afford high drug-to-antibody ratio (DAR>10) and significantly improves its potency, bystander effect and anti-drug resistance performance. As an effort to deliver high-quality NC18 materials, we carefully optimized the polymer-based conjugation process and developed a novel analytical method for DAR analysis.



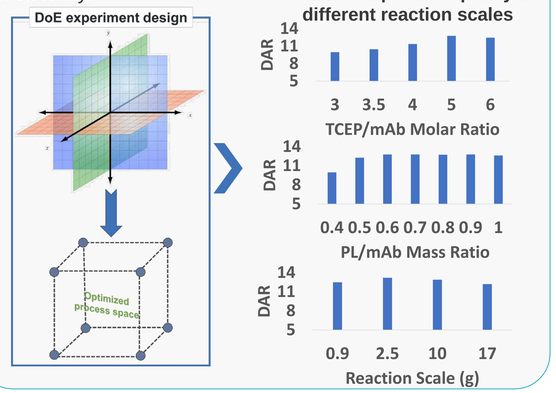

- AF-HEA cytotoxic payload
- A polymeric linker design
- Controllable bystander effect & superior PK profile

## Conjugation Process Optimization

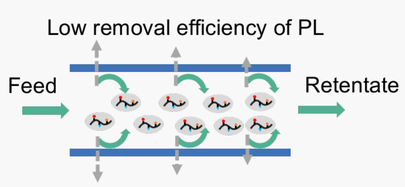


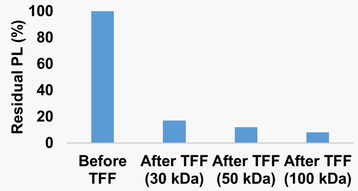

**Challenge:** The heterogeneity(MI & PI) of the polymer-based payload-linker (PL) structures poses challenges to generate consist batch-to-batch materials.

**Our solution:** DoE experiments to evaluate the PL molecular weight, MI/PI, and the mass ratio between mAb and the PL were performed to acquire the optimized process space with the improved performance to assure the batch-to-batch consistency.



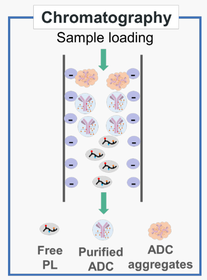

## Chromatography Process Optimization




**Challenges:** (1) The large-size polymer payload-linker could not be easily removed through a TFF process; (2) Conjugation of polymer payload-linker could induce ADC aggregation.

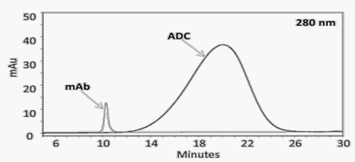
**Our Solution:** A cation exchange chromatography method was developed to remove the unconjugated payload-linker molecules as well as ADC aggregates from the conjugation.




**Significant improvement of SEC monomer percentage and efficient removal of free payload-linker species.**

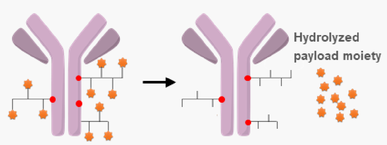


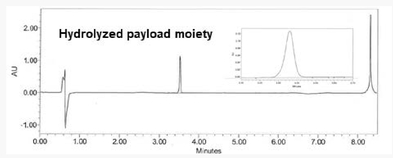

## Dedicated Method for DAR analysis




**Challenge:** Traditional analytical methods (eg HIC or MS) are not applicable for DAR analysis.

**Our Solution:** The DAR value of NC18 was determined via the absolute quantitation of the payload moiety hydrolyzed from the ADC molecules.

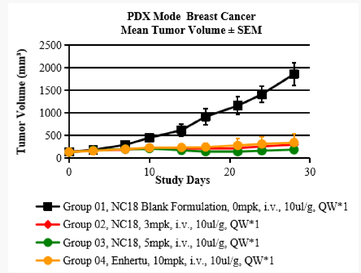


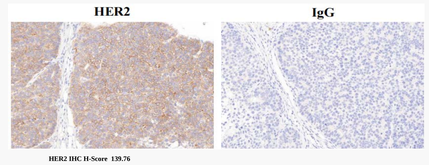




$$\mathbf{DAR} = \frac{(C_{ADC-H_2O} - C_{ADC-ACN})/1000 \times V_{\mathbf{load}}}{M_{drug}} \div \frac{C_{mAb} \times V_{\mathbf{load}}}{M_{mab}}$$

## Toxicology Study Data

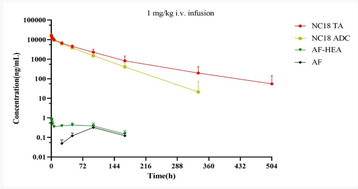
**Pharmacodynamics**




- NC18 3mg/kg TGI(%) was 84.25%
- NC18 5mg/kg TGI(%) was 90.01%
- Enhertu 10 mg/kg TGI(%) was 83.18%
- All doses did not result in body weight loss.

**Pharmacokinetics**




- No apparent sex-related differences were noted in the pharmacokinetic parameters of NC18 total antibody, ADC, AF-HEA or AF.
- The exposure to the test article in cynomolgus monkeys showed nonlinear pharmacokinetics within the doses administered in this study.
- The ratios of exposure levels of NC18 total antibody, ADC, AF-HEA and AF were higher than those of doses (calculated on $AUC_{0\text{-}672h}$) at the dose range of 0.2 mg/kg~1 mg/kg.

**Toxicology**

- No skin toxicity and neurotoxicity commonly seen with tubulin inhibitors were observed.
- No abnormalities were found in ECG parameters.
- No lung lesion occurring.
- The ophthalmic toxicity was only observed in rats manifested by the increased mitotic figures in the corneal epithelium.
- Slight hepatotoxicity and nephrotoxicity were observed in cynomolgus monkeys.

## Conclusions

- Optimization of the conjugation process allowed for the generation of high quality NC18 ADC materials with a good batch-to-batch consistency;
- A chromatography process was developed for the removal of unconjugated payload-linker and the ADC aggregates from the conjugation process;
- A dedicated analytical method was developed for the measurement of the DAR values of the polymer linker-based ADC molecules;
- The polymeric conjugation platform afforded high DAR ADCs with the improved potency & PK profile.