

# Site-specific dual conjugation enabled by an integrated *in vivo* / *in vitro* antibody production platform

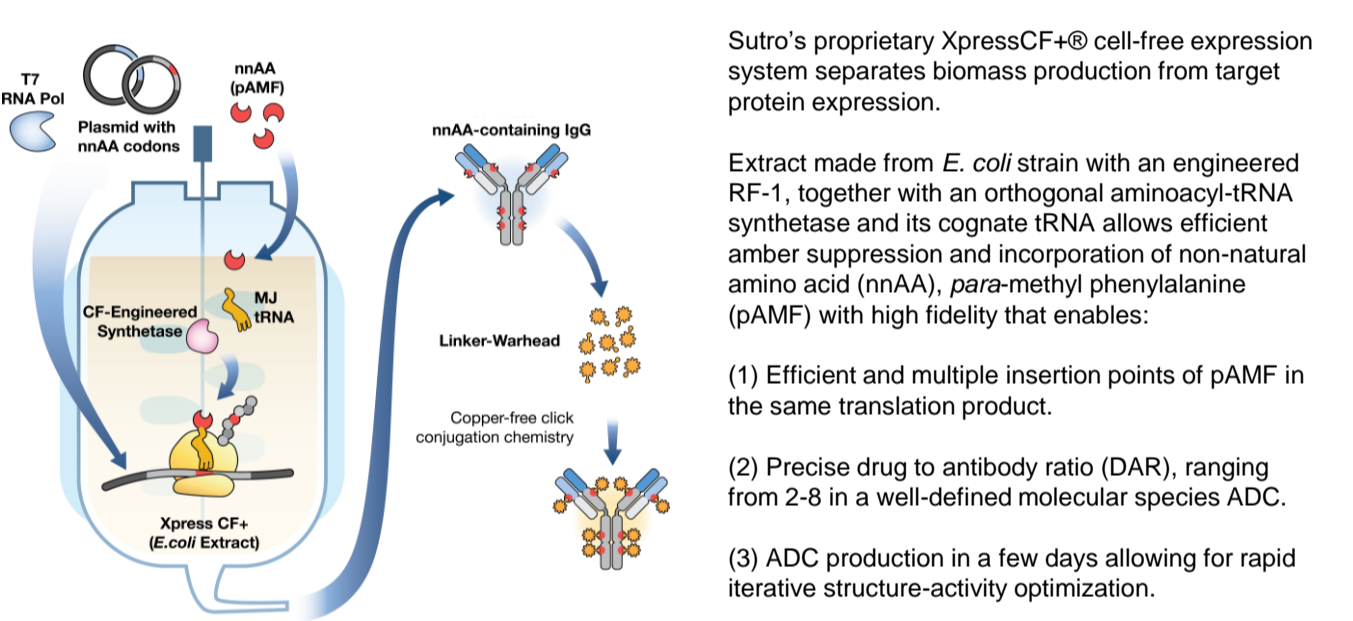
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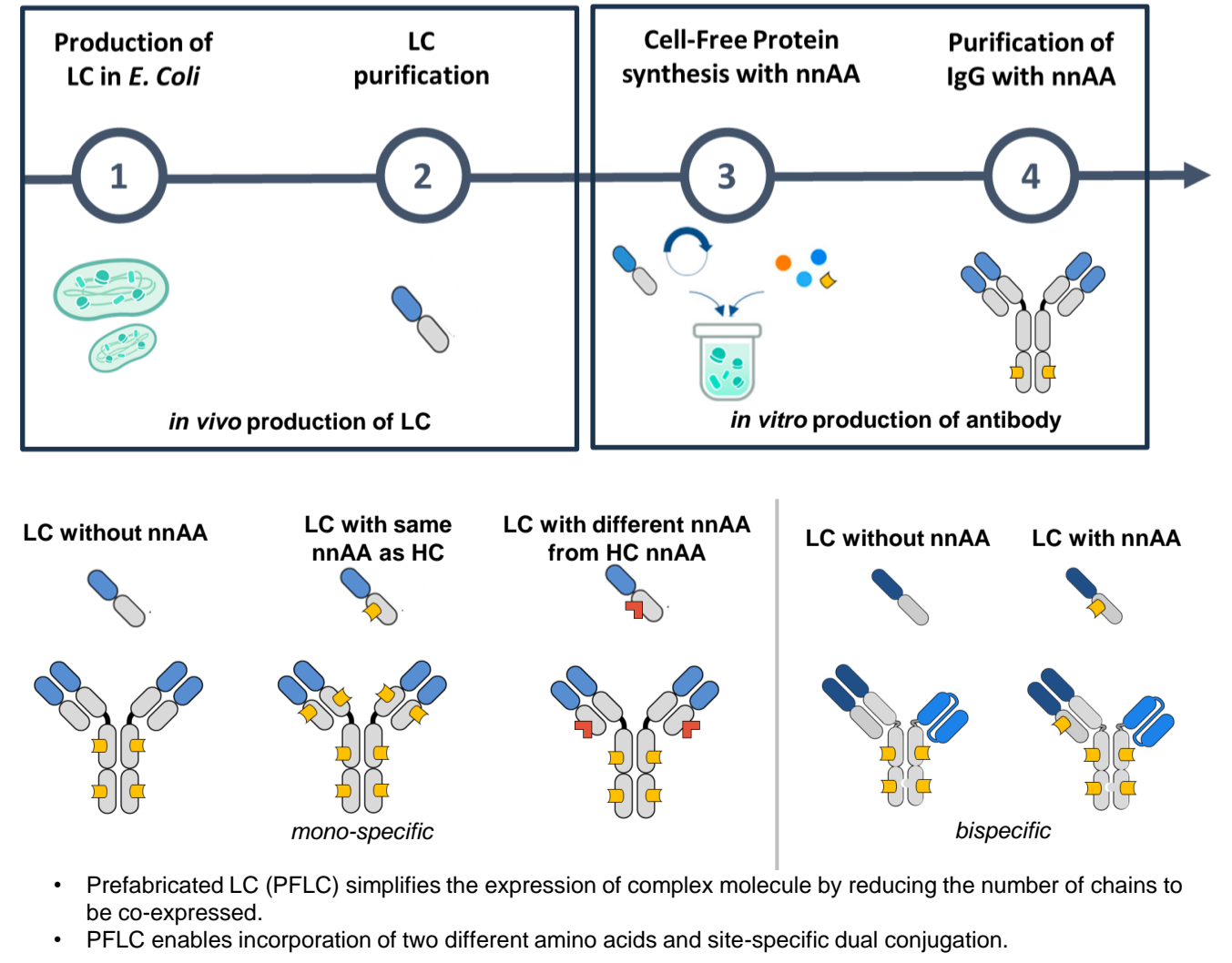
## Abstract

ADC has demonstrated its potential as a safe and effective therapy to combat cancer. With the advancement of technology, many novel modalities are in development to further improve safety and efficacy of ADCs. We describe here Sutro's unique integrated *in vivo*/*in vitro* platform that enables modular antibody production processes and novel product concepts. The integrated *in vivo*/*in vitro* antibody production platform decouples light chain and antibody production, and therefore allows incorporation of two non-natural amino acids with orthogonal conjugation handles. Immunostimulatory ADC (iADC) that harbors immunostimulant and toxin on one antibody was produced using the integrated *in vivo*/*in vitro* antibody production platform. Single dose efficacy study demonstrated superior anti-tumor response. PD analysis demonstrated both innate and adaptive immune compartments were activated.

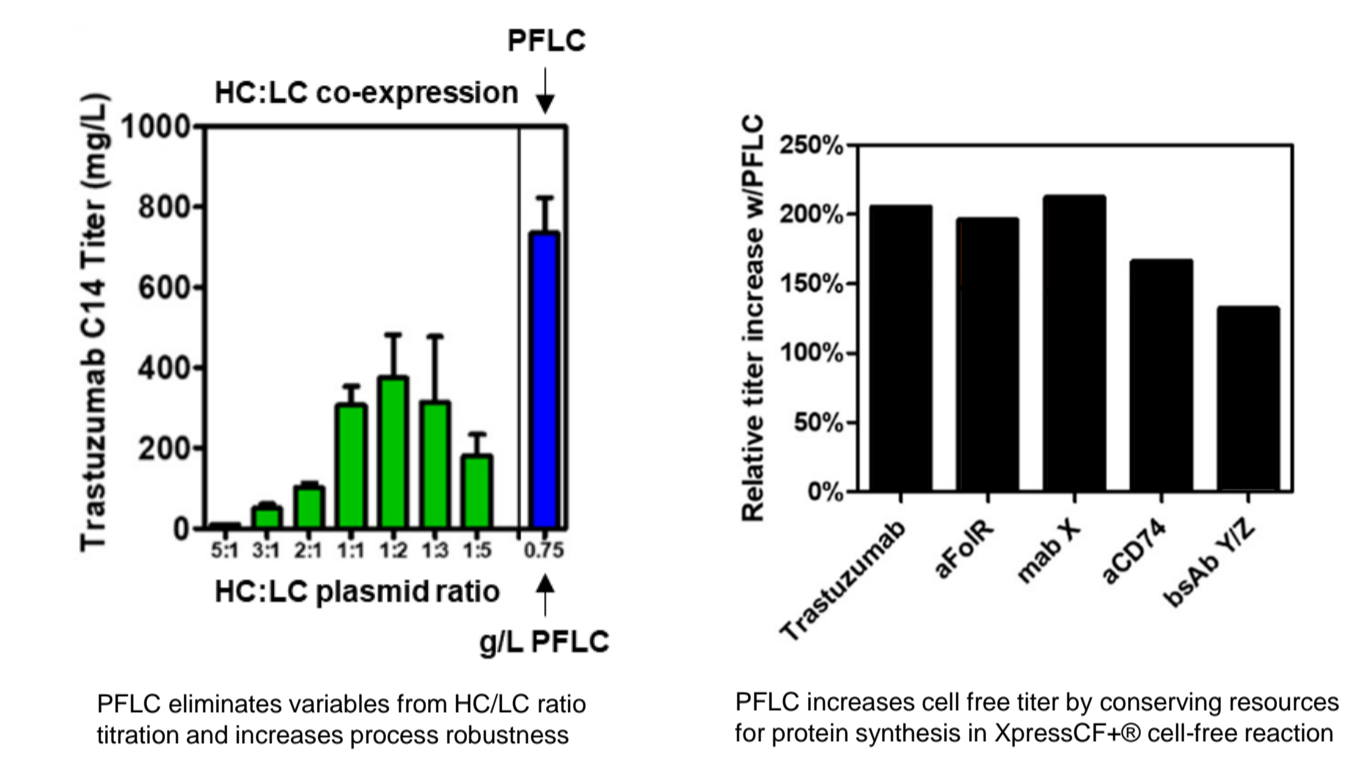
## Figure 1: XpressCF+® cell free expression systems allow efficiency incorporation of non-natural amino acid and enable site-specific conjugation



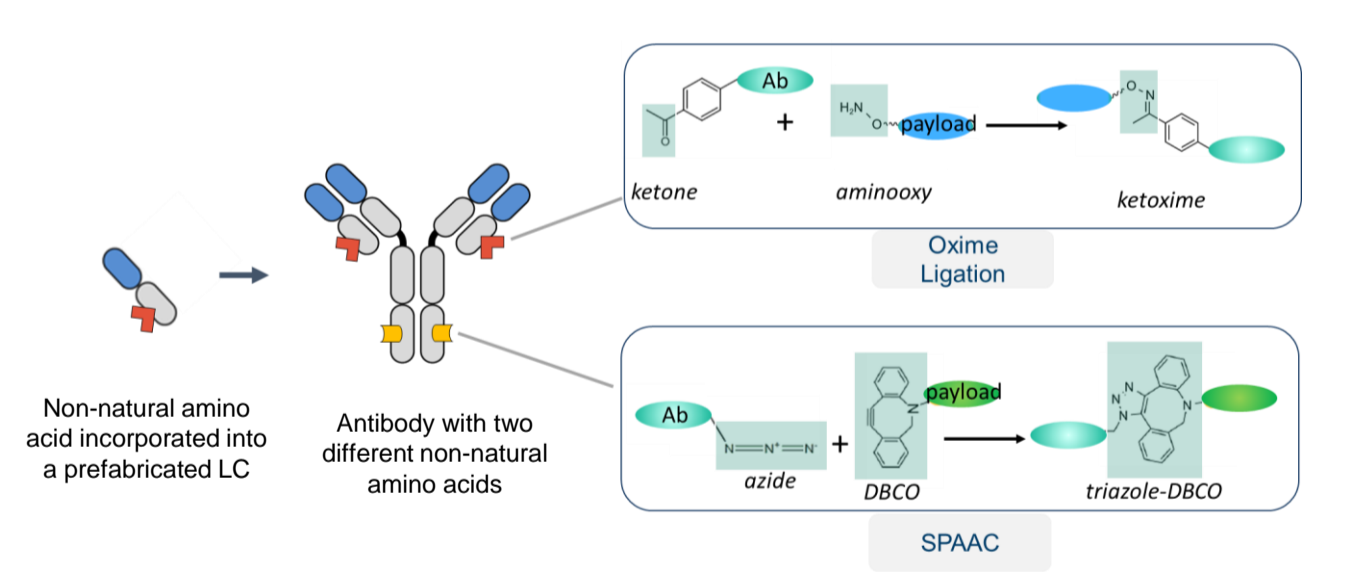
## Figure 2: XpressCF+® Cell free expression systems allow de-coupling of *in vivo* LC production from *in vitro* antibody production



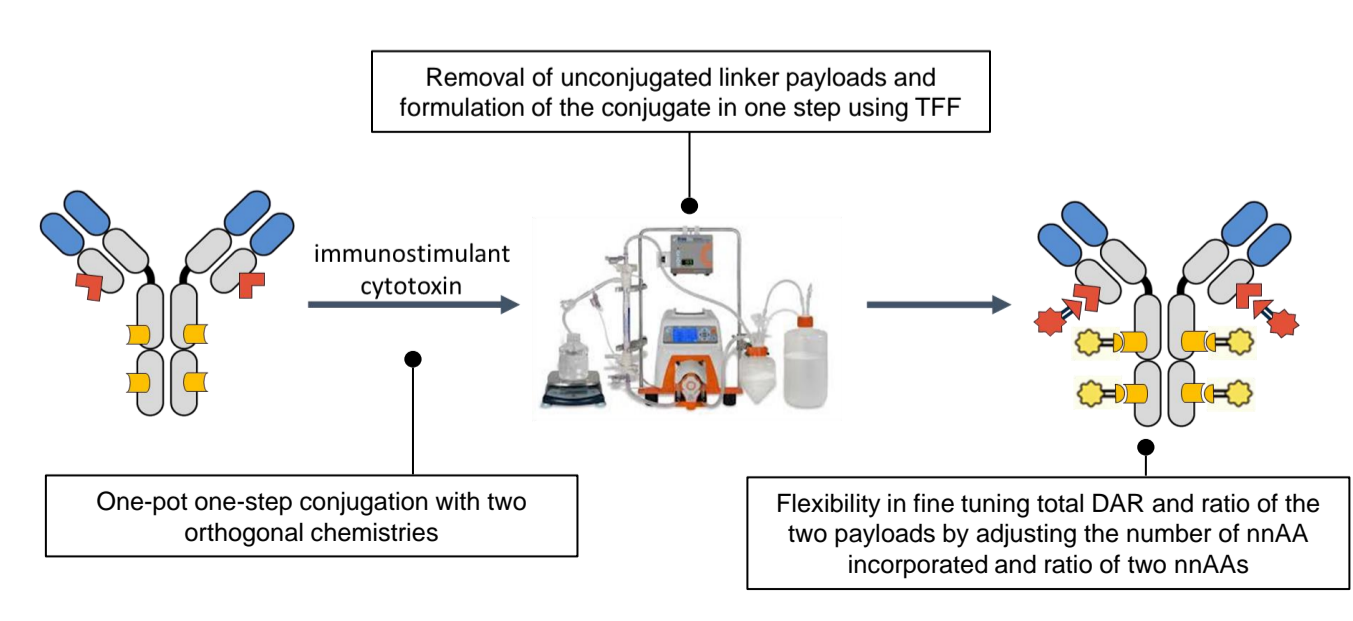
## Figure 3: PFLC improves the robustness and titer of XpressCF+® expression system



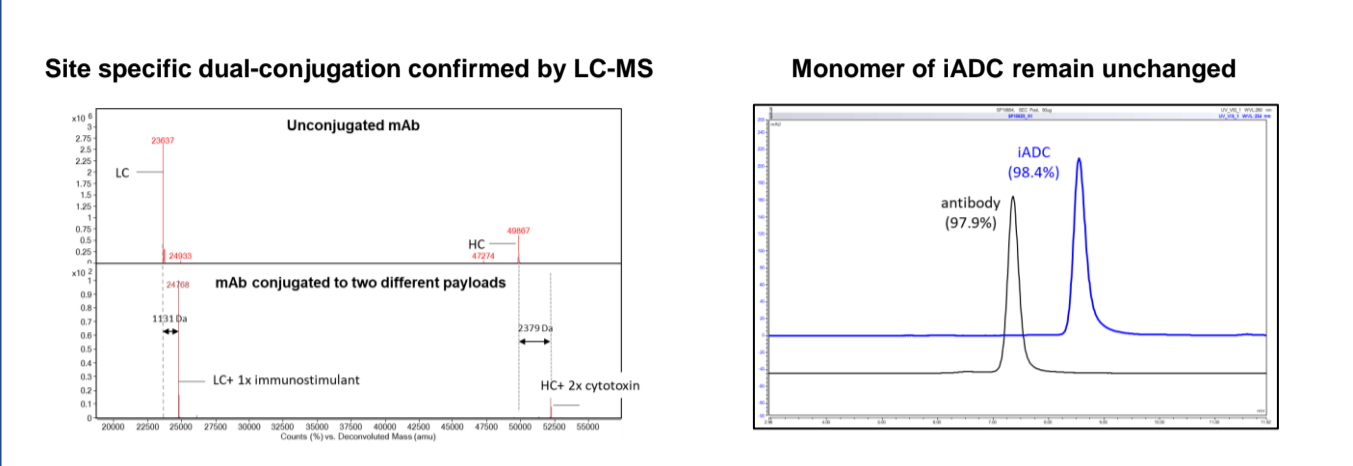
## Figure 4: Site-specific dual conjugation enabled by para-acetyl phenylalanine containing PFLC and XpressCF+® expression system



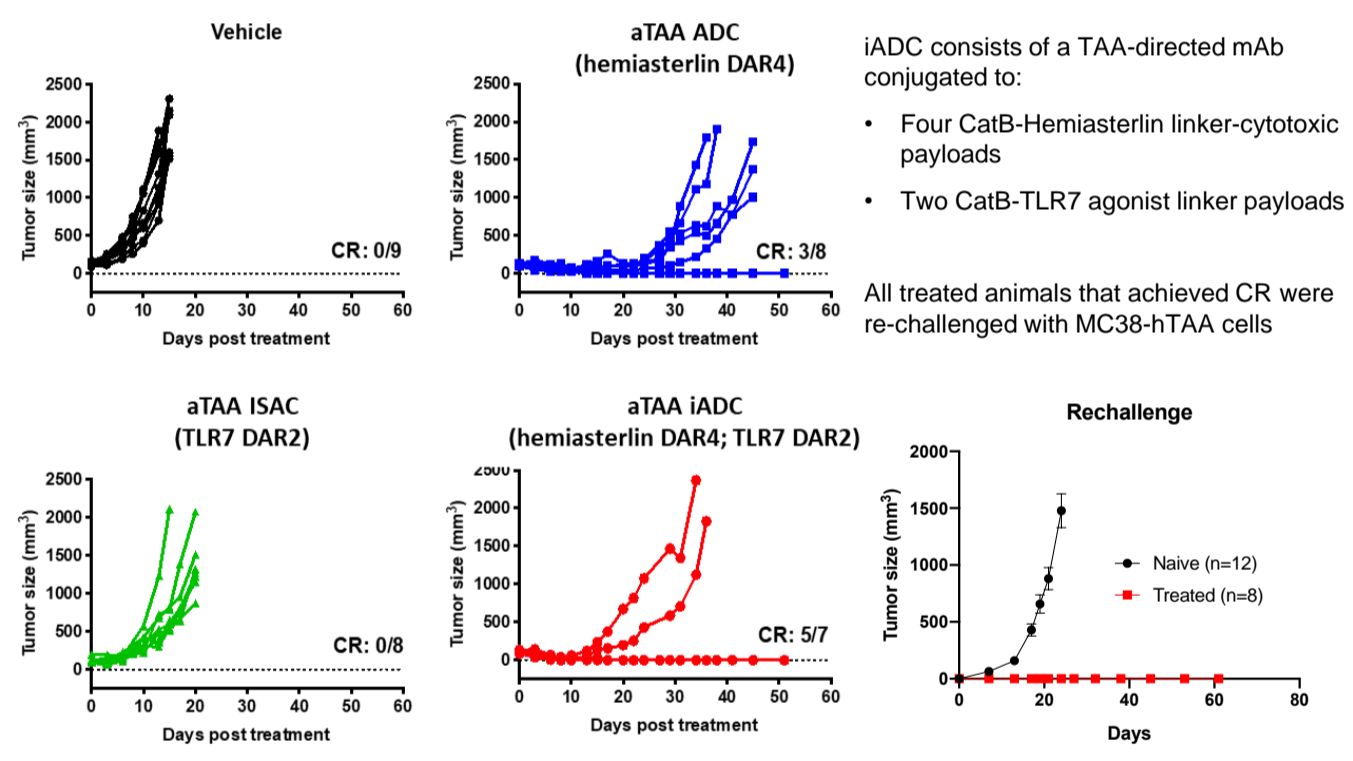
## Figure 5: Immunostimulatory ADC (iADC) produced in one-pot one-step conjugation reaction without post-conjugation purification step required



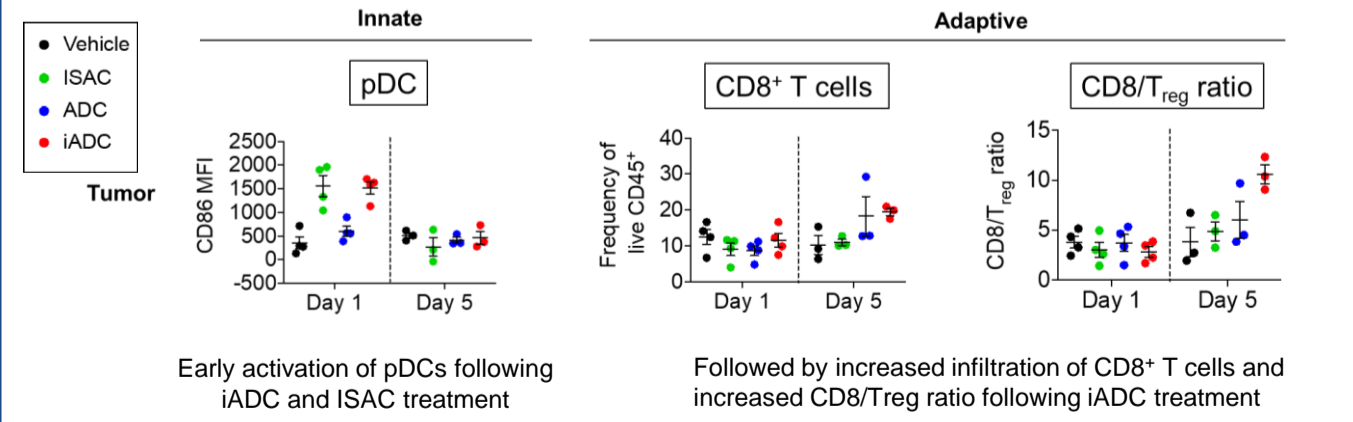
## Figure 6: Immunostimulatory ADC (iADC) produced in one-pot one-step conjugation reaction without post-conjugation purification step



## Figure 7: Superior anti-tumor response with single dose of iADC



## Figure 8: iADC engaged both innate and adaptive immune compartments in hTAA-MC38 tumor bearing mice



## Conclusion

- XpressCF+® Cell free expression systems allow de-coupling of LC expression from HC expression and antibody production.
- Prefabrication of LC (1) improves the robustness of cell free reaction, (2) increases antibody titer, and (3) enables site-specific dual conjugation.
- iADCs are produced by one-pot one-step conjugation of immunostimulant and cytotoxin linker payloads without additional purification steps.
- Single dose iADC demonstrated superior anti-tumor response by engaging both innate and adaptive immune compartments.