# 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



Sutro's proprietary XpressCF+® cell-free expression system separates biomass production from target protein expression.

Extract made from *E. coli* strain with an engineered RF-1, together with an orthogonal aminoacyl-tRNA synthetase and its cognate tRNA allows efficient amber suppression and incorporation of non-natural amino acid (nnAA), para-methyl phenylalanine (pAMF) with high fidelity that enables:

(1) Efficient and multiple insertion points of pAMF in the same translation product.

(2) Precise drug to antibody ratio (DAR), ranging from 2-8 in a well-defined molecular species ADC.

(3) ADC production in a few days allowing for rapid iterative structure-activity optimization.

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



titration and increases process robustness

250°

200%

150%

100%

50%

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



 Prefabricated LC (PFLC) simplifies the expression of complex molecule by reducing the number of chains to be co-expressed

PFLC enables incorporation of two different amino acids and site-specific dual conjugation.

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



PFLC increases cell free titer by conserving resources for protein synthesis in XpressCF+® cell-free reaction

## Site specific dual-conjugation confirmed by LC-MS Unconjugated mAb mAb conjugated to two different payloads 0.9-0.8-0.7-0.6-0.5-11310 HC+ 2x cytotoxi

#### Monomer of iADC remain unchanged



#### 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



increased CD8/Treg ratio following iADC treatment

#### Conclusion

iADC and ISAC treatment

- 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 addition purification steps.
- Single dose iADC demonstrated superior anti-tumor response by engaging both innate and adaptive immune compartments.