Evaluation of Conjugation Process Conditions

Compatibility of ADC formulation with conditioning regiments

To evaluate whether the formulation buffer components are incompatible with the lysine conjugation chemistry, both bulk pH adjustment and buffer exchange by TFF were carried out. Conjugated ADCs were analyzed for CAR using a UV-based Fe(III) chelation assay.

The reaction time, Df excess, ADC concentration and temperature are important parameters to optimize during process development.

An optimized process results in a consistent Df-to-Antibody ratio. Inset shows the effect of different Df-to-ADC ratios used in the reaction on the resulting conjugated Df.

In Vitro and Vivo Results

Binding Affinity: ADC2

Potency of ADC1:

Antigen binding and cytotoxicity are not impacted following DFO conjugation

ADC2 and DI-ADC2 showed concentration-dependent binding with EC50 of 0.44 nM and 0.51 nM, respectively

Cell killing potency remained the same (Cell Titer Glo assay)

Radiolabeling and Immunoreactivity:

ADC: PET/CT Imaging of Pancreatic Tumors:

Sequential PET images from a single mouse scanned at the indicated time. Top panels show the mouse that was injected with 89Zr-DI-ADC and the bottom panels shows the mouse that was injected with radiolabeled parent mAb, 89Zr-DI-mAb. Images are scaled the same.

Imaging/biodistribution studies revealed 2-fold higher uptake of ADC in tumors compared to the unconjugated mAb

ADC1: PET/CT Imaging of High and Low Antigen Expressing NHL Tumor Models:

Sequential PET images from a single mouse scanned at the indicated time. Representative DI-ADC1 exhibited excellent targeting in both low and high antigen expressing tumor models in vivo showing that low antigen expressing targets can be visualized with PET

ImmunoPET Tracers

Schematics: Desferrioxamine (DF) isothiocyanate is conjugated on lysine residues of an ADC resulting in a randomly distributed chelator cage with an average CAR of 1.8. Zr-89 positron emitting radionuclide with a T1/2 of 3.1 days was then added resulting in a radio-tracer with radiochemical purity of >97% and specific activity of 5-7 µCi/µL. The Immunoreactivity was assessed using SEC-based assay to be greater than 75%.

Df Conjugation Did Not Perturb The DAR

RP-HPLC shows that DAR has not changed.

Additional species and peak tailing are due to the Df conjugation (arrows)

Df Conjugation Did Not Increase Aggregation

Reduced and non-reduced SDS-PAGE demonstrate a similar pattern between the “parent” ADC and DI-ADC.

A slight band retardation is consistent with DI-conjugation

Conclusions

- Process conditions were optimized to allow efficient conjugation of multiple ADCs. The desired chelator was consistently achieved and ADC aggregation was minimized
- The DAR, potency and binding affinity were not perturbed as shown with a panel of assays including RP-HPLC, SE-HPLC, binding by FACS and cell cytotoxicity prior to radiolabeling and by retention of immunoreactivity post-radiolabeling.
- Representative DI-ADC showed excellent targeting of the HPAF-II tumors but the tumor accumulation was 2-fold higher (215% vs. 125% DI(0) than in the “parent” unconjugated mAb. Representative DI-ADC1 showed that both PET imaging using radiometal labeled ADCs is a promising approach to determine tumor targeting and tissue biodistribution in real time and for use as a patient selection tool

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