A comprehensive discovery and engineering workflow for developing innovative anti-Nectin-4 antibody drug conjugates

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Abstract

The novel ETx-22, a nectin-4-targeting antibody-drug conjugate (ADC), addresses limitations of existing ADCs, like enfortumab vedotin (EV). Leveraging the Beacon Optofluidic system, it becomes possible to select antibodies targeting nectin-4-expressing tumor cells with minimal keratinocyte binding. Humanized and conjugated to an advanced linker and exatecan, ETx-22 shows strong in vitro and in vivo antitumor efficacy, including in EV-resistant and low-nectin-4 models, suggesting that ETx-22 could be a valuable therapy to treat patients whose urothelial cancer has progressed on EV therapy.

Figure 1: Antibody generation with Bruker Beacon® single B cell platform

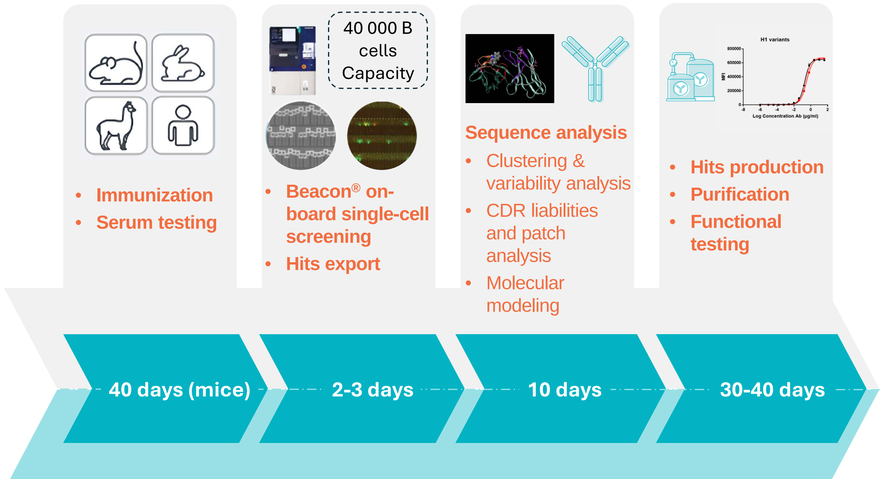
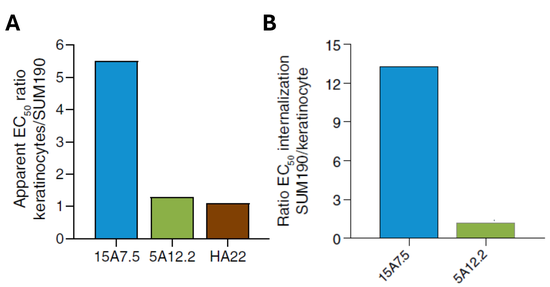
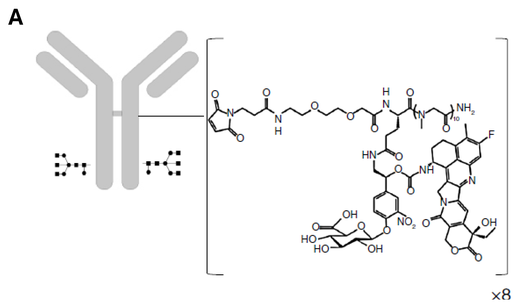


Figure 2: Epitope characterization and activity of the 15A7.5 antibody

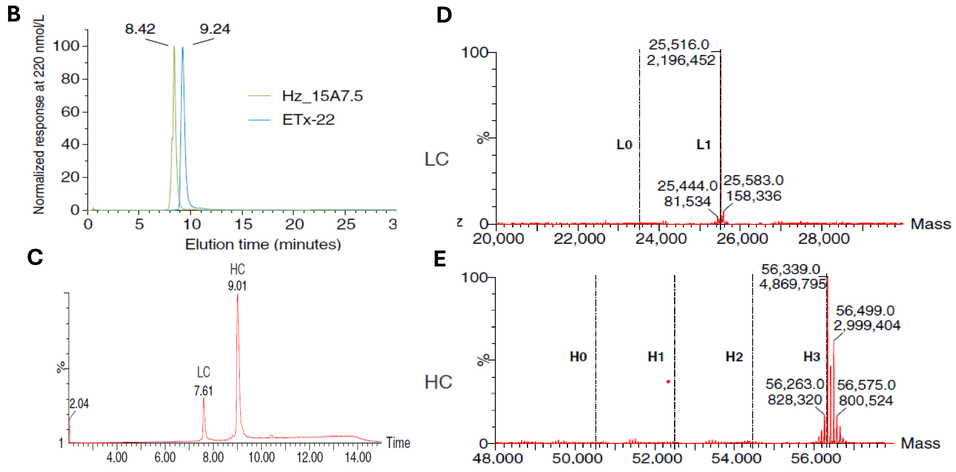


15A7.5 mAb binds preferentially to the SUM190 cancer cell line expressing nectin-4, while 5A12.2 and HA22 (EV mAb) bind equally to human keratinocytes and tumor cells (Fig. 2A). After conjugation with a pH-sensitive fluorochrome (Promega), 15A7.5 showed effective tumor internalization but minimal uptake in keratinocytes (Fig. 2B)

Figure 3: Synthesis and characterization of ETx-22 ADC

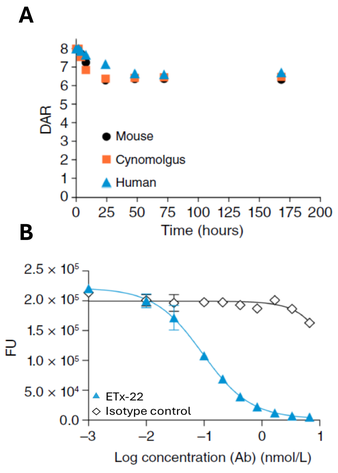


mAb 15A7.5 - maleimide–β-glucuronide–exatecan–PSARLink™ linker (Mablink Bioscience) at DAR 8, Fc fragment was engineered to reduce FcγR binding (Fig. 3A).



- Hydrophobic interaction chromatography showed PSAR enhances ADC hydrophilicity (Fig. 4B)
- LC/MS analysis (Fig. 4C). revealed ETx-22 has one payload per light chain (Fig. 4D) and three per heavy chain (Fig. 4E), yielding a DAR of 8.

Figure 4: in vitro efficacy of ETx-22

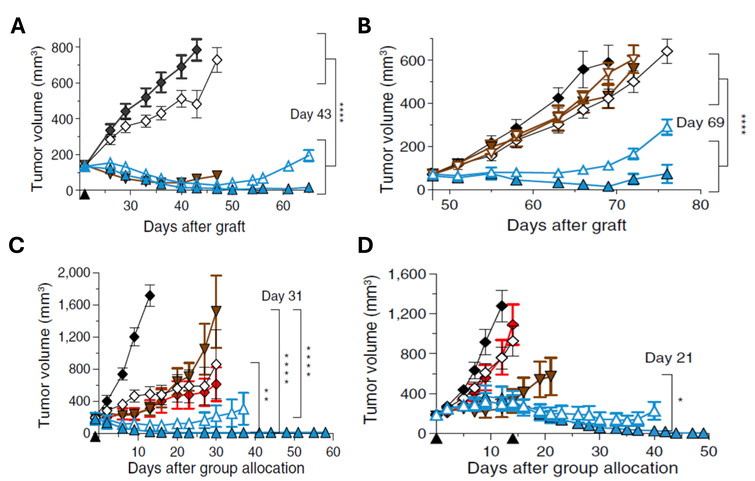


Ex vivo DAR stability was assessed by LC/MS after serum incubation (Fig. 3A).

ETx-22 showed strong in vitro cytotoxicity against nectin-4–transfected HCT116-2G10 cells (IC50 = 0.1 nmol/L) at 8 days (Fig. 3B).

- ETx-22, 4 mg/kg (△) or 8 mg/kg (▲)
- Isotype control, 8 mg/kg (◇)
- Enfortumab vedotin, 4 mg/kg (▽) or 8 mg/kg (▼)
- Chemotherapy control group (◆) / cisplatin plus gemcitabine
- ADC diluent (◆)

Figure 4: in vitro and in vivo efficacy of ETx-22



- In immunodeficient mice bearing SUM190 tumors, ETx-22 antitumor effect outlasted EV (Fig. 4A) and was effective in EV-resistant SUM190R CDX models, unlike EV (Fig. 4B).
- In bladder PDX models (PDX-B521 and PDX-BCLU-003, Urosphere), ETx-22 induced more durable effects than EV and even complete regression at higher doses (Fig. 4C, D).

Conclusion

- Etx-22 is a second-generation ADC against nectin-4 with optimized linker payload stability, higher DAR, and differentiated payload.
- 15A7.5 an anti–nectin-4 mAb was chosen due to its observed selectivity toward nectin-4–expressing tumor cells in comparison with skin keratinocytes potentially leading to less in vivo–associated clinical signs of skin toxicity.
- Our results suggest that ETx-22 antitumor activity and tolerability were increased compared to an ADC approved to treat urothelial carcinoma.

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