Patient-derived Antibody APN-497444 Targets a Tumor-selective Glycan and Displays Potent Activity as an ADC in Vivo

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INTRODUCTION

ADC linker-payload technologies have made significant advances in recent years but are limited by the availability of antibodies binding novel, tumor-specific targets with favorable internalization kinetics. We describe here Atreca's ADC Discovery Engine, which enables consistent and large-scale identification of such antibodies against novel and tumor-specific targets by analyzing the immune repertoires of cancer patients. One antibody generated by our platform, APN-497444, recognizes a novel, tumor-specific glycan present on the surface of tumor cells from solid tumors in multiple indications. Weaponized as an ADC, APN-497444 displays robust anti-tumor activity following a single dose in a CRC xenograft model, even though the antibody has not yet been optimized.

Atreca's Discovery Engine Provides a Rich Starting Point for ADC Candidates

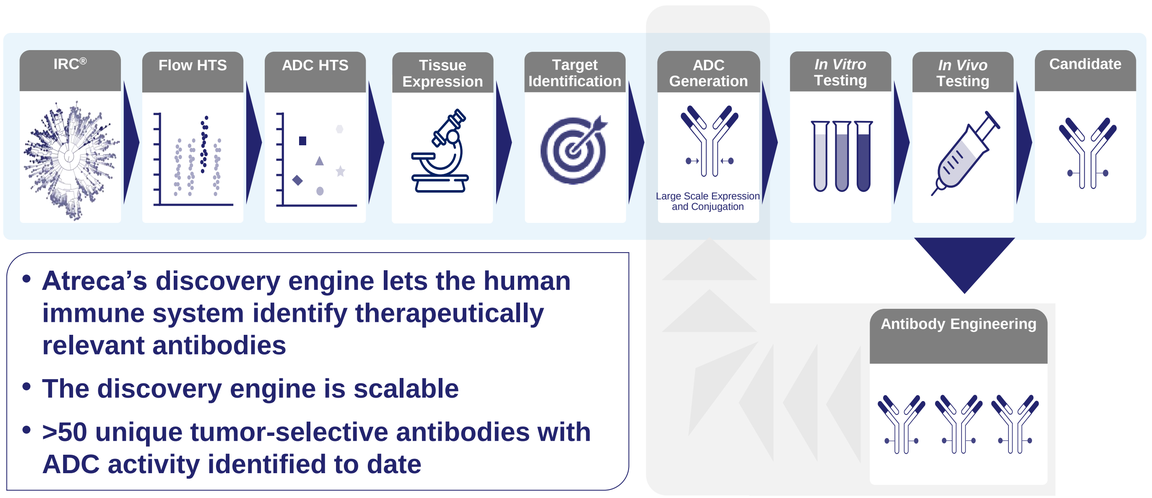
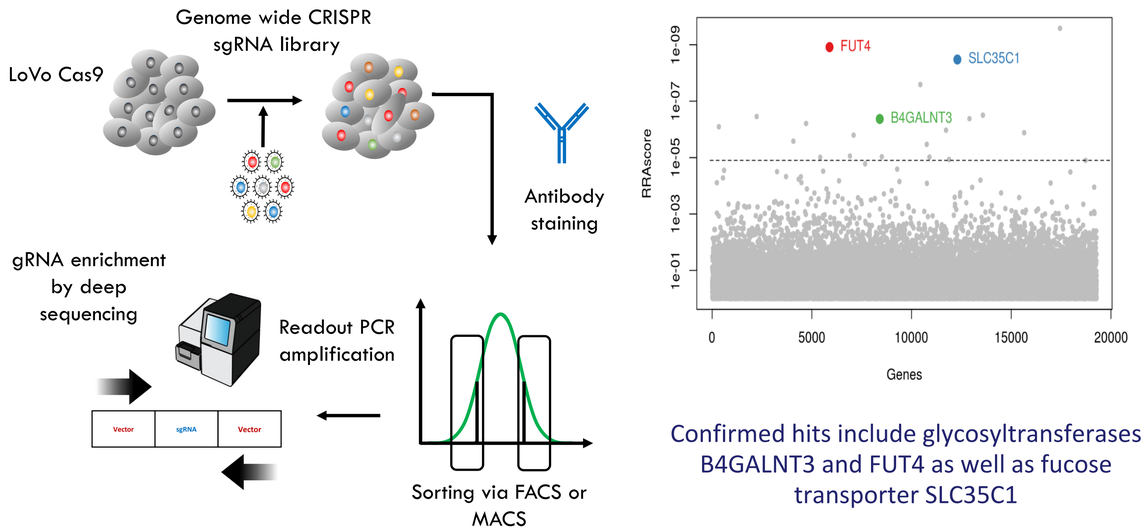


Figure 1: Atreca's ADC Discovery Engine. Sequences of antibodies expressed by single plasmablast B cells in patients undergoing an active anti-tumor response to immunotherapy are used to identify novel antibodies with therapeutic potential. Antibodies selected by *in silico* analysis are synthesized and screened for binding to panels of cancer cell lines, and positive hits are assessed for activity as ADCs as well as their binding to tumor *vs.* normal tissues. Targets of promising antibodies are identified using a variety of assays. ADCs are then optimized to improve potency and developability and further evaluated *in vitro* and *in vivo.*

Functional Genomics Screen Identifies B4GALNT3 and FUT4 Glycosyltransferases as Genes Required for APN-497444 Binding to Tumor Cells



Confirmed hits include glycosyltransferases B4GALNT3 and FUT4 as well as fucose transporter SLC35C1

Figure 2: Target identification. A functional genomics screen was performed in an APN-497444 flow-positive CRC cell line expressing the Cas9 nuclease. Cells were transfected with a genome-wide CRISPR sgRNA library and assessed for disruption of APN-497444 binding by flow cytometry. Two glycosyltransferases, B4GALNT3 and FUT4, and a fucose transporter, SLC35C1 were among the top hits from the screen.

Target Validation or APN-497444 Confirms a Role For B4GALNT3 and FUT4

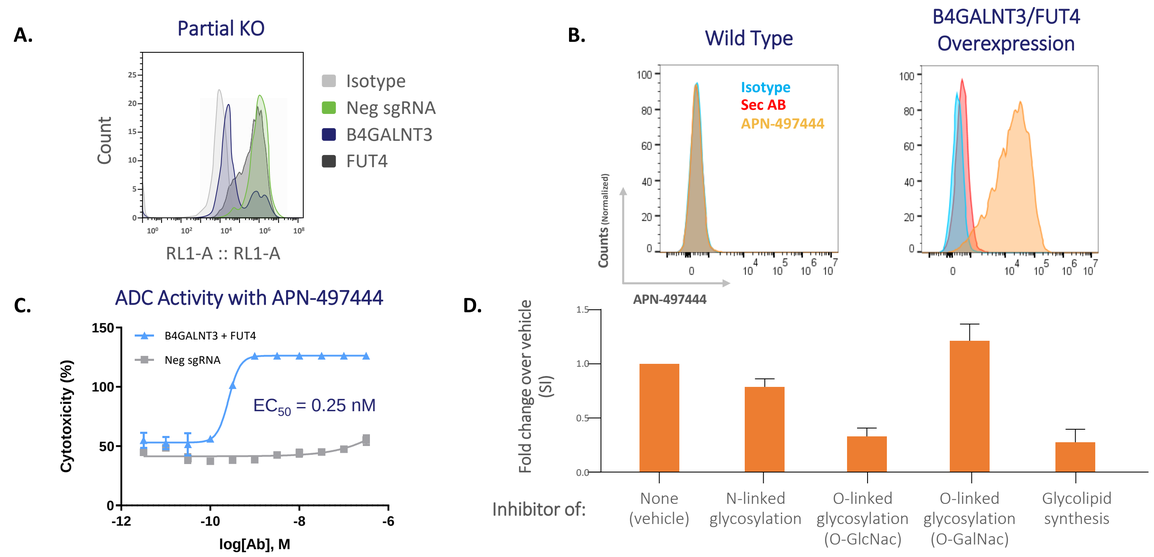
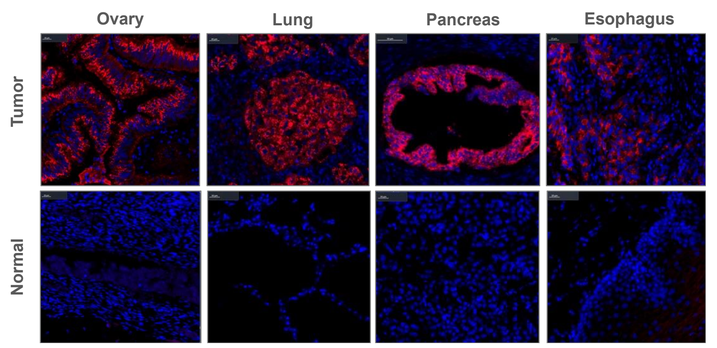


Figure 3: APN497444 target confirmation. A. The requirement for B4GALNT3 and FUT4 in generating the epitope recognized by APN497444 was confirmed through individual gene knockdown in LoVo cells (partial). **B., C.** Overexpression of both B4GALNT3 and FUT4 in a target negative cell line (A459) leads to APN-479444 reactivity and sensitizes these cells to ADC killing. **D.** Inhibitors of O-linked glycosylation and glycolipid synthesis reduce APN-497444 binding.

APN-49744 Exhibits Strong Immunoreactivity Across a Range of Cancers



Indication	Percent reactive	Total cores scored
Ovarian cancer	71%	7
Lung cancer	60%	10
Colorectal cancer	60%	20
Pancreatic cancer	50%	18
Esophageal cancer	50%	10
Prostate cancer	22%	18
Thyroid cancer	20%	20

No significant immunoreactivity across a panel of 27 normal tissues

Figure 4: Tissue binding assessed by immunofluorescence. Tissue reactivity was assessed through immunohistochemistry with fluorescence detection across a range of tumor and normal tissues. We observe strong immunoreactivity in ovarian, NSCLC, CRC, and pancreatic cancer with negligible binding to normal tissues.

A Single Dose of APN-497444 Demonstrates Dose-responsive Anti-tumor Activity Using Zymeworks ZymeLink™ Linker-payload Technology

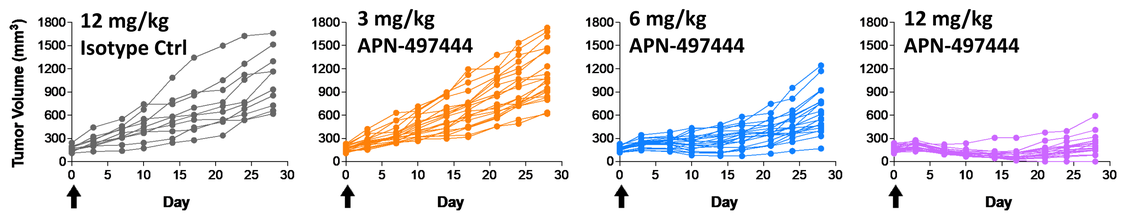


Figure 5: In vivo efficacy. Mice bearing LoVo xenograft tumors were treated with a single dose of APN-497444 ADC, which is weaponized with the Zymeworks ZymeLink™ Auristatin-based linker payload technology on Day 0 at 3, 6, and 12 mg/kg. All doses were well-tolerated with no significant body weight.

CONCLUSIONS AND FUTURE DIRECTIONS

- Atreca's ADC Discovery Engine efficiently identifies antibodies with ADC potential binding novel targets, such as APN-497444, which binds a tumor-specific glycan
- APN-497444 requires expression of both B4GALNT3 and FUT4 for binding to cells
- Dose-dependent *in vivo* activity was observed using the Zymelink™ linker-payload
- Lead optimization of APN-497444 is underway