ATRC-301: a novel EphA2-targeting ADC binding a unique epitope

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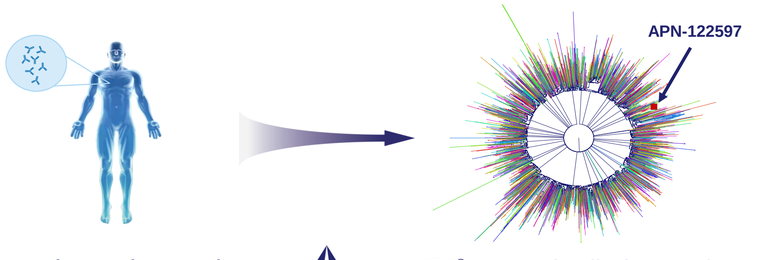
Introduction

EphA2 is a receptor tyrosine kinase that is highly expressed in tumors but only expressed at low levels in normal adult tissue, thus rendering it an attractive tumor target. Here we describe the identification and development of ATRC-301, an ADC that uses an optimized version of an anti-EphA2 antibody originally isolated from a cancer patient. ATRC-301 binds EphA2 via a novel epitope at sub-nanomolar potencies and possesses potent ADC activity in vitro and in vivo. In initial rat toxicology studies, single doses up to and including 60 mg/kg were well tolerated with no significant safety signals, including around coagulation, suggesting a wide therapeutic window for ATRC-301.

Program Highlights

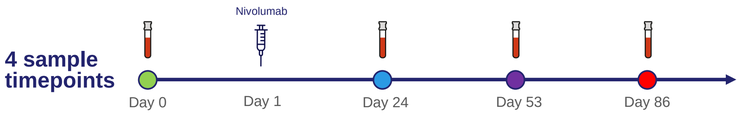
- Atreca anti-EphA2 antibodies target a novel epitope
- Epitope residues conserved across human and toxicology species
- ≥50% epitope prevalence in patient tumor samples from 12 different cancer indications but clean in normal tissue
- Multiple Fv sequences generated that demonstrate a range of potencies
- Dose responsive in vivo efficacy at exposures that are well tolerated in initial safety studies
- ATRC-301 currently advancing in IND enabling studies, IND targeted for 2H23
- Atreca owns worldwide rights

Discovery



Lung adenocarcinoma patient with anti-tumor immune response after treatment with nivolumab

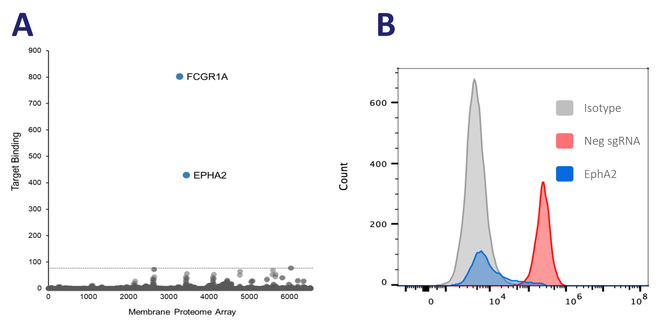
IRC®-generated antibody repertoire Small clonal family identified with APN-122597 as it's most divergent member



Lineage:

- Anti-EphA2 clonal family of eighteen plasmablasts from four timepoints with thirteen unique antibody clones was identified
- Clonal family was present at the first timepoint and expanded following nivolumab treatment
- APN-122597 (original lead) was observed at fourth timepoint

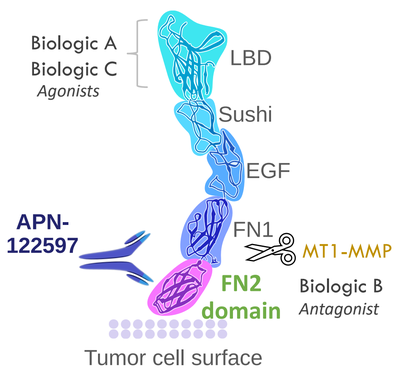
Target and Epitope



APN-122597 binds to EphA2

(**A**) Target identified using Integral Molecular membrane protein array against ~6000 proteins individually expressed on the cell surfaces.

(**B**) Loss of APN-122597 binding to A549 lung cancer cells after EphA2 knock-out *in vitro*.

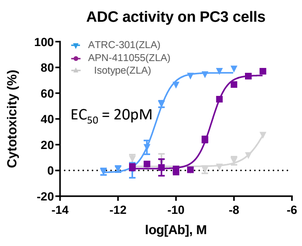


APN-122597 targets a novel, membrane-proximal epitope in EphA2 FN2 domain

The epitope of APN-122597 was mapped in parallel using yeast-display mutagenesis screening as well as co-crystallization and identified as a conformational epitope spanning four stretches of sequence in the FN2 domain.

The epitope is differentiated from those of EphA2-targeting biologics that have entered clinical trials and is C-terminal of the MT1-MMP cleavage site

Lead Optimization and Signaling

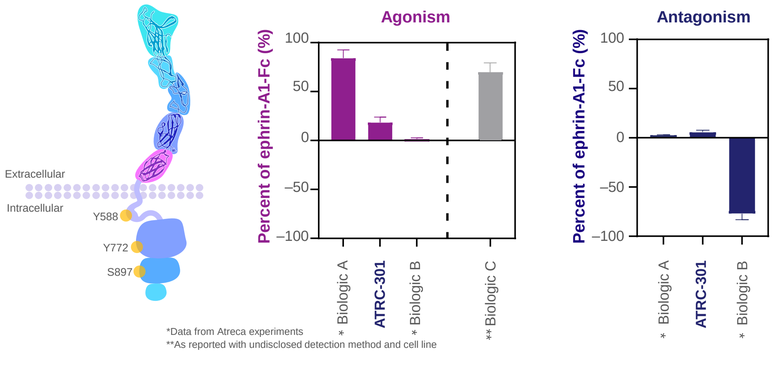


	APN-122597	APN-411055	ATRC-301
Antibody	Lead	Optimized R 3	Optimized R 4
Mutations from lead, n	0	5	9
Predicted high-risk liabilities, n	0	0	0
Predicted medium-risk liabilities, n	1	0	0
Predicted immunogenicity risk	Low	Low	Low
BVP binding	0	0	0
T_m (°C)	64.8	64.7	66.4
Monovalent binding affinity	2000 nM	1000 nM	20 nM
A549 cell binding EC_{50}	400 nM	30 nM	0.5 nM
A549 ADCC EC_{50}	30 nM	3 nM	0.006 nM

ATRC-301 generated through optimization of parental of APN-122597

Sequence- and yeast display-based lead optimization methods were used to optimize the lead molecule APN-122597.

Significant improvements in potency and developability were achieved, yielding the optimized clinical candidate, ATRC-301.

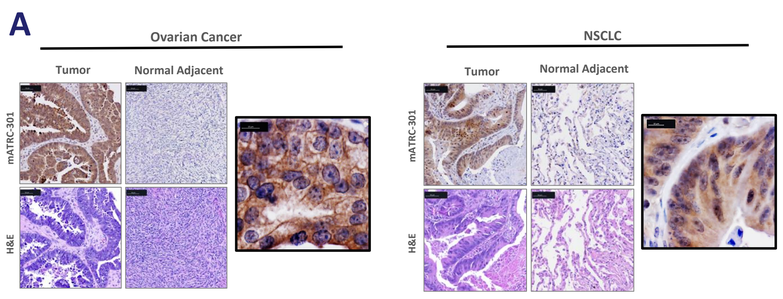


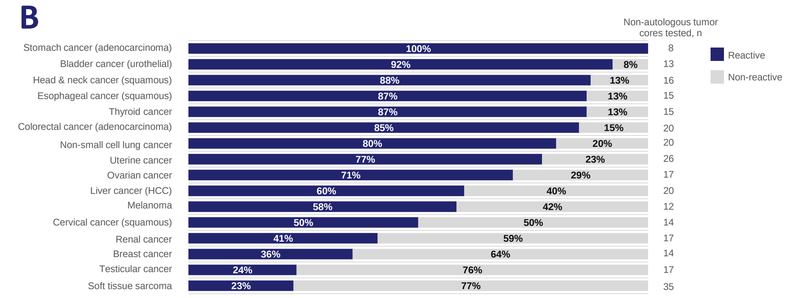
ATRC-301 minimally impacts the EphA2-EphrinA1 signaling axis

Any effect of ATRC-301 on EphA2 signaling was assessed using phospho-specific antibodies directed against three key sites in the intracellular domain.

ATRC-301 is differentiated from EphA2-targeting biologics that have entered the clinic by inducing only minimal receptor agonism and not interfering with ligand mediated receptor activation.

Epitope Prevalence



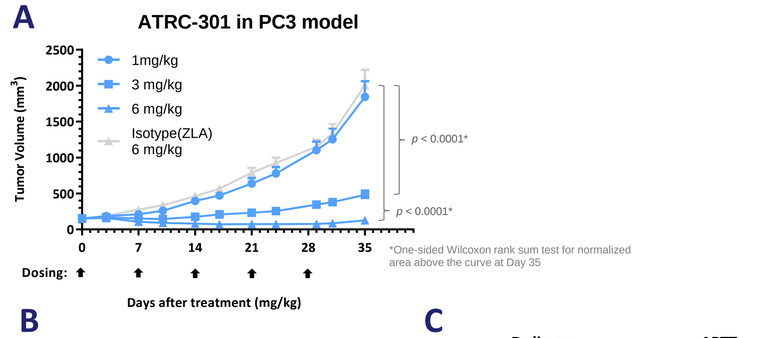


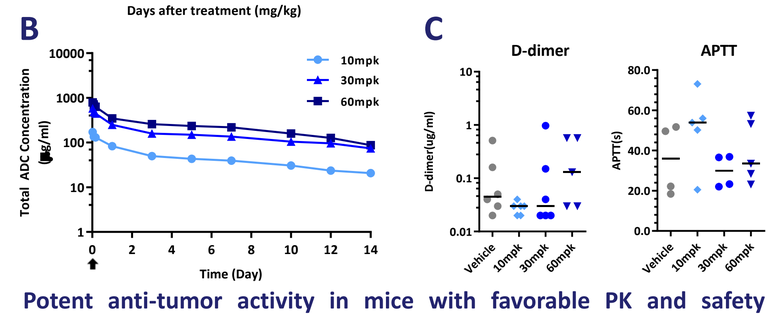
ATRC-301 selectively recognizes tumor cells in multiple indications.

(**A**) ATRC-301 staining shows strong and selective binding to malignant cells in ovarian cancer and NSCLC human FFPE samples while not binding to normal adjacent tissue.

(**B**) Prevalence data indicate reactivity to multiple relevant cancer indications and generally aligns with reported EphA2 expression. No appreciable signal, including membrane decoration, was detected by IHC in 26 normal human tissues (not shown).

In Vivo Assessments





Potent anti-tumor activity in mice with favorable PK and safety profiles in rats

(**A**) ATRC-301 leads to significant tumor growth inhibition at 3 and 6 mg/kg after weekly dosing in the PC3 xenograft model.

(**B**) ATRC-301 shows favorable PK profile with a half-life of 7.5-10 days. PK is dose-proportional at tested doses. No signs of TMDD seen despite cross-reactivity to rat.

(**C**) Single doses up to 60mpk were generally well-tolerated with no bleeding or abnormal coagulation observed.

Upcoming Milestones

- IND-enabling activities initiated for candidate and backup
- Initial NHP toxicology data expected 2H 2022
- IND filing targeted for 2H 2023
- FIH study targeted 1H 2024 in solid tumor disease including gastrointestinal and gynecological cancers