Discovery and pre-clinical development of a novel and differentiated EphA2-targeted antibody in multiple bispecific formats

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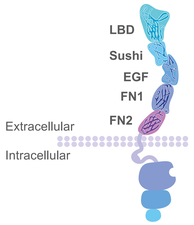
Abstract No: 1194

Background

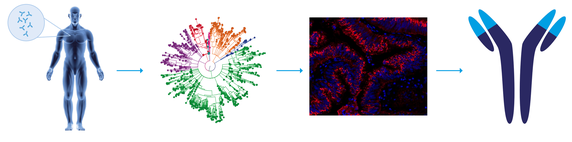
- Eph proteins are the largest family of RTKs known in humans[1]
- Eph receptors regulate cell-to-cell communication, plasticity, and patterning[1,2]
- Overexpression of EphA2 has been identified in multiple cancers including prostate, lung, esophageal, colorectal, cervical, ovarian, breast, and skin cancers[3]
- To date, clinical efforts to target EphA2 have demonstrated limited efficacy or significant toxicity[3]

FIGURE 1 – EphA2 receptor domain structure



Methods

FIGURE 2 – Discovery of EphA2-targeted antibody



Plasmablast B cells collected from a patient with an anti-tumor immune response

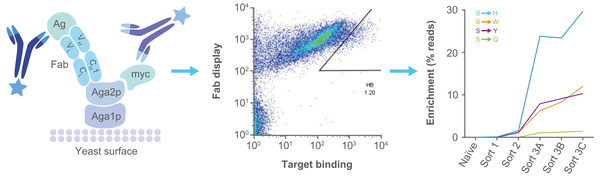
Antibody chains expressed by single plasmablasts were sequenced

Identified antibodies selectively binding to non-autologous human tumor tissues

HIT antibodies

- Optimized leads were engineered into several weaponized formats and tested for safety and anti-tumor activity

- Lead optimization used sequence- and structure-based rational mutations, combined with high-throughput yeast display selections



Yeast display identifies improved variants

Select best binders by FACS

Identify best variants by NGS

Results

- An anti-EphA2 antibody was identified from plasmablasts of a patient with NSCLC after treatment with nivolumab
  - The antibody binds selectively to the surface of tumor cell lines and to human tumors compared with normal tissue
  - Target was determined by membrane protein array
  - Epitope was determined by yeast display and X-ray crystallography; the antibody binds a novel conformational epitope on the most membrane-proximal fibronectin type-II domain; the epitope is conserved across relevant model species and distinct from biologics previously or currently in clinical development
  - The antibody minimally impacts the EphrinA1–EphA2 signaling axis; no antagonism and weak agonism

Antibody optimization

TABLE and FIGURE 3 – Antibody engineering yielded leads with significantly improved potency and reduced developability risks, exhibiting single-digit picomolar activity in cell-based assays

	APN-122597	APN-411055	APN-382558
Antibody	Lead	Optimized R3	Optimized R4
Mutations from lead, n	0	5	9
Predicted high-risk liabilities, n	0	0	0
Predicted medium-risk liabilities, n	0	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

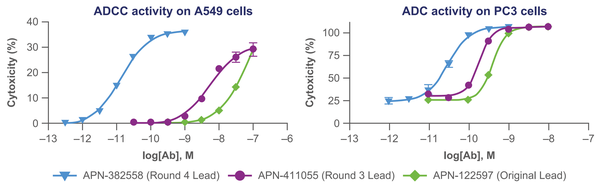
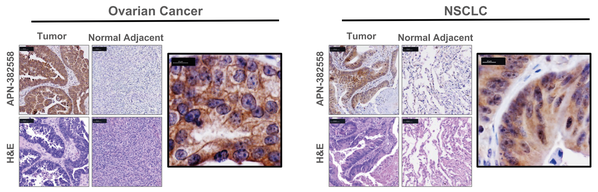


FIGURE 4 – Immunoreactivity observed in >80% of malignant cells in ovarian cancer and NSCLC but not stroma or normal adjacent tissues

- Prevalence consistent with mRNA expression reported in TCGA



Results (continued)

Antibody weaponization

- Lead antibodies were weaponized into multiple formats, which delivered potent anti-tumor activity *in vivo* without safety signals

Tumor-targeted 4-1BB bispecific antibody

- A novel tumor-targeted 4-1BB bispecific antibody significantly enhanced therapeutic index compared with untargeted 4-1BB agonist antibodies
- A weak 4-1BB agonist paired with EphA2 targeting drove cross-linking and activation at the tumor and dose-dependently inhibited tumor growth

FIGURE 5 – Tumor targeting required for activity of EphA2 x 4-1BB bispecific

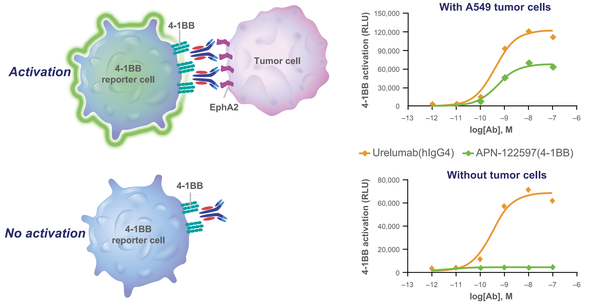


FIGURE 6 – EphA2 x 4-1BB bispecific drives tumor reduction

- CT-26 syngeneic model
- Treatment of established tumors
- Robust effects on tumor growth compared to untargeted 4-1BB
- No body weight loss or other adverse clinical signs noted

*Anti-hen egg lysozyme "targeting" 4-1BB bispecific, control for EphA2 targeting of 4-1BB.
**One-sided Wilcoxon rank sum test for normalized area above the curve at end of study.

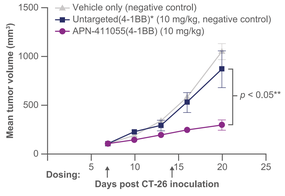
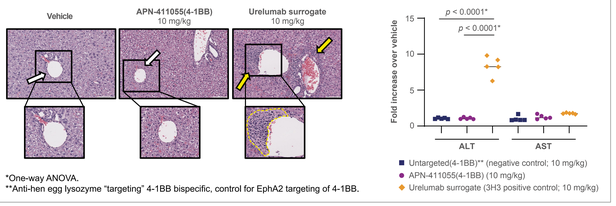


FIGURE 7 – EphA2 x 4-1BB bispecific does not show signs of liver toxicity

- No signs of liver inflammation observed with APN-411055(4-1BB) by histological analysis
- No increase in ALT observed with APN-411055(4-1BB)



*One-way ANOVA.
**Anti-hen egg lysozyme "targeting" 4-1BB bispecific, control for EphA2 targeting of 4-1BB.

Results (continued)

CD3-bispecific T-cell engager

- EphA2-targeted CD3 bispecifics exhibited sub-picomolar potency *in vitro* and robust tumor reduction *in vivo*

FIGURE 8 – Optimized lead APN-411055 as a bispecific T-cell engager demonstrates potent PC3 cell killing

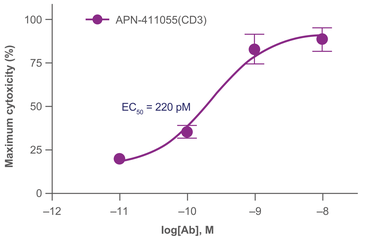
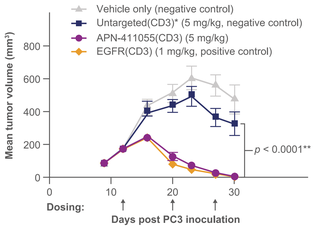


FIGURE 9 – *In vivo* tumor reduction with the optimized lead APN-411055 in a CD3 bispecific format

- Standard humanized mouse model
- Treatment of established tumors
- Weekly IP dosing
- APN-411055(CD3) treatment leads to complete tumor regression
- No significant body weight changes



*Anti-hen egg lysozyme "targeting" 4-1BB bispecific, control for EphA2 targeting of 4-1BB.
**One-sided Wilcoxon rank sum test for normalized area above the curve at Day 30.

ADC, ATRC-301

- ATRC-301, an ADC version, is entering IND-enabling studies
- More information about ATRC-301 can be accessed using this link

CLICK HERE

Conclusions

- Atreca's EphA2 program leverages a patient-derived antibody and a novel epitope to deliver differentiated biologics for this potentially high-value target
- Optimized leads with high potency and developability exhibit anti-tumor activity in multiple formats, including 4-1BB and CD3 bispecifics
- These data demonstrate the power of Atreca's antibody discovery platform to uncover unique antibodies and their epitopes

References

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2. Singh A, et al. *Front Biosci (Landmark Ed)*. 2012;17(2):473–497.
3. Xiao T, et al. *J Hematol Oncol*. 2020;13(1):114.

Acknowledgments

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Abbreviations: ADC, antibody–drug conjugate; ADCC, antibody-dependent cellular cytotoxicity; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BVP, baculovirus particles; C, constant; CD3, cluster of differentiation 3; EC_{50}, half maximal effective concentration; EphA2, erythropoietin-producing hepatocellular receptor A2; EGF, epidermal growth factor-like; Fab, fragment antigen-binding; FACS, fluorescence-activated cell sorting; FN1/2, fibronectin type III domains 1 and 2; H&E, hematoxylin and eosin; hIgG4, human immunoglobulin G4; HIT, heparin induced thrombocytopenia; IP, intraperitoneal; LBD, ligand-binding domain; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; R, round; RLU, relative light unit; RTK, receptor tyrosine kinases; TCGA, The Cancer Genome Atlas; T_m, melting temperature; V, variable.