Background Eph proteins are the largest family of receptor tyrosine kinases in humans and are normally involved in cell-to-cell communication, plasticity, and patterning. EphA2 represents an attractive therapeutic target due to its overexpression in many cancers. Unfortunately, to date, clinical efforts to target EphA2 have demonstrated limited efficacy or significant toxicity.

Methods We sequenced the antibody chains expressed by single plasmablast B cells from patients with cancer and identified antibodies selectively binding to non-autologous human tumor tissues. The target and epitope of an antibody of interest were identified by FACS, yeast display, and crystallography methods. Lead optimization used sequence- and structure-based rational mutations, combined with selections from high-throughput yeast display screening. Optimized leads were engineered into several weaponized formats and tested for safety and anti-tumor activity.

Results An anti-EphA2 antibody was identified from plasmablasts of a patient with lung cancer after treatment with nivolumab. This antibody binds to the surface of tumor cell lines and selectively to human tumors compared with normal tissue. The antibody binds a novel conformational epitope on the most membrane-proximal fibronectin type-II domain, and this epitope is conserved across relevant model species. The epitope targeted is non-overlapping with biologics previously or currently in clinical development exhibiting neither agonist nor antagonist activity.

Antibody engineering yielded leads with significantly improved potency and developability, exhibiting single-digit picomolar activity in cell-based assays. Optimized leads demonstrated tumor-selective binding across multiple tumor types and labeling of plasma membranes and cytoplasmic puncta.

Antibody weaponization in multiple formats delivered potent anti-tumor activity in vivo without safety signals. A novel 4-1BB bispecific significantly enhanced therapeutic index compared with untargeted 4-1BB agonist antibodies. A weak 4-1BB agonist paired with tumor targeting drove cross-linking and activation at the tumor and dose-dependently inhibited tumor growth while avoiding the alanine transaminase increases observed with a urelumab surrogate. In addition, a CD3-bispecific T-cell engager was generated that exhibited sub-picomolar potency in vitro and robust tumor reduction in vivo. Separately, an antibody–drug conjugate version, ATRC-301, is entering IND-enabling studies and is described elsewhere.

Conclusions Our 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 a 4-1BB bispecific. These data demonstrate the power of our antibody discovery platform to uncover unique antibodies and their epitopes.

Acknowledgements Jiang Liu, Niv Chowdhury, Tapan Shah, Mark Whidden, Bamini Balaji, Shayla Wyman, Kelsey Hart, Alan Liu, Ming Han Ho, Jason Tong, Jason Heisler, Gary Bolton, Lance Kate, Blair Cain