Activity of the erythropoietin-producing hepatocellular A2 receptor (EphA2) targeting Bicycle® Toxin Conjugate (BTC™) BCY6033 in EGFR inhibitor resistant non-small cell lung cancer (NSCLC) patient derived xenografts

Kenneth Ngo1, Elena V. Ivanova2, Tyler J. Teceno3, Carly Campbell2, Johanna Lahdenranta2, Stephen J. Blakemore2, Gavin Bennett3, Pasi A Jänne1, Cloud P. Paweletz2, Prafulla C. Gokhale3

1Dana-Farber Cancer Institute, Belfer Center for Applied Cancer Science, Boston, MA; 2Bicycle Therapeutics, Plc., Cambridge, United Kingdom

Background

- EphA2 is a receptor tyrosine kinase that contributes to multiple biological processes e.g. cell migration, adhesion, proliferation and differentiation. It is a transmembrane protein expressed on the cell surface which is highly expressed in many cancer types including NSCLC, TNBC, pancreatic, urothelial, etc.
- BCY6033 is a bicyclic peptide targeting EphA2, linked to a cytotoxin Monomethyl auristatin E via a molecular spacer and cleavable linker designed to target EphA2 expressing tumors.
- Recent literature indicates that tumor EphA2 expression may be upregulated in EGFR mutant tumors following 1st, 2nd, and possibly 3rd generation EGFR inhibitors [1].
- We have generated >150 NSCLC patient derived xenograft (PDX) models of various mutation subtypes of clinical relevance.

Methods

- TMA was constructed using FFPE samples from 69 PDX models (NSCLC n=61, SCLC-transformed n=5, de novo SCLC n=3). Of the 69 models, 35 were EGFR mutant.
- EphA2 expression on the TMA samples was performed using the tissue microarray (TMA) constructed using FFPE samples from 69 PDX models.

Results

- Establishment and utilization of NSCLC patient-derived xenograft models

Heterogeneous EphA2 expression observed in PDX models

BCY6033 demonstrates potent anti-tumor activity and retains sensitivity to out-growing tumors in the DFCI-161 and DFCI-220 PDX models

Conclusions

- BCY6033 demonstrates potent anti-tumor activity in EphA2 expressing non-small cell lung cancer PDX models.
- Tumor out-growth >50 days after treatment stop continue to express EphA2 and retain sensitivity to BCY6033 treatment.
- BT5528 (EphA2 targeting Bicycle® with valine-citrulline cleavable linker and a cytotoxin MMAE payload) is currently being evaluated in a Phase I/II clinical trial as a monotherapy in EGFR inhibitor resistant NSCLC.

Acknowledgement

We extend sincere gratitude and appreciation to the Robert and Renee Belfer Family Foundation and Expect Miracles Foundation for their generosity in making this research possible.

Figure 1: BCY6033, a Bicycle toxin conjugate is a fully synthetic ~4.4 kDa molecule consisting of a bicyclic peptide targeting the tumor antigen EphA2 (Kd: 3.4 ± 0.3 nM), linked to the cytotoxin MMAE via a molecular spacer and cleavable (ValCit) linker.

Figure 2: NSCLC patient derived xenograft (PDX) models at DFCI.

Figure 3: Patient derived xenograft (PDX) development platform for NSCLC model generation at DFCI.

Figure 3: EphA2 IHC TMA of 69 PDX models. Each model contained at least two specimens per repeat for evaluation. DFCI-161 and 120 were selected based on relative EphA2 expression.

Figure 4: EphA2 IHC TMA of 69 PDX models. Each model contained at least two specimens per repeat for evaluation. DFCI-161 and 120 were selected based on relative EphA2 expression.

Figure 5: Female NSG mice implanted subcutaneously with either DFCI-161 or DFCI-220 PDX tumors. Animals were randomized into treatment groups with n=8/group. Mice were treated with either vehicle control or BCY6033, 3 mg/kg once weekly for 4 weeks. B, Body weight data shows that BCY6033 was well-tolerated. C, Individual tumor volume plot. D, Out-growing tumors from BCY6033 treated group were randomized again to receive either vehicle or BCY6033 at the same dosing schedule. Tumor samples were collected and stained for EphA2 via IHC to examine protein expression levels.

Figure 6: Representative IHC images of EphA2 expression from vehicle and BCY6033 re-challenged tumors and H-score analysis for membrane and cytoplasmic EphA2 staining of study tumors.

Clinical annotation of DFCI-161 and DFCI-220

Models | Procedure Type | Mutational Profile | Previous Treatment | Histologic Subtype
--- | --- | --- | --- | ---
DFCI-161 | Pleural effusion | EGFR L858R, MET amplification | Erlotinib | Adenocarcinoma
DFCI-220 | Core biopsy | EGFR exon 19 del | Erlotinib | Adenocarcinoma

EphA2 expression maintained in BCY6033 treated and re-challenged tumors enabling for continued BCY6033 response

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