Dana-Farber Cancer Institute

Robert and Renée Belfer Center for Applied Cancer Science

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

HARVARD MEDICAL SCHOOL

bicycle therapeutics

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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 space and cleavable linker designed to target EphA2 expressing tumors.

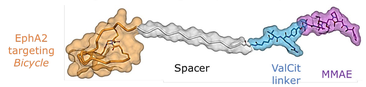

Figure 1: BCY6033, a Bicycle toxin conjugate is a fully synthetic ~4.4 kDa molecule consisting of a bicyclic peptide targeting the tumor antigen EphA2 (K_D: 3.4 ± 0.3 nM), linked to the cytotoxin MMAE via a molecular spacer and cleavable (ValCit) linker.

- Recent literature indicates that tumor EphA2 expression may be up regulated in EGFR mutant lung models 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

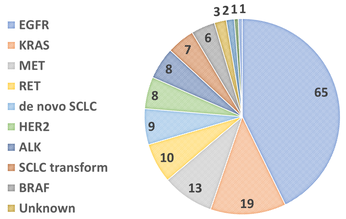

Figure 2: NSCLC patient derived xenograft (PDX) models at DFCI

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 α-EphA2 (R&D Systems) primary antibody. Tumor membranous and cytoplasmic H-score was assigned by a pathologist and a score of ≥50 was considered positive.
- EphA2 expressing EGFR mutant PDX models, DFCI-161 and DFCI-220 were implanted as tumor fragments in female NSG mice.
- Established tumors were treated with BCY6033 administered intravenously once a week.

Results

Establishment and utilization of NSCLC patient-derived xenograft models

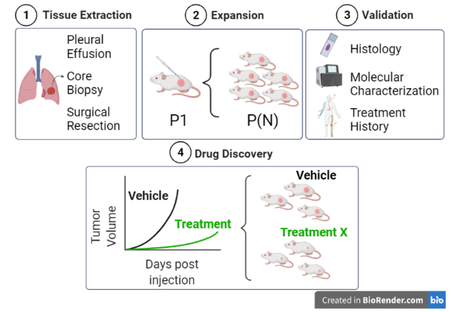

Figure 3: Patient derived xenograft (PDX) development platform for NSCLC model generation at DFCI

Heterogeneous EphA2 expression observed in PDX models

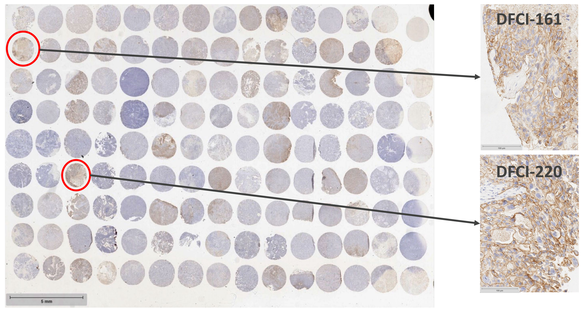

Figure 4: EphA2 IHC TMA of 69 PDX models. Each model contained at least two specimens per model for evaluation. DFCI-161 and 220 were selected based on relative EphA2 expression.

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

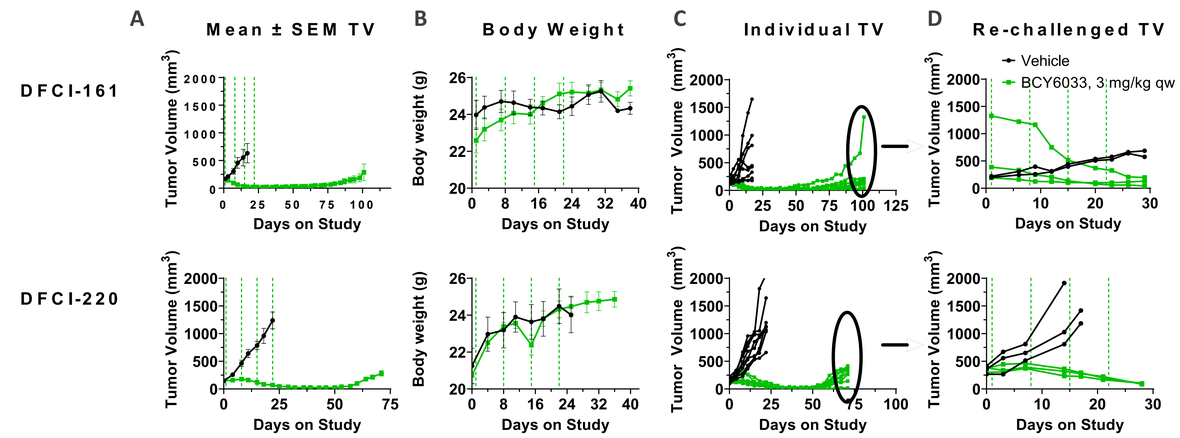

Figure 5: A, Female NSG mice implanted subcutaneously with either DFCI-161 or DFCI-220 PDX tumors. Animals were randomized into treatment groups with n=8/group. Mice were treated with either vehicle control of BCY6033, 3 mg/kg once weekly IV for 4 weeks. **B,** Body weight data shows that BCY6033 was 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.

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

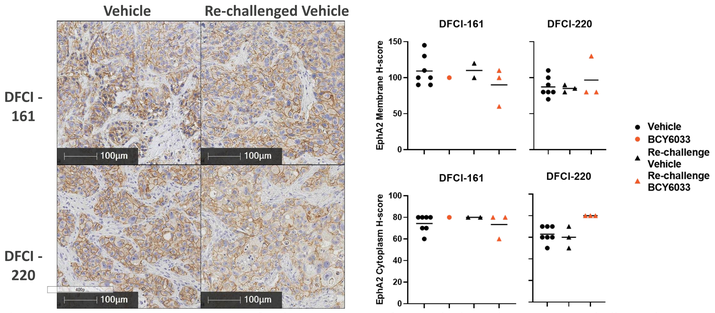

Figure 6: Representative IHC images of EphA2 expression from vehicle and BCY6033 re-challenged tumors and H-score analysis for membrane and cytoplasmic EphA2 -staining of study tumors

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 and in combination with nivolumab

Acknowledgement

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[1] Amato, Katherine R., et al. "EPHA2 blockade overcomes acquired resistance to EGFR kinase inhibitors in lung cancer." Cancer research 76.2 (2016): 305-318.