ABX9xx: A multi-specific Centyrin that synergizes to attenuate intracellular signaling in cMET/EGFR positive tumor cells

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Russ Addis, Robert Kolakowski, Swapnil Kulkarni, Josh Gorsky, Rebecca Meyer, Yao Xin, Steve Nadler, Karyn O'Neil

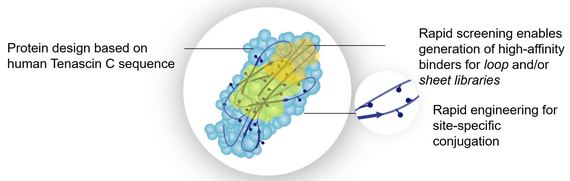
Aro Biotherapeutics Company

ABSTRACT

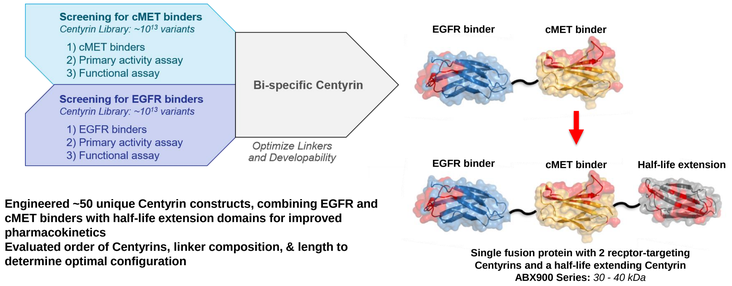
ABX900 series molecules are multi-specific Centyrins targeting the well validated EGFR/cMET pathways. Centyrins are small, single chain proteins based on a human FN3 domain that can be engineered to have high affinity for a selected target and are easily linked to form multi-specific binders (1). The standard of care for patients with EGFR mutant non-small cell lung cancer (NSCLC) is treatment with small molecule tyrosine kinase inhibitors (TKIs) including recently approved third generation molecules (e.g. Tagrisso). Despite initial promising responses with TKIs, most patients progress within 12-14 months due to resistance. A predominant resistance mechanism dependent on *MET* amplification and signaling has been described from both *in vitro* experiments and clinical data. Our data indicate that targeting cMET and EGFR with a multi-specific receptor-binding inhibitor that attenuates intracellular signaling will provide a significant efficacy advantage and reduced side effect profile compared to small molecule TKI combinations. Exploiting the potential for avidity on tumor tissue, a multi-specific ligand-blocking inhibitor of cMET and EGFR is also anticipated to provide improved selectivity for tumor tissues that overexpress both receptors compared to normal tissue with lower receptor expression. ABX900 series activity on cMET, EGFR, and downstream signaling proteins (e.g. ERK and AKT) was confirmed on tumor cell lines from a variety of tumor types (lung, head & neck, glioblastoma) *in vitro* including those carrying clinically observed mutations in cMET and EGFR. ABX900 series inhibited EGFR phosphorylation, independent of EGFR mutational status, with higher potency than Tagrisso. In addition, ABX900 series inhibited ERK phosphorylation with higher potency than capmatinib, a potent cMET inhibitor currently in clinical trials. ABX900 series demonstrated full receptor occupancy of cMET and induced EGFR internalization and degradation. Notably, ABX900 series molecules are effective on *MET*-amplified lines (e.g. HCC827-GR5), an attribute not seen in other EGFR/cMET-targeting biologics currently under development. Our lead molecules contain a serum protein-binding Centyrin for half-life extension in order to improve pharmacokinetics. Together, the data provide a strong rationale for advancing ABX900 series into clinical development for NSCLC and other cancers where EGFR and MET are drivers of tumor progression.

MULTI-SPECIFIC CENTYRIN ENGINEERING

Small bi- or multi-specific proteins with flexible architectures, optimized for delivery of complex drug payloads, including RNA drugs



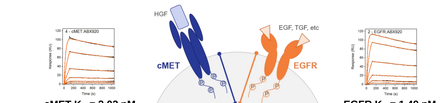
VERSATILE HUMAN PROTEIN SCAFFOLDS
~100 amino acids
SMALL, SIMPLE, SOLUBLE, STABLE



- **Engineered ~50 unique Centyrin constructs, combining EGFR and cMET binders with half-life extension domains for improved pharmacokinetics**
- **Evaluated order of Centyrins, linker composition, & length to determine optimal configuration**

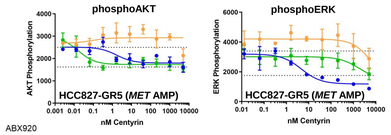
Single fusion protein with 2 recptor-targeting Centyrins and a half-life extending Centyrin
ABX900 Series: *30 - 40 kDa*

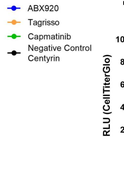
POTENT INHIBITION OF cMET & EGFR

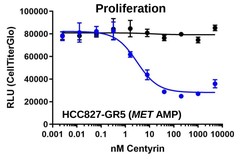


cMET K_D = 2.02 nM

EGFR K_D = 1.49 nM



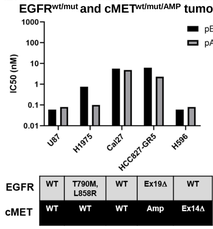




Rationale for cMET:EGFR Therapeutic

- cMET pathway activation is a resistance mechanism to EGFR TKI therapeutics
- EGFR and cMET driven signaling is implicated in multiple solid tumor types (e.g. NSCLC, HNCC, gastric, GBM)

ABX900 series inhibits downstream signaling in EGFR$^{wt/mut}$ and cMET$^{wt/mut/AMP}$ tumor cells



EGFR	WT	T790M, L858R	WT	Ex19Δ	WT
cMET	WT	WT	WT	Amp	Ex14Δ

✓ **Blocks Proliferation**
✓ **Blocks Phosphorylation of MET, EGFR, ERK1/2, AKT**
In a range of solid tumor types:

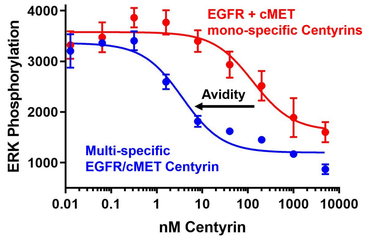
Cell Line	Tumor Type	EGFR status	cMET status
H1975	NSCLC	T970M; L858R	WT
HCC827-GR5	NSCLC	Ex19del	AMP
H596	NSCLC	WT	Ex14del
CAL27	SCCHN	WT	WT
U87-MG	GBM	WT	WT

HCC827-GR5, a Gefitinib-resistant and *MET* amplified lung cancer line (2), was obtained from Dr. Pasi Janne of the Dana Farber Cancer Institute, Harvard Medical School

THE AVIDITY EFFECT OF A SINGLE CHAIN MULTI-SPECIFIC CENTYRIN

Multi-specific Centyrins show greater potency than their component mono-specific units

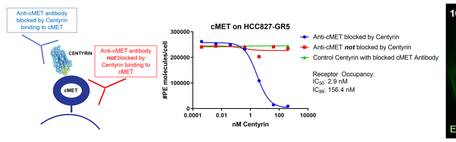
Avidity enhances binding on cells that express both receptors, increasing potency and reducing off-target effects



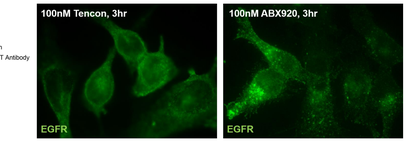
Biologic	IC50 values (nM) in HCC827-GR5			
	pMET	pEGFR	pERK	pAKT
EGFR binder	436.4	47.1	247.5	>1000
cMET binder	4.1	>1000	>1000	167.4
Mixture of EGFR and cMET binders	4.1	17.5	276.7	723.9
Tri-specific Centyrin	**0.5**	**4.8**	**6.3**	**2.3**

RECEPTOR OCCUPANCY & INTERNALIZATION

Receptor Occupancy



Receptor Internalization



- **Receptor Occupancy:** cMET receptor levels were measured on HCC827-GR5 cells (MET amplified) following treatment with serial dilutions of ABX900 series molecules. By comparing results with an anti-cMET antibody that is blocked by Centyrin binding to an antibody that is not blocked by Centyrin, it is apparent that ABX900 series can induce full occupancy of MET receptors.
- **Receptor Internalization:** EGFR receptor was visualized in H1975 cells (EGFR T790M, L858R) using an anti-EGFR antibody. In cells treated with the Tencon negative control Centyrin, EGFR remains localized to the cell surface. Upon treatment with ABX900 series, EGFR is internalized to the cytoplasm.

CONCLUSIONS

- An EGFR/cMET targeted Centyrin potently inhibits receptor phosphorylation, downstream substrate phosphorylation, and proliferation of EGFR and cMET driven tumor cell lines
- A multi-specific Centyrin more avidly inhibits cellular responses than combinations of mono-specific Centyrins, demonstrating functional amplification of dual receptor targeting single chain inhibitors
- A dual receptor targeted Centyrin should be more efficacious *in vivo* than combinations of mono-specific Centyrins (or combinations of EGFR and cMET TKIs) and may prevent the emergence of secondary mutations
- ABX900 series molecules demonstrate potency in *MET*-amplified tumor cells, a result not observed in other EGFR/cMET bispecific molecules under development
- An EGFR/cMET targeted multi-specific Centyrin should be efficacious in patients whose tumors are driven by the EGFR and/or cMET pathways
- A multi-specific EGFR/cMET Centyrin may have broad utility beyond NSCLC and can be clinically developed in a stratified patient population in which tumors are primarily driven by EGFR and cMET

REFERENCES

1. Diem, M. et al. "Selection of high-affinity Centyrin FN3 domains from a simple library diversified at a combination of strand and loop positions." *Protein Engineering, Design and Selection*, 2014, **27**, 419–429.
2. Engelman, J.A. et al. "MET Amplification Leads to Gefitinib Resistance in Lung Cancer by Activating ERBB3 Signaling." *Science*, 2007, **316**, 1039-1043.

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