EO1001: A first-in-class irreversible pan-ErbB inhibitor with excellent brain penetration

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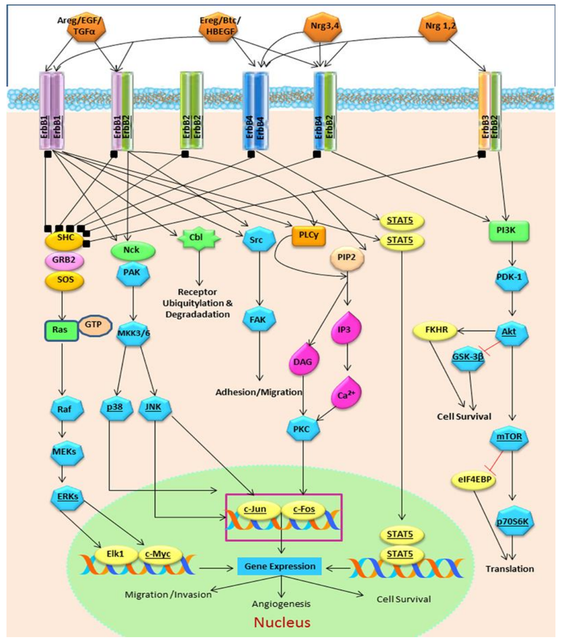
Background: ErbB receptor tyrosine kinases: EGFR (ErbB1), HER2 (ErbB2, neu), HER3 (ErbB3) and HER4 (ErbB4) are part of a complex network activating signaling pathways involved in cell growth and survival. Mutations causing errant ErbB activation is an oncodriver in many cancers including NSCLC. Inhibitors targeting ErbB mutations have transformed outcomes for patients; however, resistance to treatment develops rapidly. The various ErbB receptors have overlapping roles in oncogenesis and crosstalk between ErbB family members is associated with acquired resistance and metastases. For example, amplification of HER2 is a well-established mechanism of acquired resistance to EGFR-TKIs. The development of next-generation agents targeting multiple ErbB receptors has shown promise but have been limited by toxicity and poor brain penetration. Up to 80% of NSCLC patients will experience a brain lesion associated with their disease.; treatment-resistant phenotypes metastasizing to the brain have become an important driver of morbidity and mortality and patients have limited therapeutic options. New agents are needed to address this important and growing unmet medical need.



The ErbB family receptors and their main cell signaling pathways: the Ras/MAPK, the PI3K/AKT and the PLCγ pathways. *Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/The-ErbB-family-receptors-and-their-main-cell-signaling-pathways-the-Ras-MAPK-the_fig2_259530587*

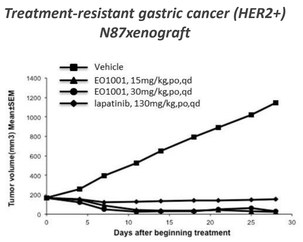
***In vitro* testing:** EO1001 exhibits excellent and balanced equipotent activity against all three important ErbB receptors including EGFR, HER2 and HER4 with low nM activity (0.4 to 7.4 nM), with high specificity vs. off-target receptors.

Target	IC_{50} EO1001	Target	IC_{50} EO1001	Target	IC_{50} EO1001
ErbB1/EGFR	0.40 nM	EGFR (d746-750)	2.62 nM	ABL1	113.80 nM
ErbB2/HER2	4.18 nM	EGFR (L858R)	0.39 nM	BLK	21.43 nM
ErbB4/HER4	2.08 nM	EGFR (T790M)	4.35 nM	JAK3	133.20 nM
		EGFR (L858R, T790M)	7.42 nM	LCK	45.40 nM

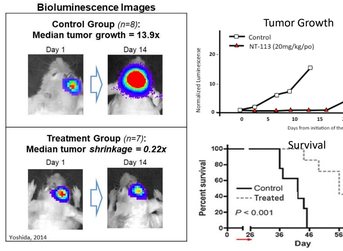
***In vivo* studies:** Following oral administration, EO1001 treatment resulted in a statistically significant improvement in outcomes compared to positive and negative controls in erbB-positive mouse orthotopopic models including N87 (Her2+), H1975 (EGFR/T790M), GBM12 (EGFR+), GBM39 (EGVRvIII+). EO1001 rapidly enters the CNS at high concentrations relative to plasma and inhibits signaling downstream of mutant ErbB receptors in tumor tissue. Treatment with EO1001 was generally well-tolerated with no gastrointestinal side effects observed at efficacious doses in mouse xenograft models.

In vivo studies *(14-days treatment)*

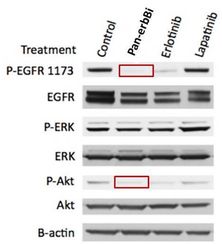
Treatment-resistant gastric cancer (HER2+) N87xenograft



EGFRvIII+ GBM30 xenograft

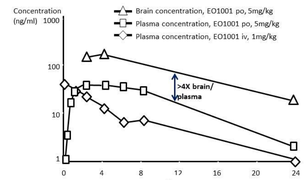


EO1001 inhibits signaling via EGFR, HER2, HER4 and AKT following oral dosing @ <1μM



EO1001 CNS Exposure

CNS penetration of EO1001 was evaluated following daily oral dosing at 5mg/kg in rats. EO1001 rapidly enters the CNS and penetrates brain tissue at a ≥4-fold higher concentration in brain vs. plasma within two hours of dosing and enters tumor tissue at high concentration with long observed tumor exposure.



Brain PK: brain/plasma concentration after oral administration at 5 mg/Kg in rat *Brain conc. (ng/g), n=3* *Plasma conc.(ng/g), n=3* ***Brain: Plasma ratio***

Time (hr)	*Mean*	*SD*	*Mean*	*SD*
2	*156*	*37.8*	*39.0*	*11.2*	***4.0***
4	*173*	*42.0*	*39.3*	*14.1*	***4.4***
24	*17.6*	*7.7*	*2.0*	*1.2*	***8.7***

Days post dosing	Weight (mg)	Amount (ng)	Concentration (ng/mL)
Day 33			
Contra-lateral hemisphere	140	23.5	168.0
Right brain adjacent to the tumor	160	39.5	247.0
Tumor	10	6.73	**673.00**
plasma			**468**
Day 43			
Contra-lateral hemisphere	130	16.6	128.0
Right brain adjacent to the tumor	130	24.3	187.0
Tumor	50	10.8	**216.0**
plasma			**128**
Day 57			
Contra-lateral hemisphere	150	7.48	49.90
Right brain adjacent to the tumor	170	10.3	60.6
Tumor	40	4.26	**107.00**
plasma			**91.5**

PK and toxicity results: Preclinical pharmacokinetic and toxicology studies have been completed. EO-1001 exhibits a half-life of 16-20 hours in rodent models. Toxicities typical of the ErbB inhibitor class, including gastrointestinal effects, weight loss and decreased activity were observed at higher dose groups in both rodent and non-rodent species. Extrapolation to human dosing suggests an attractive therapeutic window in comparison to other agents in the class.

Pharmacokinetic observations

		Cmax (ng/mL)	T1/2 (hr)	CL (L/hr)	Vz (L)	AUC0-t (ng*hr/mL)	AUC0-inf (ng*hr/mL)	F (%)
Mouse PK parameters								
dosing	IV 2mg/Kg	302	20.7	1.40	12.6	1342	1426	100
	PO 8mg/Kg	453	15.8	1.72	11.9	5619	5802	100
Rat PK parameters								
dosing	IV 1mg/Kg	44.4	7.48	6.31	20.5	135	158	100
	PO 5mg/Kg	39.3	14.86	9.00	58.1	542	555	70.1

Summary of repeat dose toxicity studies

(multiple ascending daily dose)

Observations in rat (14d dosing)

- No observed adverse event level (NOAEL): 5 mg/kg/Day
- MTD: >5, <15 mg/kg/day
  - Mortality observed at 15 & 30 mg/kg/day
- Clinical observations at 15 & 30 mg/kg/day: Watery feces (diarrhea), ocular discharge (red), swollen (lip, nose), material around eyes and nose (red), emaciated, posture hunched & decreased activity.

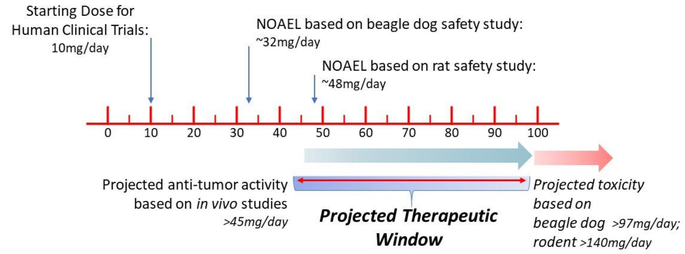
Observations in beagle dog (28d dosing)

- No observed adverse event level (NOAEL): 1 mg/kg/Day
- Control and low dose ell tolerated, clinical signs equivalent between groups 1 and 2
- Group 3 (High Dose)
  - Dosing stopped after 7 days due to adverse signs, animals rapidly recovered; dosing resumed on day 14-22 and was stopped again due to adverse signs
  - Observed clinical signs included GI tox typical of EGFR-targeting agents
- No observation of dermal toxicity in any group
- No treatment-related changes of organ weights in any group

EO1001 Human Safety and Efficacy Extrapolation Based on Preclinical Observations

Human Equivalent Dose for 60kg Subject

(calculations based on Nair, 2016)



Conclusion & Next Steps:

Based on observations to date, EO1001 has the potential to be a best-in-class CNS-penetrating pan-ErbB inhibitor that is amenable for use as a single agent and in combination treatment regimens.

Regulatory filings have been initiated to allow first-in-man clinical testing with EO1001.

Continued characterization of EO1001 activity against specific ErbB mutations will be undertaken in parallel with clinical evaluation