Tarloxotinib Exhibits Potent Activity in *NRG1* Gene Fusion Positive Cancers

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Introduction

- Despite substantive cancer genome sequencing efforts, a majority of solid tumors still lack therapeutically tractable genetic alterations
- *NRG1* gene fusions are oncogenic drivers that may be clinically actionable
- *NRG1* fusions result in overexpression of chimeric transmembrane proteins containing the EGF-like domain or cleaved soluble EGF-like domain that serves as the ligand for HER3 leading to HER2/HER3 heterodimer formation and activation of the MAPK, PI3K/AKT and NF-kB pathways
- *NRG1* fusions are enriched in invasive mucinous adenocarcinoma (IMA) of the lung and are reported in 27-31% of patients and are mutually exclusive with KRAS mutations[1,2]
- *NRG1* fusions have been reported in a variety of cancers with an overall incidence of 0.2% in solid tumors[3,4]
- Initial reports of activity with HER-directed therapies afatinib[5,6], GSK2849330[3], lumretuzumab and erlotinib[7] provided clinical concept validation
- There are no approved therapies for NRG1 fusions highlighting the therapeutic gap for patients with *NRG1* fusions.

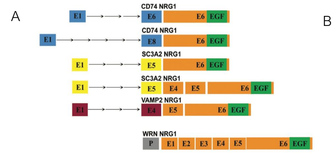


Figure 1. ***NRG1*** **gene fusions encode chimeric proteins**

A. Schematic diagram showing selected *NRG1* fusion variants in lung tumors[9]. Specific NRG1 exons fused with the 5' gene partner are colored in orange. The 5' partner gene are represented with different colors and only the first and the exon fused with NRG1 are represented. The NRG1-EGF domain of the chimeric gene is colored in green. *Exons are not to scale*

B. Schematic representation of wild-type NRG1 III-β3 and predicted CD74–NRG1 fusion protein in the cellular membrane[1]

- Hypoxia in solid tumors contributes to the development of resistance to radiotherapy, cytotoxic therapy, targeted therapies and immunotherapy
- Tarloxotinib is a hypoxia-activated prodrug (HAP) that releases a potent irreversible pan-ErbB TKI (tarloxotinib-E) under pathophysiological hypoxia present in solid tumors. Tumor selective release increases dose intensity and significantly enhances the tolerability due to reduced WT EGFR-mediated side effects compared to approved EGFR TKIs
- STEAP4 is a transmembrane reductase that is identified as the major contributor of the conversion of tarloxotinib to tarloxotinib-E in hypoxic tumors[8]

Figure 2. Tarloxotinib conversion to its irreversible pan-ErbB inhibitor. Addition of a hypoxia trigger (blue) to tarloxotinib-E significantly reduces the potency of the prodrug, allowing for administration of a higher relative dose

CLU-NRG1 patient-derived xenograft model

- OV-10-0050, an ovarian PDX model with outlier expression of NRG1 mRNA
- *CLU–NRG1* fusion results from the intragenic fusion of exon 2 of *CLU* with exon 6 of *NRG1*, retaining the EGF-like extracellular domain

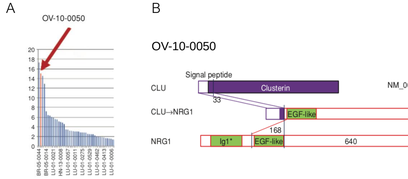
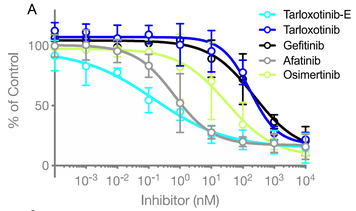


Figure 3. A. High expression of *NRG1* mRNA in various PDX models **B.** Schematic representation of *CLU-NRG1* fusion[3]

Potent *in vitro* activity of tarloxotinib-E in *DOC4-NRG1* fusion breast cancer cell line



B

Compound	MDA-MB-175 DOC4-NRG1 IC_{50} (nM)
Gefitinib	404
Tarloxotinib	307
Osimertinib	37
Afatinib	1.2
Tarloxotinib-E	0.3

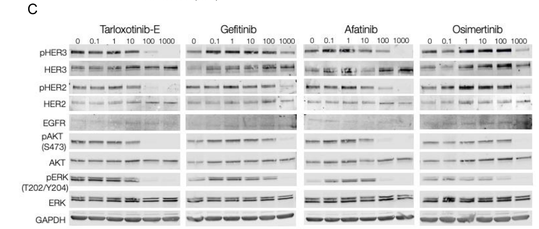


Figure 4. Activity of tarloxotinib-E in *DOC4-NRG1* fusion breast cancer cell line.

A. Dose response curves of cell proliferation of MDA-MB-175VIII (breast cancer, *DOC4-NRG1* fusion) Cells were treated with afatinib, gefitinib, tarloxotinib (pro-drug) and tarloxotinib-E (active drug) for 72 hours and measured by MTS. Experiments were done in triplicate; mean ± SEM is plotted.

B. Table summarizing IC_{50} values of the proliferation experiment.

C. MDA-MB-175VIII cells were treated with the indicated doses of tarloxotinib-E (active drug), gefitinib, afatinib or osimertinib for 2 hours, lysed and analyzed by immunoblot. Experiments were done in triplicate. Phospho-antibodies used: pEGFR (Y1068), pAKT (S473), pERK (Y202/204), pHER2 (Y1221/1222), pHER3 (Y1289).

Hypoxia and STEAP4 levels in *CLU-NRG1* PDX model

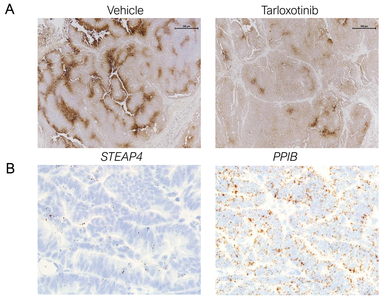


Figure 5. Hypoxia and *STEAP4* levels in CLU-NRG1 PDX model

A. Hypoxia in OV-10-0050 PDX tumors. Mice bearing subcutaneous tumors were treated with vehicle or tarloxotinib (48mg/kg). Pimonidazole (60mg/kg) was administered 60 min before sacrifice and 23 hr after tarloxotinib dosing. Excised tumors were formalin-fixed, paraffin-embedded and stained for hypoxia-dependent pimonidazole binding with DAB, HRP substrate (brown).

B. *STEAP4* ISH staining (RNASCOPE) in OV-10-0050 PDX tumors with score of 2. PPIB housekeeping gene used as a control.

Potent *in vivo* antitumor activity of tarloxotinib in *CLU-NRG1* fusion ovarian cancer PDX

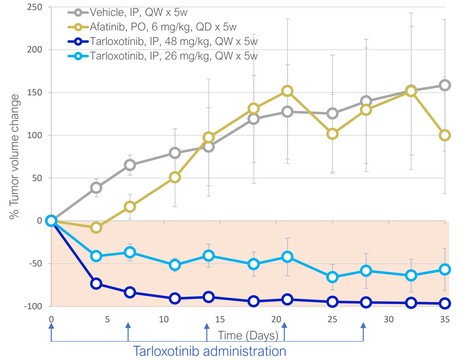


Figure 6. Tarloxotinib inhibits tumor growth of *CLU-NRG1* patient derived xenograft model. Percent changes from baseline tumor volume in nude mice implanted subcutaneously with OV-10-0050 PDX and treated with vehicle, afatinib (6mg/kg, daily, PO), tarloxotinib (48mg/kg, once weekly, IP) and tarloxotinib (26 mg/kg, once weekly, IP). Tarloxotinib 48 mg/kg and 26 mg/kg correspond to human equivalent doses of 150 mg/m^2 and 75 mg/m^2 respectively.

Tarloxotinib and tarloxotinib-E exhibit sustained tumor exposure in CLU-NRG1 fusion ovarian cancer PDX

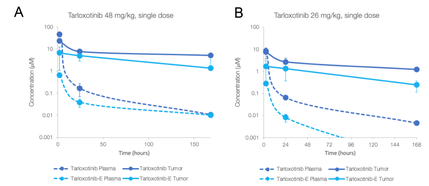


Figure 7. Plasma and tumor pharmacokinetics of a single dose of tarloxotinib in OV-10-0050 tumor bearing mice depicting the profiles of tarloxotinib and tarloxotinib-E when tarloxotinib was administered at 48 mg/kg (**A**) or 26 mg/kg (**B**).

HER2/HER3 Pathway inhibition 2h post tarloxotinib

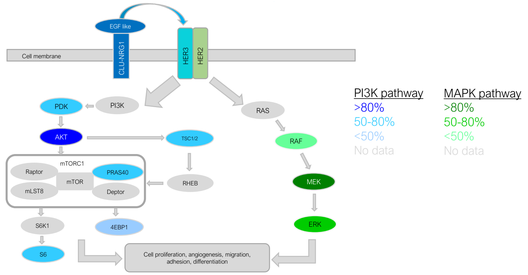


Figure 8. *NRG1* fusion pathway[10] changes in the OV-10-0050 PDX model at 2 hours post tarloxotinib (48 mg/kg) dosing.

Tarloxotinib induces sustained downregulation of multiple cancer signaling pathways in *CLU-NRG1* PDX model

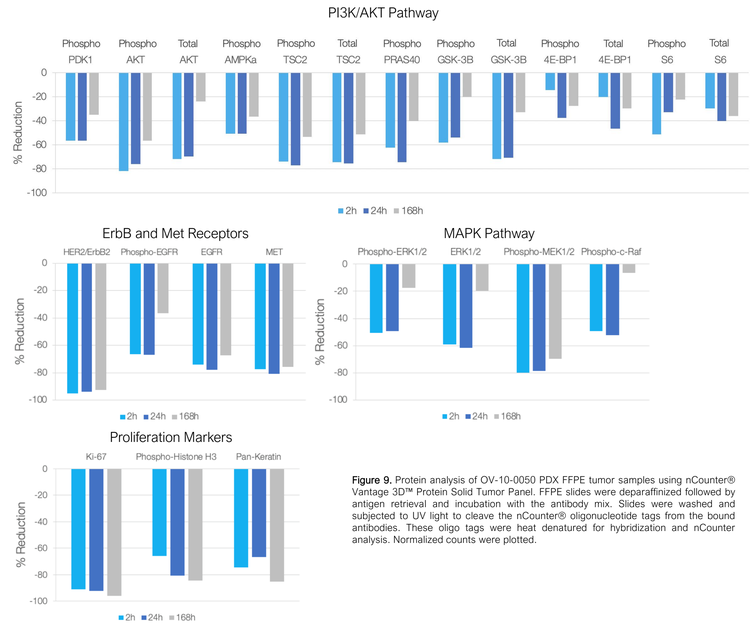


Figure 9. Protein analysis of OV-10-0050 PDX FFPE tumor samples using nCounter® Vantage 3D™ Protein Solid Tumor Panel. FFPE slides were deparaffinized followed by antigen retrieval and incubation with the antibody mix. Slides were washed and subjected to UV light to cleave the nCounter® oligonucleotide tags from the bound antibodies. These oligo tags were heat denatured for hybridization and nCounter analysis. Normalized counts were plotted.

Conclusions

- Tarloxotinib-E (active drug) inhibits *in vitro* proliferation of MDA-MB-175VIII cells harboring a *DOC4-NRG1* fusion
- Tarloxotinib-E demonstrated >100x higher activity compared to the pro-drug tarloxotinib
- Tarloxotinib-E inhibits HER2 and HER3 phosphorylation and downstream signaling *in vitro* in MDA-MB-175VIII cells
- Tarloxotinib significantly regressed tumors in *CLU-NRG1* ovarian PDX model at both doses of 48 mg/kg and 26 mg/kg in a dose dependent manner
- Significant hypoxia is present in *CLU-NRG1* tumors. Moderate levels of STEAP4 reductase which mediates the conversion of tarloxotinib prodrug to tarloxotinib-E active drug detected in *CLU-NRG1* tumors
- Tarloxotinib and tarloxotinib-E clears quickly in plasma, but shows prolonged tumor retention
- In the *CLU-NRG1* PDX model, single dose of tarloxotinib led to significant reductions in total and phosphoproteins in the MAPK and PI3K/AKT pathways for up to 7 days
- Tarloxotinib is currently in a phase 2 clinical trial (RAIN-701, NCT03805841) for EGFR exon 20 and HER2 mutation positive NSCLC
- *NRG1* fusion positive cancers represent an attractive clinical trial opportunity for tarloxotinib

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