Tucatinib, a Selective Small Molecule HER2 Inhibitor, is Active in HER2 Mutant Driven Tumors

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Background

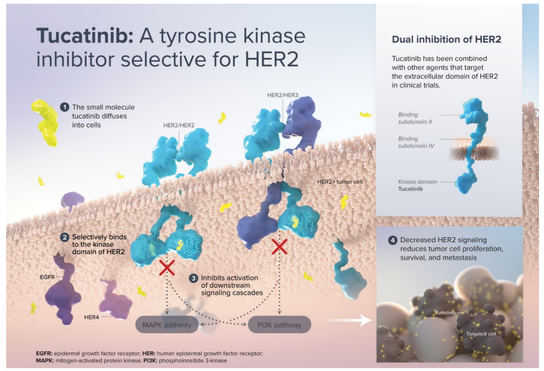
Tucatinib is an, oral, reversible, small molecule tyrosine kinase inhibitor that is highly selective for the kinase domain of HER2 without significant inhibition of EGFR and has demonstrated activity in multiple HER2 amplified preclinical tumor models alone, and in combination with trastuzumab (1)

Tucatinib was recently approved for use by the U.S. Food and Drug Administration in combination with trastuzumab and capecitabine for adult patients with advanced unresectable or metastatic HER2-positive breast cancer, including patients with brain metastases based on the results of HER2CLIMB, a randomized, double-blind, placebo-controlled trial (2)

[1]Kulukian et al., Preclinical Activity of HER2-Selective Tyrosine Kinase Inhibitor Tucatinib as a Single Agent or in Combination with Trastuzumab or Docetaxel in Solid Tumor Models Mol Cancer Ther 2020;19:976–87

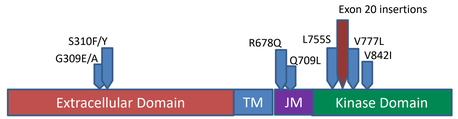
[2]Murthy RK et al. Tucatinib, trastuzumab, and capecitabine for HER2-positive metastatic breast cancer. N Engl J Med 2020;382:597–609.

Tucatinib proposed mechanism of action



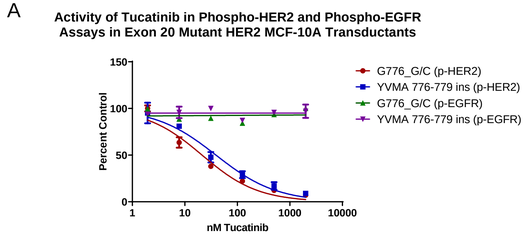
HER2 Mutations as Oncogenic Drivers

- HER2 hotspot somatic mutations have been identified in multiple diseases and shown to function as oncogenic drivers in preclinical tumor models
- Activating mutations are found in the extracellular domain, juxta-membrane (JM) domain and kinase domain of HER2 and each of these mutations shows a pattern of enrichment in subsets of cancers



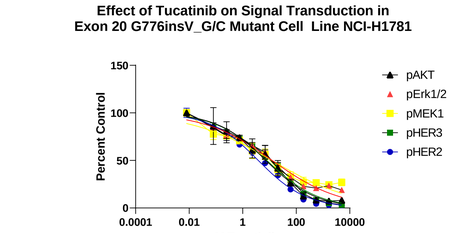
Tucatinib is a Potent and Selective Inhibitor of Exon 20 HER2 Mutant Kinase Signaling

A



	IC50
G776_G/C (p-HER2)	21.33 nM
YVMA 776-779 ins (p-HER2	39.19 nM
G776_G/C (p-EGFR)	NA
YVMA 776-779 ins (p-EGFR)	NA

B



	pHER2	pHER3	pMEK1	pErk1/2	pAKT
IC50	3.825	8.021	12.33	12.70	7.404

C

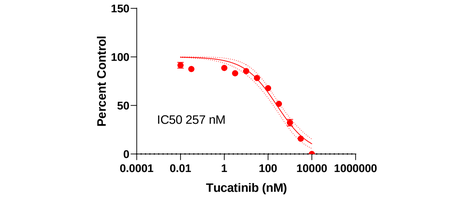
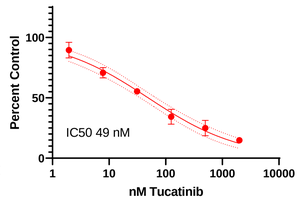


Figure 1. (**A**) Dose response of tucatinib in an MCF10A cell line clone expressing the HER2 exon 20 insertion mutation G776insV_G/C. HER2 phosphorylation was determined by Luminex assay using total tyrosine phosphorylated HER2 as an endpoint with tucatinib titrated from 1-2000 nM in 4-fold dilutions. **(B)** The effect of tucatinib on NCI-H1781 cell downstream signaling was determined by measuring phosphorylation of HER2, HER3, AKT, ERK1/2, and MEK1 using a multiplexed antibody capture assay employing Luminex technology. HER2, HER3 (total Tyr), ERK1/2 (Thr185; Tyr187), MEK1 (Ser222) and AKT (Ser473) were measured in cell extracts after 2-hour tucatinib treatment. Tucatinib was titrated using 3-fold dilutions starting from 0.08 nM to 5000 nM **(C)** The effect of tucatinib on NCI-H1781 cells was determined using the Cell Titer-Glo Luminescent Cell Viability Assay. Tucatinib was titrated using 3-fold dilutions starting from 0.0001 μM to 10,000 nM

Tucatinib is Effective in HER2 L755S Mutant Patient Derived Xenograft Models

A



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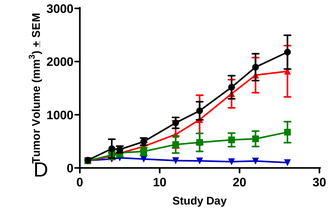

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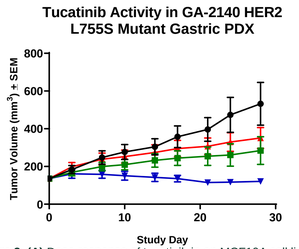


D

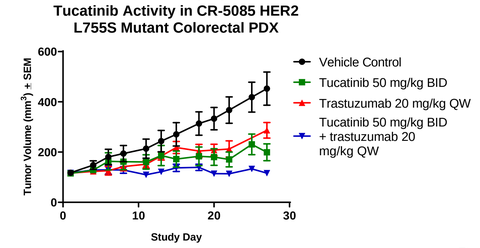
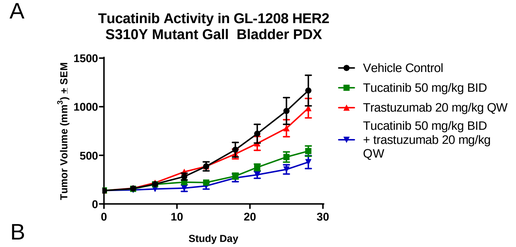


Figure 2. (A) Dose response of tucatinib in an MCF10A cell line clone expressing the HER2 L755S mutation. HER2 phosphorylation was determined by Luminex assay using total tyrosine phosphorylated HER2 as an endpoint with tucatinib titrated from 1-2000 nM in 4-fold dilutions . **(B-D)** Activity of tucatinib in L755S mutant HER2 PDX tumor models. Tucatinib was administered at 50 mg/kg twice daily for the duration of the study, while trastuzumab was dosed at 20 mg/kg weekly.

Tucatinib is Active in S310Y Mutant HER2 PDX Models

A



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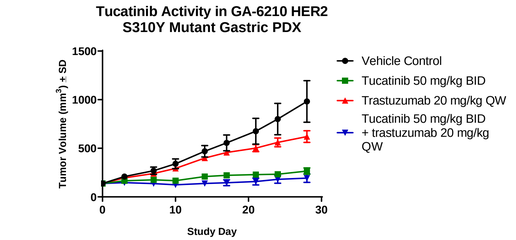
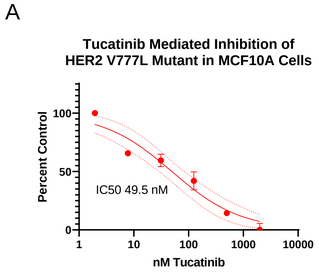


Figure 4. Activity of tucatinib in S310Y mutant HER2 PDX tumor models. **(A&B)** Tucatinib was administered at 50 mg/kg twice daily for the duration of the study, while trastuzumab was dosed at 20 mg/kg weekly.

Tucatinib Induces Tumor Regression in a V777L HER2 Mutant PDX Model

A



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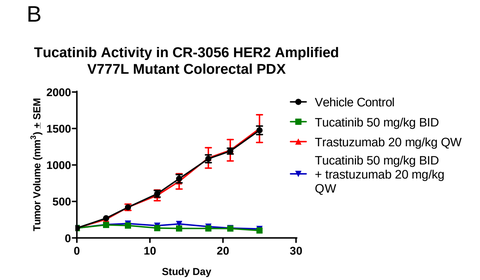

Figure 3. (A) Dose response of tucatinib in an MCF10A cell line clone expressing the HER2 V777L mutation. HER2 phosphorylation was determined by Luminex assay using total tyrosine phosphorylated HER2 as an endpoint with tucatinib titrated from 1-2000 nM in 4-fold dilutions . **(B)** Activity of tucatinib in V777L mutant HER2 PDX colorectal tumor model. Tucatinib was administered at 50 mg/kg twice daily for the duration of the study, while trastuzumab was dosed at 20 mg/kg weekly.

Conclusions

Tucatinib is a potent inhibitor of mutant HER2 signaling in vitro

- In cell signaling assays, tucatinib exhibits potent inhibition of HER2 phosphorylation in exon20 insertion mutants without measurable effects on EGFR phosphorylation
- In the exon 20 G776 InsV_G/C mutation background tucatinib potently blocked downstream signal transduction and inhibited cell proliferation in vitro
- In the HER2 L755S and V777L mutants tucatinib potently blocked HER2 phosphorylation.

Tucatinib is active in HER2 mutant PDX tumor models

- Tucatinib induced tumor regressions in L755S and V777L driven PDX models alone, and in combination with trastuzumab. In S310Y mutants tucatinib induced tumor growth delay as a single agent and in combination with trastuzumab

These data demonstrate that selective inhibition of HER2 mutants by tucatinib results in the regression or inhibition of tumor growth in patient derived xenograft models and support the evaluation of tucatinib in clinical studies in HER2 mutant driven cancers

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