Preclinical Characterization of a Novel EGFR Sparing ErbB2 Inhibitor with Activity Against Oncogenic ErbB2 Mutations

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Background

- ErbB2 is a receptor tyrosine kinase that belongs to a family of four receptors EGFR, ErbB2, ErbB3, and ErbB4, also known as HER1, 2, 3, 4[1]
- ErbB2 is a well-established oncogenic driver with no known direct activating ligand
- Receptor activation induces dimerization with a marked preference for ErbB2 as a partner[2]
- Phosphorylation of the ErbB kinase domain activates PI3K/Akt and the Ras/Raf pathways which regulate cell growth, survival and differentiation[3]

Figure 1. HER Receptors: Heterodimer Formation and Signaling

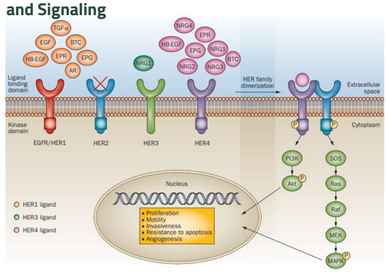


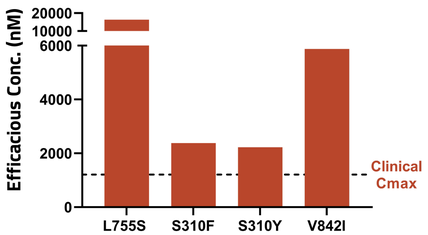
Table 1. ErbB2 Non-Exon 20 Mutational Frequency in Solid Tumors from TCGA

Most Common Cancers With ErbB2 Alterations	Main Point Mutation by Cancer Type	Yearly US Prevalence[4]	Non-Exon 20 Mutational Frequency[5]	Estimated Yearly Cases Non-Exon 20 Mutation
Bladder Urothelial Carcinoma	S310F/Y	83,000	11%	9100
Uterine Corpus Endometrial Carcinoma	V842I R678Q	66,000	6.8%	4500
Stomach Adenocarcinoma	S310F/Y	26,000	5%	1300
Breast	L755S	284,000	0.9%	2500

- ErbB2 amplifications and mutations occur in a mutually exclusive fashion (80-90% of cases) and represent independent drivers of human cancer pathogenesis (**Table 1**)[6]
  - Activating mutations in the ErbB2 gene have been identified in multiple cancers and demonstrate a tumorigenic role similar to that of ErbB2 amplification
  - Emerging mutations result in both acquired and cross resistance

- The non-selective dual EGFR/ErbB2 inhibitors are active against ErbB2 point mutations, however, inhibition of EGFR leads to dose limiting toxicities that include severe rash, diarrhea and mucositis
- Tucatinib, the first-generation selective EGFR sparing ErbB2 inhibitor, does not reach clinical plasma concentrations to cover the IC_{90} efficacious concentration for prevalent ErbB2 mutations

Figure 2. Tucatinib Lacks Coverage of Prevalent ErbB2 Mutants at the Approved Clinical Dose[7]



In-house cell IC_{50} values were generated and used for this calculation

Goal:

Identification of a potent, mutant active, EGFR-WT sparing ErbB2 inhibitor would address a clear unmet medical need for the treatment of patients with ErbB2 mutations and patients with ErbB2 resistance mutations on currently approved HER2 TKIs including tucatinib

1. Arteaga et al. 2012. Nat Rev Clin Oncol. 9:16-32. 2. Tai et al. 2010. J Control Release.146(3):264–275. 3. Yarden and Pines. 2012. Nat Rev Cancer.12:553–563. 4. American Cancer Society. Cancer Facts & Figures 2021. Atlanta: American Cancer Society; 2021. 5. The AACR Project GENIE Consortium. AACR Project GENIE: Powering Precision Medicine Through An International Consortium, Cancer Discovery 2017. 6. Subramanian et al. 2019. The Oncologist 24:e1303-e1314. 7. Filho et al. 2020. Ann Oncol. 31(9):1231-1239.

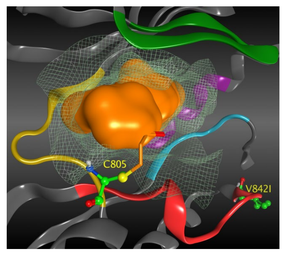
Results

Table 2. Early Lead Compound CGT1786: Selective EGFR Sparing ErbB2 Inhibitor with Cellular Activity Against Oncogenic Mutations

Compound	Cellular pErbB2 Inhibition IC_{50} (nM) in Engineered Cell Lines					
	ErbB2-WT	S310F Urothelial	S310Y Urothelial	V842I Uterine	L755S Breast	EGFR-WT pEGFR inhibition
CGT1786	27	21	18	41	50	4200

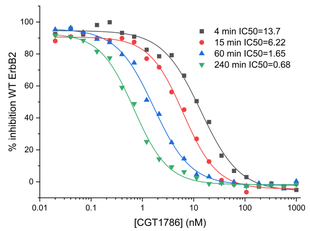
- Potent on WT and prevalent ErbB2 point mutations including L755S measuring pErbB2 inhibition
- Showed >150-fold selectivity for ErbB2 over EGFR, sufficient to avoid EGFR-related toxicity

Figure 3. Proprietary Crystal Structure of ErbB2 V842I Drove Inhibitor Design



- 2.3 Å resolution crystal structure of mutant ErbB2 V842I with inhibitor bound
- Covalent bond from inhibitor to Cys805 is highlighted, the remainder of the compound is masked as an orange surface
- Proprietary crystal structures of ErbB2 were used to optimize inhibitors for potency and selectivity

Figure 4. Inactivation Kinetics of CGT1786 are Comparable to Approved Agents

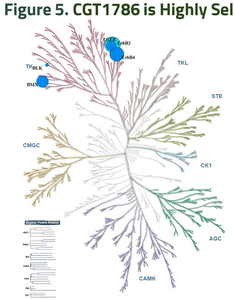


- CGT1786 exhibits time dependent inhibition of ErbB2-WT, which is consistent with covalent inactivation
- CGT1786 inactivates ErbB2 at rates comparable with the approved ErbB family inhibitors neratinib and afatinib indicating it has clinically efficacious rates of ErbB2 inactivation

Compound	ErbB2 K_{inact}/K_I (M^{-1} s^{-1})	EGFR K_{inact}/K_I (M^{-1} s^{-1})	Inactivation Ratio (ErbB2/EGFR)
CGT1786	1.25E+05	2.41E+03	51.8
afatinib	1.81E+05	7.92E+06	0.02
neratinib	2.16E+05	1.18E+06	0.2

K_{inact}/K_I were determined at Enzymlogic, Madrid, Spain, using COVALfinder® technology

Figure 5. CGT1786 is Highly Selective Across 371 Kinases Tested



- CGT1786 was screened against a panel of 371 kinases at a concentration of 500nM, 100x the ErbB2 enzyme IC_{50}
- In addition to HER family kinases, only BLK and BMX were identified as hits for CGT1786 in the kinome screen
- BLK and BMX are commonly inhibited by EGFR and BTK inhibitors and are not expected to cause toxicities
- Selectivity for ErbB2 over EGFR is >150X

Figure 6a. CGT1786 Shows Promising Pharmacokinetics in Mice

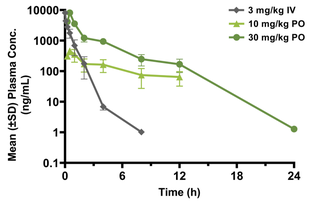
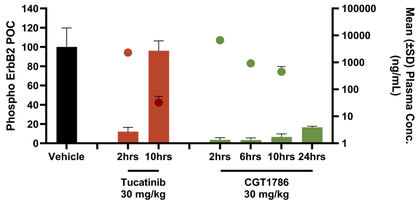
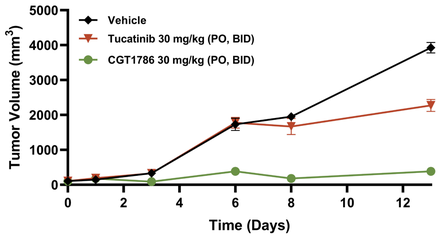


Figure 6b. CGT1786 Demonstrates Robust Inhibition of Tumor pErbB2 Levels in a 3T3 L755S Mouse PK/PD



- CGT1786 is a low clearance compound with dose proportional exposure from 10-30 mg/kg in mice (**Fig. 6a**)
- PK/PD comparing tucatinib and CGT1786 at 30 mg/kg in the 3T3 L755S breast cancer tumor model (**Fig. 6b**)
  - Tucatinib reached plasma concentrations 6x higher than clinically achievable and still only gave 88% inhibition of pErbB2 at the 2h timepoint and no inhibition at the 10h timepoint
  - **In contrast, CGT1786 showed complete pErbB2 inhibition of >95% at the 2 and 6-hour timepoints and preserves >90% inhibition at 10 hours**

Figure 7. CGT1786 Shows Superior Tumor Growth Inhibition Compared to Tucatinib in a 3T3 L755S Model



- Tumor growth inhibition (TGI) comparing tucatinib to CGT1786 in a 3T3 L755S breast cancer model with 100 mm^3 staged tumors
- Tucatinib showed minimal TGI (42%) at 30 mg/kg PO, BID
- **The 30 mg/kg PO, BID dose of CGT1786 resulted >90% TGI**
- Both compounds were tolerated in this study with no weight loss observed

Conclusions

- Treatments for patients with ErbB2 point mutations are limited
  - Dual EGFR/ErbB2 inhibitors suffer from EGFR toxic side effects
  - First generation ErbB2 selective inhibitor, tucatinib, cannot cover the point mutations at clinical Cmax
- A next generation selective EGFR-sparing ErbB2 inhibitor would address a clear unmet medical need
- Proprietary crystal structures of mutant ErbB2 were used to optimize compounds for potency and selectivity
- CGT1786 – Early Lead Compound
  - >150-fold selective for EGFR WT and is potent on prevalent point mutations
  - Exhibits inactivation kinetics comparable to approved agents
  - Highly selective across a panel of 371 kinases
  - Demonstrates robust inhibition of tumor phospho ErbB2 levels in a 3T3 L755S breast cancer model
  - Shows superior tumor growth inhibition compared to tucatinib in a 3T3 L755S model

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