Mechanisms of resistance to anti-HER2 therapies in brain metastatic derivatives of inflammatory HER2-positive breast cancer models

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Abstract

Background: HER2-positive (HER2+) breast cancer (BC) is an aggressive subtype with 30-50% incidence of brain metastases in metastatic patients. The HER2-selective tyrosine kinase inhibitor (TKI) tucatinib (Tuca), the pan-HER TKI neratinib (Nrb), and the novel HER2 antibody drug conjugate (ADC) T-DXd are effective, including in treating brain metastases and the aggressive inflammatory BC. High EGFR expression has been shown to reduce the efficacy of T-DXd [1]. **Materials and Methods:** GFP/Luc tagged SUM190-BR3 "parental", a brain-tropic derivative of the HER2+/ER- inflammatory SUM190 cell model (PIK3CA H1047R mutation and p53 null), was used [2]. A long-term highly Tuca resistant (TucaR) derivative of this line was developed through exposure to increasing doses of Tuca up to 1uM. Characterization of these resistant and naïve cells was performed by Western blot (WB), DNA-seq, and RNA-seq. Drug efficacy studies involved six-day methylene blue-based cell growth and IC_{50} assays. The Akt inhibitor (i), capivasertib, T-DXd, the EGFR-specific TKI gefitinib, or monoclonal antibody (mAb) cetuximab, and the HER2-specific monoclonal antibody pertuzumab were assessed to therapeutically overcome resistance. To characterize the *in vivo* tumorigenicity and metastatic capacity of our resistant model, immunocompromised mice were injected with cells via subcutaneous or intracardiac routes and monitored for primary tumor growth and metastasis by bioluminescence imaging. **Results:** WB analysis of the TucaR model shows elevated levels of phosphorylated and total EGFR, suggesting pathway reactivation at time of resistance. TucaR cells remain sensitive to Nrb. Capi is effective as a single agent in the TucaR model (p<.0001). Both Gef and Cetux improve the efficacy of T-DXd in our models.

Objective

We aim to understand the roles and therapeutic importance of *PIK3CA* mutations and high-EGFR in resistance to anti-HER2 therapies in a TucaR brain metastatic inflammatory HER2+ BC model.

Drug	Class	Target	Abbreviation	Concentration
Tucatinib	TKI	HER2	Tuca	1uM
Neratinib	TKI	EGFR, HER2, & HER4	Nrb	200nM
Capivasertib	KI	Akt	Capi	1uM
Gefitinib	TKI	EGFR	Gef	1uM
T-DXd	ADC	HER2	T-DXd	5ug/mL
Cetuximab	mAb	EGFR	Cetux	10ug/mL
Pertuzumab	mAb	HER2	Pertuz	10ug/mL

Results

The SUM190-BR3 parental cells are partially intrinsically resistant to Tuca, but not Nrb

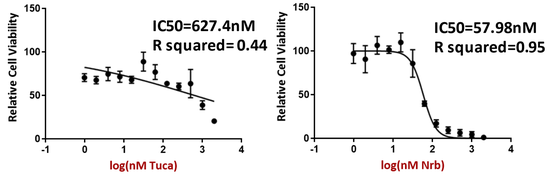


Figure 1: The SUM190-BR3 parental cells are partially intrinsically resistant to Tuca (IC_{50}>600nM), but not Nrb (IC_{50}<60nM).

TucaR is associated with HER layer reactivation, while Capi inhibits mTOR signaling

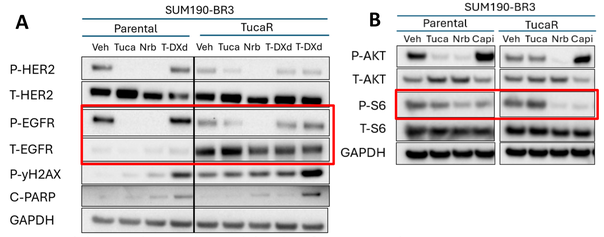


Figure 2: (A) WB shows elevated levels of HER2, and even more so EGFR, suggesting EGFR/HER2 signaling activation in the TucaR model. Inhibition with T-DXd is associated with an increase in markers of DNA damage and cell death. RNA-seq finds high EGFR in the TucaR cells. (B) When treated with Capi, P-S6 (mTOR) signaling is reduced more strongly in the TucaR cells vs. parental. Parental and TucaR cells were treated for 24 hours. The final column in (A) is T-DXd for 72hrs.

Nrb and Capi are effective as single agents in TucaR cells

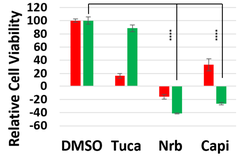


Figure 3: TucaR cells remain highly sensitive to Nrb, suggesting the importance of sequencing, and Capi, inhibiting constitutive Akt signaling downstream from the *PIK3CA* mutation, is effective as a single agent.

High-EGFR TucaR cells are less sensitive to T-DXd

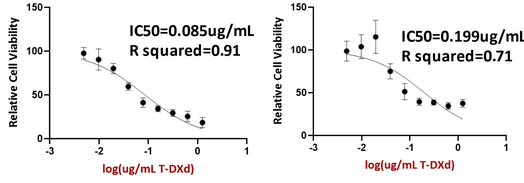


Figure 4: The TucaR cells have a T-DXd IC_{50} about 2.3x higher than the parental. Recent studies have suggested that high-EGFR is associated with reduced sensitivity to antibody drug conjugates such as T-DXd [1].

High-EGFR TucaR cells are sensitive to EGFRi

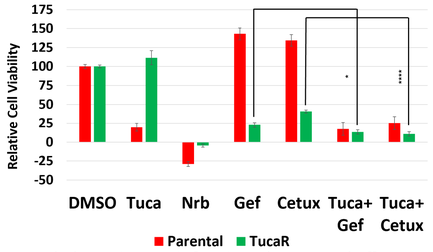


Figure 5: Blocking EGFR via Gef or Cetux has minimal effect on parental cell viability, but significant inhibition on TucaR. The parental cells are sensitive to Tuca, but adding EGFRi does not improve efficacy of Tuca. In TucaR cells, adding Tuca to EGFRi has minimal effect.

An EGFR inhibitor is superior to pertuzumab when combined with T-DXd in a high-EGFR model

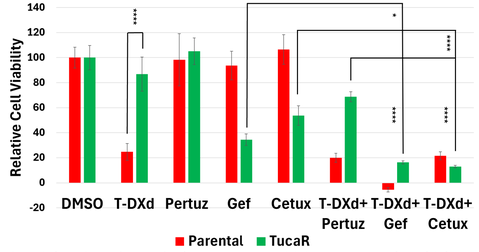


Figure 6: High EGFR (TucaR) decreases sensitivity to T-DXd, which can be overcome through inhibition of EGFR. Interim results from the DESTINY-Breast09 trial find T-DXd plus pertuzumab to be highly effective. Our preliminary results indicate that T-DXd plus an EGFRi is superior to T-DXd plus pertuzumab in our high-EGFR TucaR model. In parental cells, T-DXd +Pertuz is as good as T-DXd +Cetux, but benefit from adding Gef to T-DXd.

EGFR remains high, but heterogeneous, in TucaR when grown *in vivo*

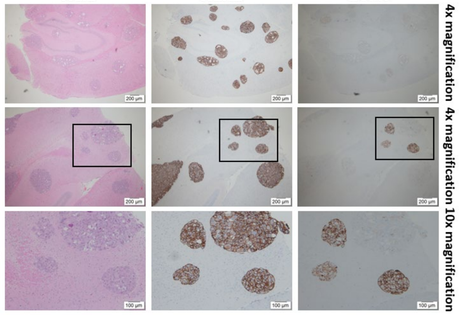


Figure 7: TucaR cells retain their brain tropism when injected into Nude mice via intracardiac injection. Immunohistochemistry of HER2 and EGFR show that the TucaR cells have high EGFR in brain metastases, although with heterogeneity, compared to the SUM190-BR3 parental.

Conclusions

- Signaling and therapeutic findings suggest that high EGFR and *PIK3CA* mutations are potentially implicated in resistance to Tuca in the SUM190-BR3 TucaR model
- The high-EGFR TucaR cells are less sensitive to T-DXd, and our preliminary data suggests that EGFR inhibitors improve the efficacy of T-DXd
- Extended studies are warranted to determine the roles of high EGFR and *PIK3CA* mutations in treatment efficacy and patient outcome
- Future work seeks to overcome resistance to T-DXd

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Funding

This study was supported by research funding from the breast cancer research program breakthrough awards CDMRP W81XWH-17-1-0580 (to R.S) and W81XWH-17-1-0579 (to M.F.R); Breast Cancer Research Foundation BCRF-145 (to R.S and C.K.O), Adopt-A-Scientist foundation, and the National Institute of General Medical Sciences of the National Institutes of Health under Award Number T32GM136554. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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