# Aberrant HER2 signaling is a therapeutic target in a subset of castration-resistant prostate cancer

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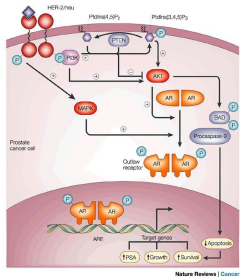
## Background

- While most men with metastatic prostate cancer (PCa) initially respond to androgen-deprivation therapy and second-line hormonal agents, resistance is ultimately universal and subsequent survival is limited.
- HER2 is a transmembrane receptor tyrosine kinase. HER2 dimerization (primarily with HER3) initiates signaling to promote cellular processes critical to survival, proliferation, and metastasis.
- HER2 signaling in PCa promotes androgen receptor (AR) transcriptional activity and contributes to persistent AR signaling in a subset of castration-resistant prostate cancer (CRPC).[1-3]

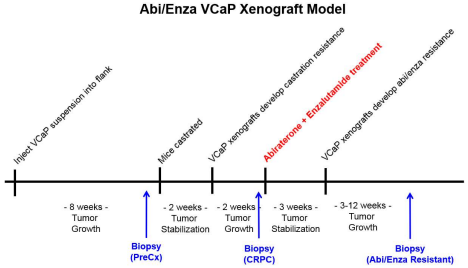


- Aberrant HER2 signaling in metastatic CRPC (mCRPC):
  - HER2 amplification is rare in PCa, but increased HER2 mRNA expression is found in 10% of the Stand Up To Cancer (SU2C) mCRPC cohort.[4]
  - In addition, the HER2 splice variant d16HER2, which results in a self-dimerizing, constitutively active protein, has been identified in 7% of mCRPC samples in the SU2C cohort and in circulating tumor cells from 6/15 (40%) patients with mCRPC (unpublished data).
  - In a trial of neoadjuvant abiraterone at our institution, approximately 20% of abiraterone-resistant PCa showed increased HER2 signaling, as detected by phospho-ErbB3 immunohistochemistry (IHC).[1]
- Here, we present preclinical and clinical data to support signaling via canonical HER2/HER3 interactions and the d16HER2 variant as potential oncogenic drivers of CRPC. We also show preclinical evidence that enhanced HER2 signaling may be targeted with irreversible HER2-targeted inhibitors to inhibit tumor growth.

## Methods

**Preclinical Studies**:

- Human-derived VCaP prostate cancer xenografts were serially biopsied prior to castration, at castration-resistance, and after resistance to abiraterone + enzalutamide (Abi/Enza).
- Biopsies were submitted for RNA sequencing and RT-PCR, IHC, and reverse-phase protein array (RPPA) for gene/protein expression changes.
- Castration-resistant VCaP and LuCaP-70CR xenografts were treated with reversible EGFR/HER2 tyrosine kinase inhibitor (TKI) lapatinib (100 mg/kg) or irreversible pan-HER TKIs afatinib (20 mg/kg) or neratinib (20 mg/kg).

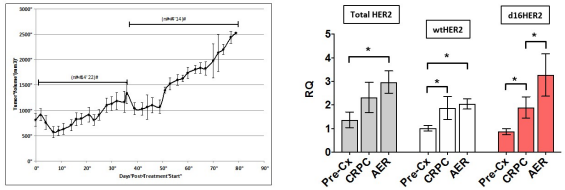



**Clinical Studies**:

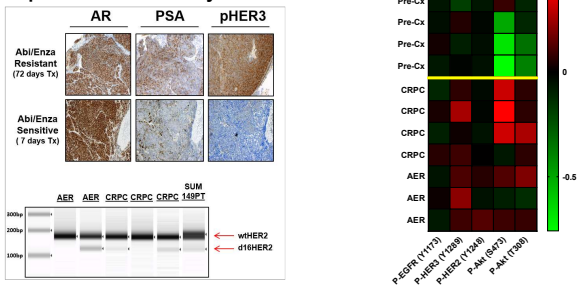
- Transcript levels of total HER2 and d16HER2 variant were determined for TCGA and SU2C PCa datasets.[4,5]
- IHC for phospho-HER2 (Tyr1221/1222) and phospho-HER3 (Tyr1289) were performed on metastatic samples from 49 heavily-treated mCRPC patients and compared to 18 hormone-naïve patient prostatectomy samples.

## HER2 Signaling Increases with Disease Progression

- VCaP PCa xenografts exhibit increased activation of HER2/HER3 signaling and increased expression of d16HER2 with progression through castration and Abi/Enza therapy.

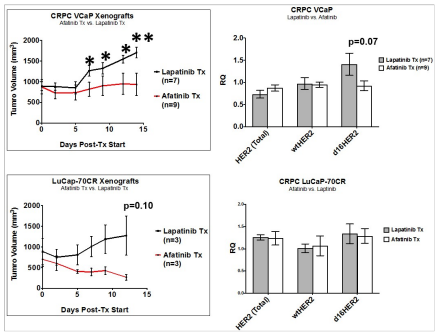


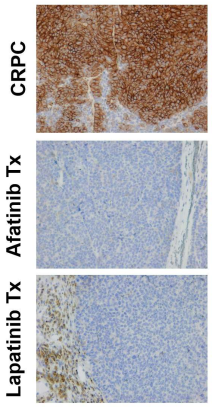

- Increases in pHER2 and pHER3 were observed by IHC and RPPA in serial biopsies of individual tumors, and increases in total HER2, wtHER2, and d16HER2 transcripts were observed by RT-PCR.



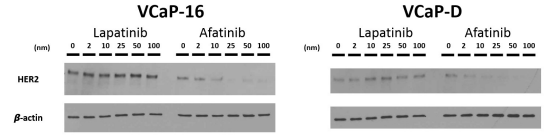

## HER2 TKIs Inhibit CRPC Growth

- Castration-resistant VCaP and LuCaP-70CR xenografts rapidly become resistant to lapatinib within 5 days, while irreversible pan-HER TKI inhibited tumor growth for at least 15 days.



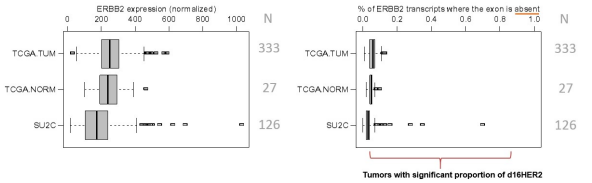




- Afatinib is more effective at decreasing total HER2 levels than lapatinib in both enzalutamide-resistant VCaP-16 and control VCaP-D.

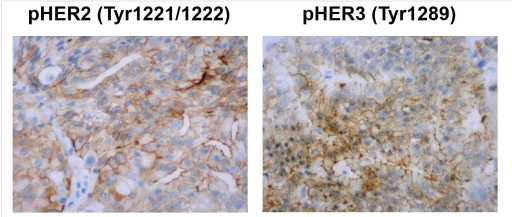



## Aberrant HER2 in Clinical PCa Samples

- Analysis of TCGA (mostly hormone-naïve PCa) and SU2C (mCRPC) RNAseq datasets shows enrichment of tumors expressing d16HER2 in mCRPC.




- pHER2/pHER3 IHC in patient tumor specimens showed 9/49 (18%) mCRPC tumors with concurrent pHER2/pHER3 staining compared to 1/18 (6%) of hormone-naïve PCa. An additional 4/49 (8%) mCRPC tumors stained positive for pHER2 alone, possibly representing d16HER2 activity.




| | pHER2 (+) | pHER3 (+) | pHER2/pHER3 (+) | pHER2 Only |
|---|---|---|---|---|
| N = 49 | 13/49 (26%) | 9/49 (18%) | 9/49 (18%) | 4/49 (8%) |

## Conclusions

- HER2 overexpression and d16HER2 expression are associated with PCa progression, castration-resistance, and resistance to second-line hormonal therapy with abiraterone/enzalutamide.
- HER2-targeted therapy with irreversible TKIs is more effective at inhibiting CRPC growth than the reversible TKI lapatinib.
- Our data suggest that a subset of CRPC with aberrant HER2 signaling representing 20-25% of mCRPC patients may have molecularly targetable disease with HER2-targeted therapy.
- Further studies to investigate potential treatment with an irreversible pan-HER TKI such as neratinib in biomarker-selected mCRPC patients after resistance to abiraterone and/or enzalutamide is warranted.

## Acknowledgements

Supported by the Congressionally Directed Medical Research Programs - Prostate Cancer Research Program and Prostate Cancer Foundation.

## References

1. Gao et al., *Clin Cancer Res* 2016.
2. Shiota et al., *Oncotarget* 2015.
3. Feldman et al., *Nat Rev Cancer* 2001.
4. Robinson et al., *Cell* 2015.
5. Cancer Genome Atlas Research Network, *Cell* 2015.