PS2-10-02 : Liquid Biopsy Tracking of PI3K–mTOR Residual Disease Signatures in Metastatic HER2+ Breast Cancer

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Background

- Approximately **15–20%** of all breast cancers are HER2-positive.
- Associated with higher grade, faster growth, and increased likelihood of metastasis.
- HER2-targeted therapies have dramatically improved outcomes.
- *Early stage HER2+:* **~20–30%** of patients relapse within 10 years due to drug resistance.
- *Metastatic HER2+:* Nearly **all** patients relapse.
- **Critical need to understand resistance mechanisms for the development of novel therapeutic strategies to prevent recurrence.**

Materials and Methods

Stage IV HER2-positive metastatic breast cancer patients underwent liquid biopsy profiling. We applied digital pathology, global immune profiling, and flow cytometry to characterize biomarker expression and immune landscape features. Ex vivo drug screening with PI3K–mTOR inhibitors, including the brain-penetrant paxalisib, was performed to evaluate their effects on mesenchymal phenotypes, disruption of circulating tumor cell (CTC) clusters, and immune reinvigoration.

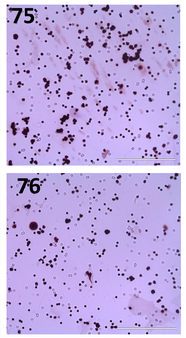
Results

1. CTC Clusters Enriched in Stage IV HER2⁺ Patients Despite Treatment Response

A *HER2+ Patient Summary*

	Clinical assessment	Treatment response
68	Widespread metastases	**Pre response*** ·1 month treatment
69	Minor brain progression	**Partial response**
72	No visible disease	Responding
73	No visible disease	Responding
74	No visible disease	Responding
75	No progressive disease	Responding
76	No visible disease	Responding
79	Progressive disease	**Not responding**

B *CTC cluster assay*



C *CTC cluster assay*

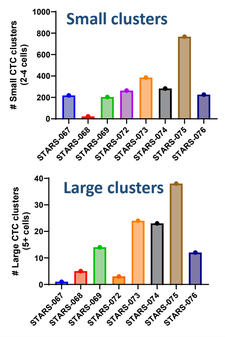
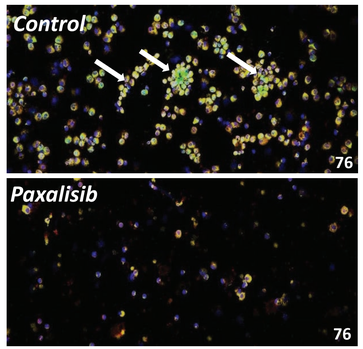


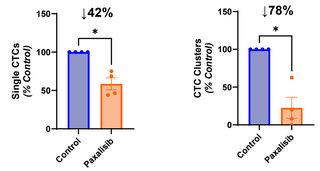
Fig. 1. (A) Clinical summary of HER2⁺ patients showing current treatment response status. (B) Representative Giemsa-stained images of CTC clusters isolated from patient blood samples. (C) Enumeration of CTC cluster subtypes, categorised as small (2–4 cells) and large (≥5 cells).

2. Paxalisib disrupts highly aggressive Vim⁺Snail⁺NRF2⁺ CTC clusters

A *Vimentin⁺Snail⁺NRF2⁺ CTC clusters*



B *Single CTCs* / *CTC clusters*



C *Phenotype*

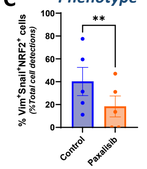
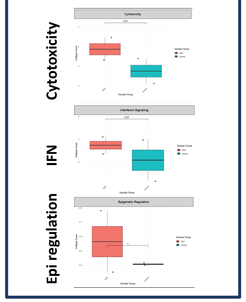


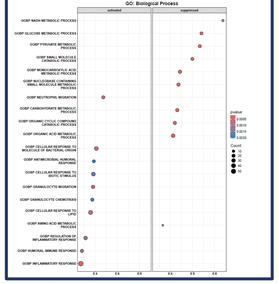
Fig. 2. (A) Representative immunofluorescence images of Vimentin⁺Snail⁺NRF2⁺ CTC clusters isolated from patient blood samples treated ± Paxalisib for 24 hr. (B) Quantification of single CTCs and CTC clusters (≥2 cells). (C) Digital pathology analysis of CTC cluster phenotypes.

3. Paxalisib-Treated CTCs Activate Cytotoxic-Linked Pathways Supporting Increased Immune Interaction

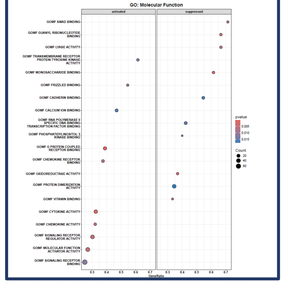
A *Pathway score analysis*



B *Biological Process enrichment analysis*



C *Molecular Function enrichment analysis*



- Paxalisib induces cytotoxicity, IFN, chemokine/cytokine activity, inflammatory response and epigenetic regulation pathways.

Fig. 3. (A-C) Nanostring RNA profiling of patient CTCs treated ± Paxalisib for 24 hr.

Results

4. Cytotoxic Dysfunction (Granzyme B/Perforin) Predicts Patient Treatment Response

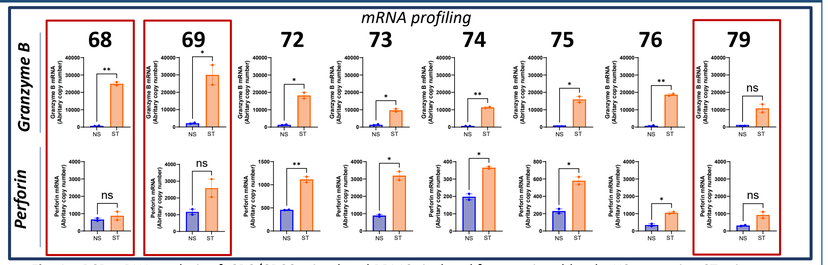
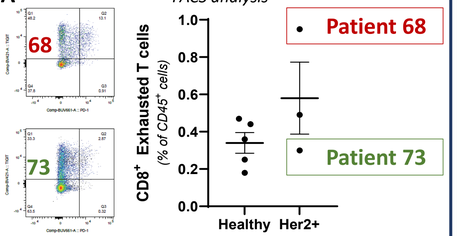


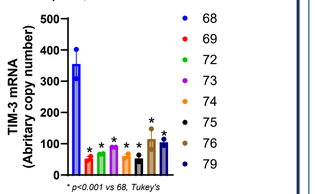
Fig. 4. qPCR mRNA analysis of CD3/CD28-stimulated PBMCs isolated from patient bloods; NS, non-stim, ST, stim.

5. Impaired Cytotoxicity Associated with Elevated T Cell Exhaustion

A *FACS analysis*



B *TIM-3 qPCR, Terminal exhaustion*



- Patient 68, ↑ T cell exhaustion compared to patient 73 with normal granzyme B and perforin induction.

*Tim3+ terminally exhausted T cells in all patients, particularly patient 68.

Fig. 5. FACS (A) and qPCR (B) analysis of PBMCs isolated from patient bloods.

Conclusions

Despite clinical response, Stage IV HER2+ patients retain a substantial burden of highly aggressive CTC clusters (key mediators of metastatic spread), likely contributing to the high rate of recurrence. Patients with partial or no response exhibited cytotoxic dysfunction, characterized by reduced induction of Granzyme B and/or Perforin. These patients also demonstrated an expanded exhausted T cell population, consistent with impaired anti-tumour immunity. Importantly, treatment with the PI3K–mTOR inhibitor, paxalisib, disrupted the therapy resistant Vim⁺/Snail⁺/NRF2⁺ CTC clusters and activated cytotoxic-linked pathways, supporting enhanced immune engagement. These encouraging findings warrant further clinical investigation.

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