Recurrent ciliary alterations under neoadjuvant selection are associated with inferior breast cancer outcomes

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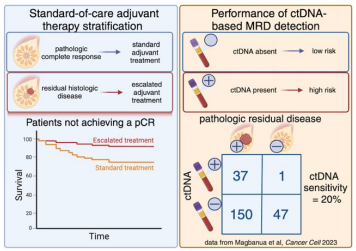
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BACKGROUND

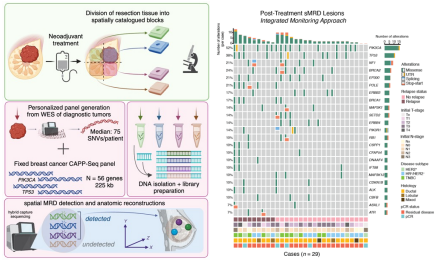
Defining post-neoadjuvant risk by detection of residual disease



- pCR status is currently used to define post-neoadjuvant risk but imperfectly predicts recurrence, resulting in over and under-treatment of many patients[1-4].
- ctDNA is falsely negative in most patients at the post-neoadjuvant timepoint who later recur, limiting its ability to inform adjuvant treatment[5-6].

APPROACH

Personalized, ultra-sensitive spatial MRD (sMRD) monitoring

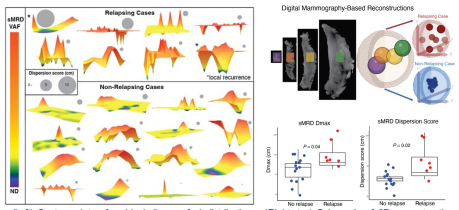


(Left) Schematic of approach; *(Right)* Co-mutation plot depicting the frequency of alterations in post-chemotherapy tissues aggregated by case, along with post-treatment pathologic evaluation and progression status.

- There is a key unmet clinical need to quantitatively evaluate chemotherapy response.
- We developed spatial MRD (sMRD), a novel personalized strategy for tissue-based MRD detection.
- We applied sMRD to 796 spatially-cataloged resection tissues from 29 breast cancers.
- We developed three-dimensional tumor bed reconstructions and described spatial patterns of chemotherapy response.
- We explored genes and pathways related to tumor bed spatial patterns.

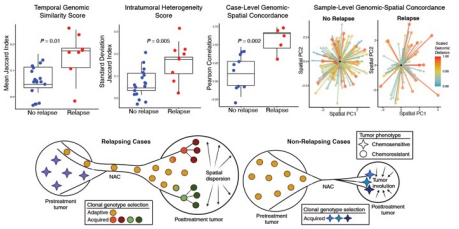
RESULTS

sMRD-defined treatment response patterns relate to recurrence



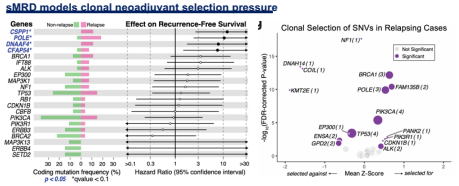
(Left) Contour plots of residual disease foci distributions; *(Right, top)* Schematic of 3D reconstruction method; *(Right, bottom)* Maximal distance between involved disease foci ("Dmax") and distribution through the tumor bed (Dispersion score), stratified by relapse status.

sMRD-defines geographic clonal evolution patterns



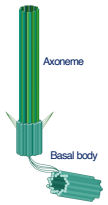
(Top) Clonal relatedness of post-treatment tumor to the pretreatment tumor, with correlation between clonal evolution and spatial dispersion. *(Bottom)* Models of neoadjuvant (NAC) clonal-genomic and spatial response by clinical outcome status.

sMRD models clonal neoadjuvant selection pressure



(Left) Recurrently mutated genes stratified by relapse status, with effect of mutations in given gene on recurrence-free survival; hazard ratios from proportional hazard model. *(Right)* Clonal distribution of single nucleotide variants (SNVs), in relapsing patients. A positive z-score represents a gene under positive selection, and a negative z-score represents a gene under negative selection.

The role of cilia in normal and tumor tissue



What are cilia and flagella?

- Cilia and flagella are microtubule-based, hair-like organelles that share structural elements – basal body, transition zone, axoneme
- Cilia – sense the environment, move cells/fluids, roles in signaling
- Flagella – locomotive, on spermatozoa, single-cell eukaryotes

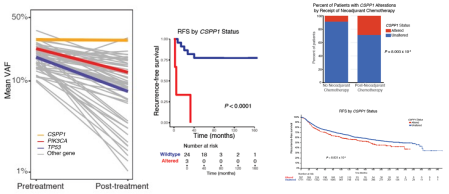
Ciliary function in *normal tissue*

- Cell antennae – for Hedgehog, WNT, PDGF, Notch pathways
- Roles in tissue patterning in embryogenesis
- Roles in cell polarity, mechanosensation
- On mammary gland lactiferous ducts – regulate branching, lactation

Ciliary function in *breast cancer*

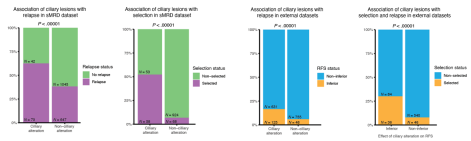
- Role in tumor initiation – percent of ciliated mammary cells reduced in early tumor formation, in pre-invasive and invasive lesions[7,8]
- Taxanes – induce elongated, morphologically abnormal cilia

CSPP1 alterations are associated with relapse and enriched post-chemotherapy



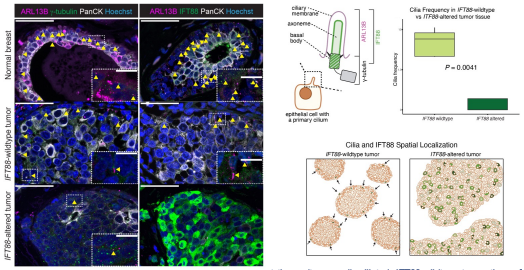
(Left) Comparison of mean VAF of selected genes before and after chemotherapy, highlighting the ciliary gene *CSPP1*; *(Middle)* Relapse-free survival by *CSPP1* mutation status in the sMRD cohort; *(Right, top)* Enrichment of *CSPP1* alterations in post-chemotherapy tumor in external datasets; *(Right, bottom)* Relapse-free survival by *CSPP1* mutation status in external datasets.

Additional ciliary alterations are associated with relapse and selection in sMRD cases and in external datasets



(Left) Association of ciliary versus non-ciliary alterations with relapse in the sMRD cohort and association of ciliary lesions with neoadjuvant selection. *(Right)* Association of ciliary versus non-ciliary alterations with relapse in breast cancer profiled in the cBioPortal, and association of ciliary alterations with post-neoadjuvant enrichment and with relapse-free survival (RFS). Inset numbers for each group and Chi-Square *P* values are provided.

Normal ciliation is lost in *IFT88*-altered tumor tissue



(Left) Immunofluorescence of ciliated, histologically-normal breast tissue (top panel); ciliated, IFT88-wildtype tumor tissue from a non-relapsing patient, with cilia highlighted at the tumor deposit periphery (middle panel); IFT88-mutated tumor tissue with minimal cilia and aberrant IFT88 expression in a relapsing patient. Images show nuclei (blue), epithelial cells (cytokeratin; white), cilia (pink), centrosomes (green, left panels), and IFT88 (green, right panels). In main images scale bar represents 50μm, in inset images scale bar represents 10μm. (Middle, top) Schematic of a primary cilia. (Right, top) Cilia frequency in post-treatment tumor of a non-relapsing IFT88-wildtype case and a relapsing IFT88-altered case, with Wilcoxon P value (two-sided) comparing cilia frequency as a percentage between patients, assessed in separate n = 19 tumor regions. (Right, bottom) Schematic of cilia and IFT88 spatial localization in an IFT88-wildtype versus -altered case. Black arrows in left panel indicate IFT88 colocalization with cilia.

CONCLUSIONS

Key findings:

1. We propose post-neoadjuvant spatial genomic features defined in the primary tumor bed reflect metastatic potential.
2. We link ciliary alterations with treatment resistance and relapse in our cohort and in external breast cancer datasets.

Implications:

1. Ciliary perturbations may not only be early breast cancer events, but also a mechanism of chemoresistance related to physical dispersion.
2. We hypothesize that cilia promote stromal invasion of tumor cells through aberrant cellular signaling, polarity, and mechanosensation.
3. Ciliary alterations may relate to altered PI3K and hedgehog pathway function and sensitivity to inhibitors[9].
4. We suggest the need for functional study of ciliary alterations.

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FUNDING

ASCO Conquer Cancer Foundation Young Investigatory Award (JDR)
Stanford Translational Research and Applied Medicine Grant (JDR)
Chan Zuckerberg Physician-Scientist Fellowship Award (JDR)
ECOG-ACRIN Paul Carbone, MD Fellowship Award (JDR)
Terri Brodeur Breast Cancer Foundation Award (JDR)
Stanford Cancer Institute Fellowship Award (JDR)
Donald E. and Delia B. Baxter Foundation (DMK)

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