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Tumor-released circulating orphan non-coding RNAs reflect treatment response and survival in breast cancer

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I-SPY The I-SPY Trials

Background

- Circulating tumor DNA (ctDNA) was recently shown to be a predictor of poor response and recurrence in breast cancer.
- ctDNA shedding from breast tumors rapidly decreases during treatment, resulting in reduced sensitivity in measuring tumor response[1].
- We recently reported the discovery of orphan non-coding RNAs (oncRNAs), as a large class of cancer specific small RNAs that are not present in healthy cells, but emerge from cancer cells[2].
- We hypothesized that oncRNAs provide an opportunity for a sensitive, rapid, and inexpensive liquid biopsy platform that does not require individualized assay development.

Annotating circulating orphan non-coding RNAs (oncRNAs):

- oncRNAs were annotated using small RNA sequencing across breast cancer cell lines, patient-derived xenograft models, and clinical samples (The Cancer Genome Atlas)[2].
- We discovered thousands of oncRNAs that are uniquely expressed in breast cancer (**Fig 1**) and largely absent in non-cancerous tissue.
- Analysis of extracellular compartment revealed that that oncRNAs are secreted by cancer cells (**Fig 2A**); and we detected oncRNAs in sera from breast cancer patients (**Fig 2B**).
- We have since annotated more than 250,000 oncRNAs across human cancers using data from the Cancer Genome Atlas.

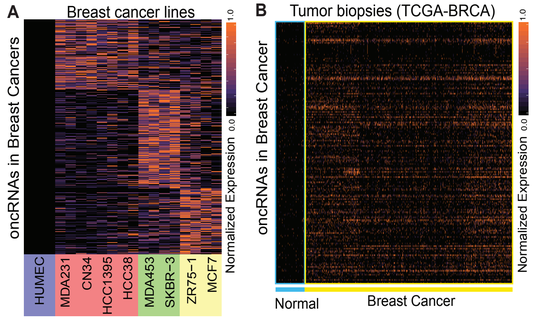

Figure 1. Discovery, annotation, and validation of cancer-specific orphan non-coding RNAs in breast cancer. (A) A heatmap representing the abundance of oncRNAs in breast cancer lines relative to HUMECs (red: TNBC, green: Her2+, yellow: luminal). **(B)** These oncRNAs were significantly expressed in breast tumor biopsies collected as part of The Cancer Genome Atlas (TCGA-BRCA), and were largely absent from the adjacent normal tissue collected from the ~200 individuals in this dataset. Figure adapted from (2).

Reference

1. Magbanua et al, *Ann. Oncol.* (2021) 32: 229.
2. Fish et al, *Nature Med* (2018) 24: 1743.

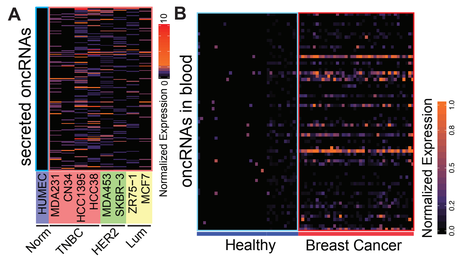

Figure 2. OncRNAs as digital circulating biomarkers. (A) OncRNA profiles in conditioned media of breast cancer cell lines **(B)** The detection of oncRNAs in sera from breast cancer patients with stage II/III disease. 35 healthy individuals from an independent study as reference. Figure adapted from (2).

Patients and Methods

- Patients received standard NAC only (n=147) or with MK-2206 (n=64) or Pembro (n=53). Sera (1mL) were collected pretreatment (T0) and prior to surgery (T3) (**Fig 3A**)
- A universal "**oncRNA fingerprinting**" test was developed by extracting total cell-free RNA and cataloguing circulating oncRNAs detected using small RNA sequencing (**Fig 3B**)

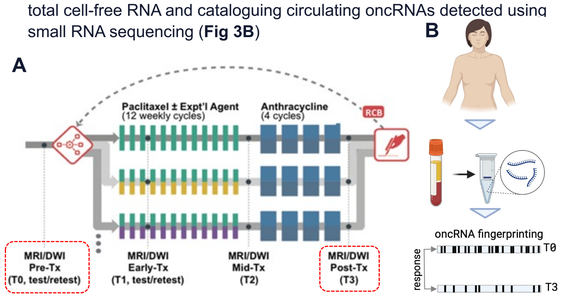

Figure 3. Study schema and sample collection in the I-SPY 2 TRIAL. (A) Study schema and sample collection in the I-SPY 2 TRIAL. from high-risk early breast cancer patients who received NAC +/- experimental agents (MK-2206 and Pembro) in the I-SPY 2 TRIAL. **(B)** A universal oncRNA fingerprinting approach, based on small RNA sequencing, was used to rapidly and robustly detect oncRNA species in ~1mL of sera.

Results

Rapid oncRNA fingerprinting in breast cancer patients:

- On average, we detected ~200 oncRNA species (per 10^6 reads mapped) in 1mL of serum from pre-treatment timepoints (T0; **Fig 4**).

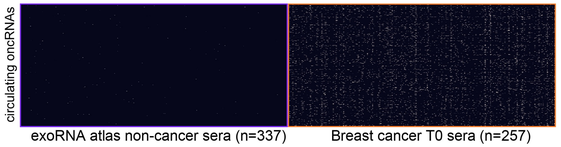

Figure 4. OncRNAs in breast cancer patients. A binary heatmap where rows indicate our annotated oncRNAs that were detected in one or more sera from breast cancer patients, and columns represent individual serum samples. (right) results for ISPY samples at T0, and (left) the same oncRNAs shown in non-cancer exoRNA atlas data.

oncRNA dynamics and clinical outcomes:

- Significantly fewer oncRNA species were detected in post-treatment samples across the arms tested (**Fig 5A**).
- oncRNA persistence in T3 relative to T0 (ΔoncRNA) was associated with higher disease burden post-treatment (**Fig 5B-C**).

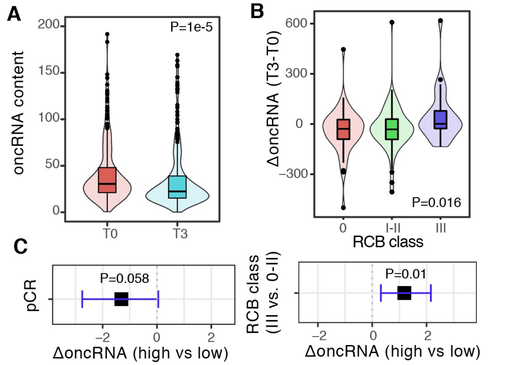

Figure 5. Changes in oncRNA content (ΔoncRNA) in response to therapies. (A) Summary of oncRNA content between T0 and T3 timepoints (Wilcoxon test). **(B)** Comparison of change in the oncRNA burden, post and pre-treatment, in every patient across the RCB classes. **(C)** Modeling short-term clinical outcomes as a function of changes in oncRNA burden. For pCR and RCB class (class III vs. class 0-II), the relative oncRNA values were first dichotomized, and logistic regression was then used to calculate coefficients, confidence intervals, and *p*-values.

- oncRNA persistence was significantly association with overall survival (HR=3.9, *P*<1e-3) and DRFS (HR=2.4, *P*=0.02) (**Fig 6A**).
- Higher oncRNA burden (ΔoncRNA) remained significant even after controlling for both pathologic complete response (pCR) and/or RCB classes in multivariate Cox proportional hazard models (**Fig 6B-C**; Log-Rank *P* of 0.004 and 0.0005, respectively).

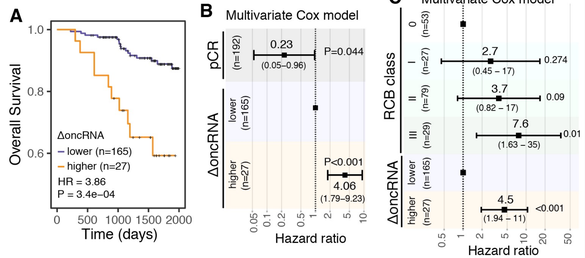

Figure 6. High oncRNA burden post-treatment is a predictor of patient survival in both univariate and multivariate models. (A) Overall survival analysis in patients with follow up information (N-192). The top 15% of ΔoncRNA values were most significantly associated with poor outcomes. The results are largely robust to the choice of threshold. Reported are the HR and Logrank *p*-values. **(B-C)** Forest plots for multivariate Cox models with ΔoncRNA and pCR or RCB class as covariates. oncRNAs remained a significant covariate even after controlling for these common clinical metrics.

Conclusion:

- oncRNAs provide a rapid (~4 days of processing), inexpensive, and robust approach to measure disease burden from <1mL of serum.
- Our results highlight that oncRNA clearance in response to treatment is prognostic across multiple arms.
- Our preliminary results indicate that even after controlling for known markers such as pCR and RCB class, oncRNAs remained prognostic.
- We will next explore integration of other key clinical predictors (e.g, MRI and tumor volume) to further evaluate the clinical utility of oncRNAs in treatment planning.

Advocate perspective: Liquid biopsies have emerged as effective, non-invasive, diagnostic tools in disease monitoring and minimal residual disease detection. While ctDNA has been shown to be a significant predictor of poor response and metastatic recurrence, small non-coding RNAs (oncRNAs), actively released into the blood by some tumors, may prove to be a more sensitive biomarker. Identifying oncRNA in blood over time (before, during and after treatment) can enable providers to predict tumor response to therapy. This simple way to get at disease burden through serum, which does not require individualizing a test for each patient, could be rapidly generated, and may provide the complementary, more sensitive information to other circulating DNA tests.

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