Identification of Biomarkers Involved in CDK4/6 Inhibitor Therapy Resistance and the Molecular Response to Treatment for Metastatic ER+/HER2- Breast Cancer

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Background

- While breast cancer in general has good 5-year survival rates, metastatic breast cancer specifically has a poor 5-year survival rate of ~25% [1, 2]
- Probability of metastatic recurrence rises every year after initial treatment ends; 20-30% of all early breast cancers will eventually experience a metastatic recurrence [2, 3]
- Primary treatment of ER+/HER2- metastatic breast cancer uses hormone therapy, but developing resistance is common and can be quick [4]
- Palbociclib, a CDK4/6 inhibitor (CDK4/6i) that halts cell cycle progression, significantly improves PFS in conjunction with standard hormone therapies used to treat ER+/HER2- metastases, but resistance is still inevitable [4, 5]
- Lack of verified biomarkers for palbociclib resistance [5]

Hypothesis

Metastatic ER+/HER2- breast cancer patients receiving therapy with CDK4/6 inhibitors present distinct molecular profiles that contribute to either acquired or intrinsic resistance to therapy. We hypothesize that second line treatment of palbociclib in combination with endocrine therapy such as fulvestrant will lead to worse outcomes compared to first line treatment of palcociclib and aromatase inhibitors such as letrozole due to different mechanisms of action that will influence the patient's molecular profile.

Methods

Analysis of Clinical Outcomes and Treatment Response

- 65 ER+/HER2- patients from MD Anderson's CDK4/6i cohort – a collection of metastatic breast cancer patients that received CDK4/6i therapy and then progressed – were selected for analysis
  - All 65 patients chosen received palbociclib as their CDK4/6i
  - All have had biopsies performed on treatment-naïve metastases; biopsies stored as FFPE slides
- The 65 patients were divided into 6 categories depending on PFS and the type of treatment received
  - Intermediate progressors could act as a unique group, or be split between early and late progressors
- The categories were used to create comparison groups for OS and PFS KM plots

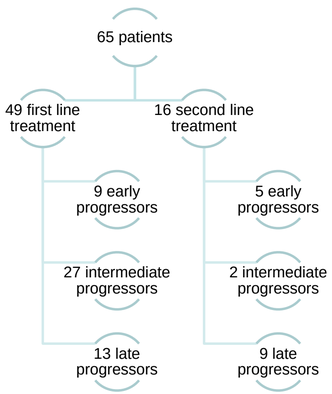

Figure 1. *Division of 65 cohort patients into the 6 categories used for comparison.* Note that early progressors can also be referred to as "intrinsic resistance" and late progressors as "acquired resistance"

Methods (cont.)

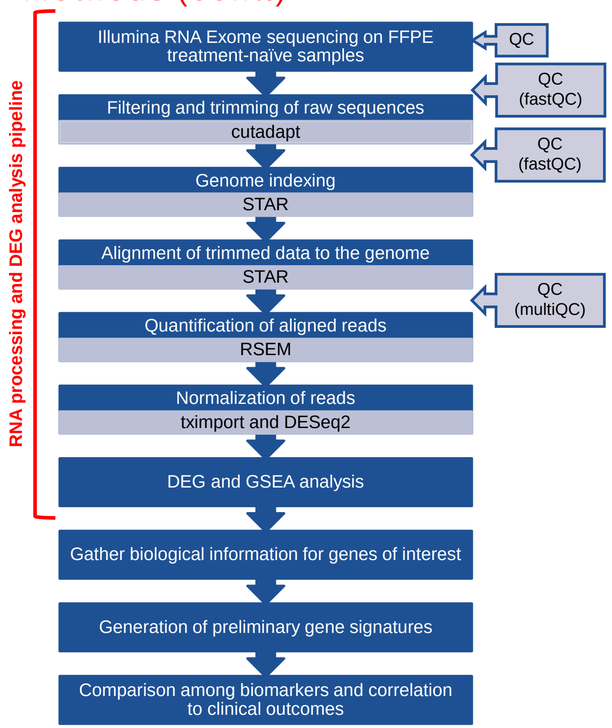

Figure 2. *Flowchart of gene analysis procedure.* Starts from Illumina sequencing and ends with testing of preliminary gene signatures. Indicates program(s) used when applicable.

Gene Analysis of Patient Cohort and Determination of Biomarkers

- RNA was extracted from patient FFPE slides and underwent Illumina RNA Exome sequencing
  - Sequencing method enriches known exon sequences; designed to work on degraded and/or low quality samples
- Sequencing data entered standard RNA processing and DEG analysis pipeline; note that while the overall steps are standard, the details, programs, parameters are not and depend on the specific research project's data and goals
- Due to low quality of samples, special emphasis placed on quality control (QC) to ensure highest sample quality possible

Results - Clinical

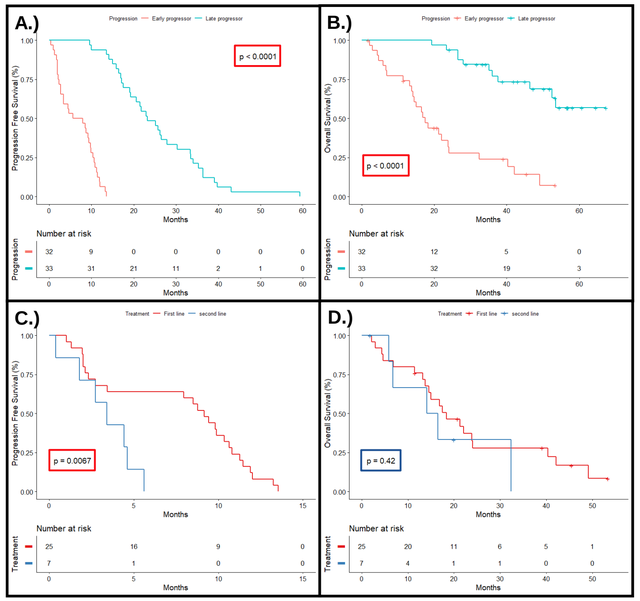

Figure 3. KM plots generated from clinical OS and PFS data using the N=65 palbociclib cohort. Significant p-values are boxed in red, non-significant are boxed in blue. All depicted plots have intermediate progressors split into early and late progressor groups rather than act as an independent third group.

A.) *PFS for early progressors vs late progressors* shows results which match expectations given that early and late progressors are defined by PFS.

B.) *OS for early progressors vs. late progressors* indicates that this significant difference extends to OS, with intrinsic resistance associated with significantly worse outcomes in patients.

C.) *PFS for early first line treatment vs early second line treatment* was the only significant KM plot among all first vs second line comparisons, and indicates that second line treatment (fulvestrant) may cause specific mutations that contribute to earlier intrinsic resistance than letrozole.

D.) *OS for early first line treatment vs early second line treatment* indicates that this difference in treatment is only applicable to PFS, and the two different treatment options will not affect overall survival.

Results - Gene Analysis

- All steps up to normalization of reads using DESeq2 have been completed
- Overall data quality appears good:
  - fastQC data reveals a majority of samples have good quality sequences with minimal overall contamination
  - Alignment rates match expectations based on previous literature using STAR alignment in conjunction with Illumina RNA Exome sequencing

Next Steps

- Complete normalization of quantified RNA-seq data using DESeq2
- Perform DEG and GSEA analysis on normalized sequencing data
- Look for patterns found in palbociclib cohort KM plots in the larger CDK4/6i cohort

Discussion

- There is a significant difference in OS based on acquired vs. intrinsic resistance
  - Suggests the existence of unique biomarkers that are characteristic of an intrinsic resistance phenotype
- While letrozole and fulvestrant might contribute to different PFS rates, they do not impact OS
- Fulvestrant might promote mutations that contribute to earlier intrinsic resistance than letrozole

References

1. NIH. (n.d.). *Cancer Stat Facts: Female Breast Cancer Subtypes*. National Cancer Institute. https://seer.cancer.gov/statfacts/html/breast-subtypes.html
2. Cardoso et al. (2018). *The Breast.* 39, 131-138.
3. Pan et al. (2017). *N Engl J Med.* 377, 1836-1846.
4. Finn et al. (2015). *Lancet Oncol.* 16(1), 25-35.
5. McCartney et al. (2019). *Front Oncol.* 9, 666.

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