**HER2 Project – Circle of Giving Grant**

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ASB Methods Log

Updated 07/02/21

**A1 Prioritizing HER2 mutations in Breast Cancer**

Mutation data for 6,664 breast cancer patient samples were downloaded from cBioPortal (1,2) across a total of 13 breast cancer studies (3–15). At the time of download, the public cBioPortal used the hg19/GRCh37 version of the reference genome. Mutations in cBioPortal are assumed to be on the (+) strand unless otherwise noted by dataset depositors. Note for data set citation, using Pan Cancer TCGA (cited hoadley and berger), waiting to hear about citation for the Metastatic Breast Cancer Project (early data available through cBioPortal.

A total of 97 ERBB2 alterations across 222 samples were collected, including single nucleotide polymorphisms and small insertions and deletions. We required SNPs to be missense or splice-site and included both frame- shift and in-frame alterations. For each alteration, we generated a primer sequence (programmatically in R) to enable primer generation for the mutation screen platform.

**A2 Candidate Resistance Driver Genes in HER2 Breast Cancer**

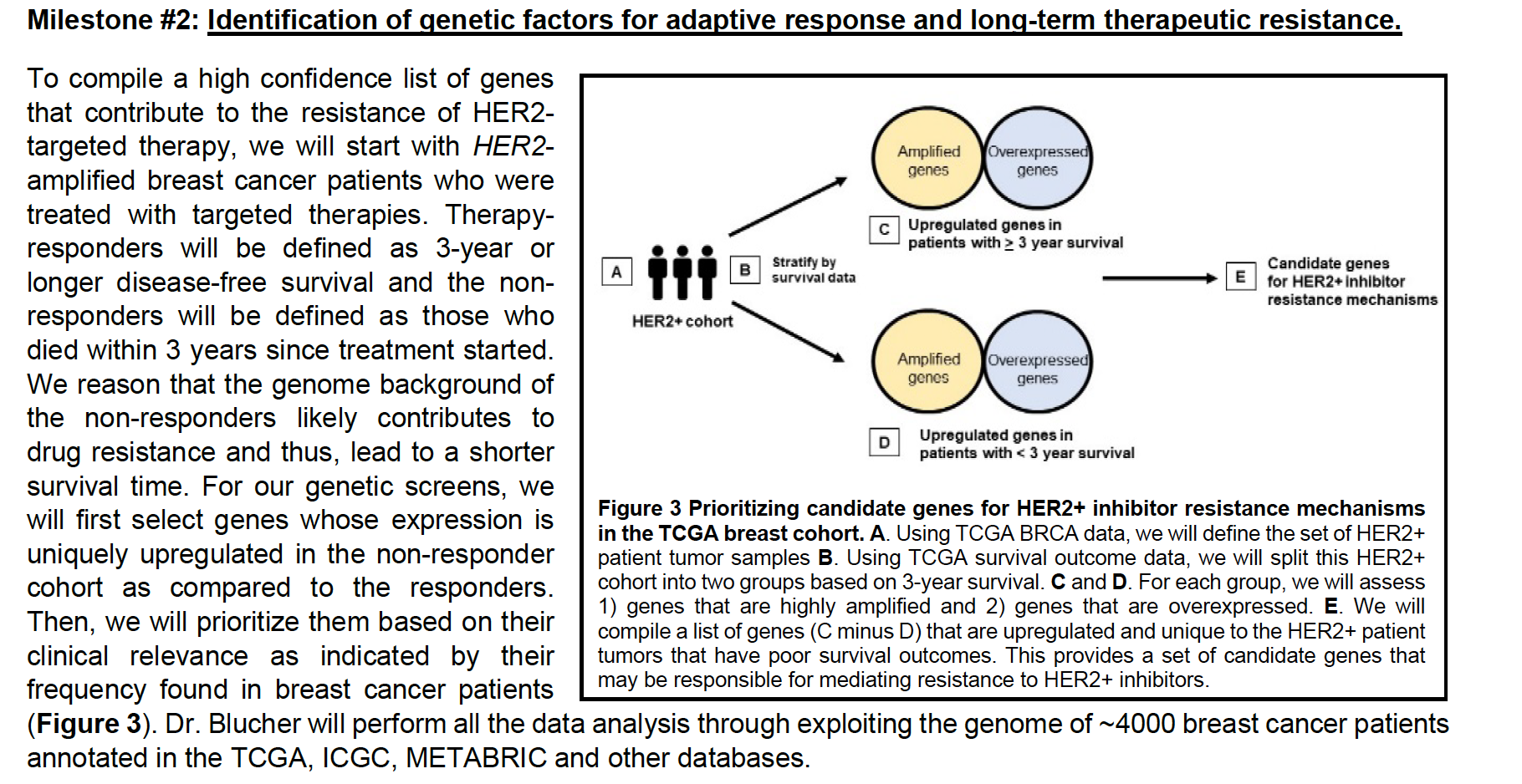


Figure 0. Fm Samuel’s grant application to OHSU Circle of Giving. Aim 2 Overview for

prioritizing candidate resistance drivers in HER2 breast cancers.

-TCGA breast~~, pull all patients with HER2 indicator via IHC~~

Gistic, ERBB2 =2, amplified; note these may be different thresholds (n=133 total)

\*can check against PAM50 if time / check against IHC\* if time

-pull survival data, plot distribution, break into <3 years versus >=3 years or similar; see if there is a break

Samples >3 years, 54 total

Samples<=3, 79 total



**Figure 1.** Histogram of survival time for TCGA BRCA HER2 positive patients. Blue dash lined indicates 3 years (1095 days).

-identify top copy number features DE between two groups

Counted number of (GISTIC==2) and compared between 2 groups with proportions test

Removed any genes with <5 samples in the Short group

Note, prioritized list does not have any CN genes passing FDR<0.20

-identify top genes DE between two groups

Using TCGAbiolinks package/ for querying RNASeq RSEEM (expected counts)

Run DE through the package, see notes in R script:

*HER2\_Aim2\_CandidateResistanceDrivers.Rmd*

~~Prioritized list of 103 genes with negative fold change (i.e. overexpressed in Short group)~~

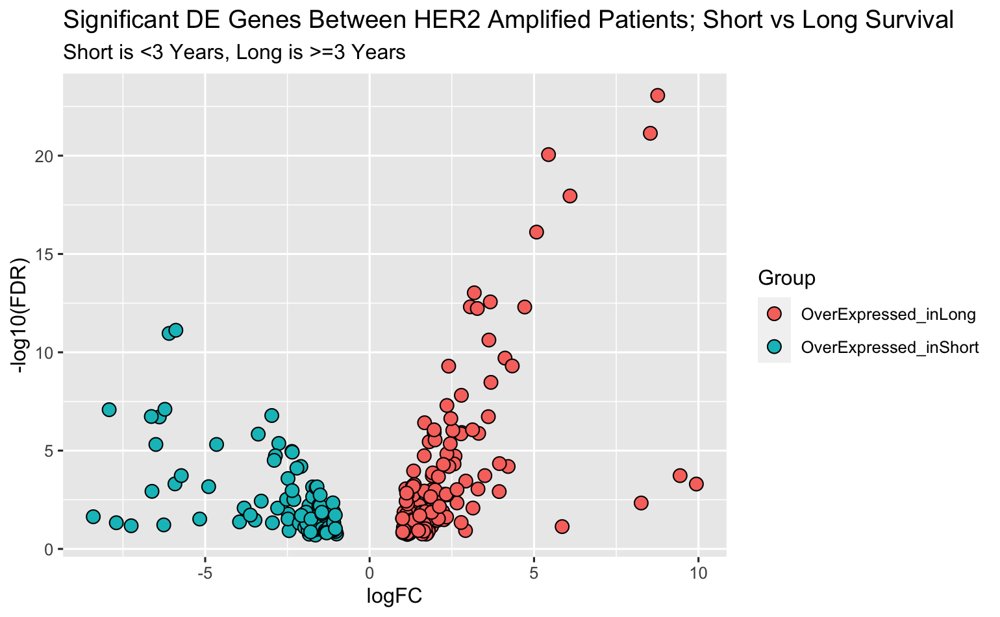
~~Run list against Samuel’s library; 83 found in library, 20 to make~~ Updated 07/02/21

Prioritized list of 135 genes that are up-regulated in the Short survival

Of these, 104 are under FDR< 0.20, but list is extended out to FDR <0.50 as Samuel has room for ~100 genes on his screening panel.

See workbook *Table\_ShortVsLong\_UppQuantNorm\_DE\_FoldChange\_FDR\_ShortHits\_LibraryAnnotation\_210625.xlsx*

Of these, 111/135 are annotated in Samuel’s existing clone library. List to Samuel 06/25/21 for Aim 2 drug screen work, he will pull clones from library and assess how many need to be made for the rest of the screen. In total, he has capacity to screen about ~100 genes.



**Figure 2.** Volcano plot showing differentially expressed genes between two groups of HER2 breast cancer patients in TCGA (TCGA BRCA). Blue indicates genes are over-expressed in the ‘Short survival’ cohort, with reported survival <=3 years.

**References**

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