# Investigation of a genomic signature for transcription factor MAF gene amplification and lack of bisphosphonate benefit in early breast cancer

ASCO®

Abstract #559

**Azadeh Nasrazadani**[1], Juan Luis Gomez Marti[2], Tara Hyder[2], Sahra Uygun[3], Lisa Eileen Blumencranz[3], Lorenza Mittempergher[4], William Audeh[3], and Adam Brufsky[2]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX; [2]University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA; [3]Agendia, Inc., Irvine, CA; [4]Agendia NV, Amsterdam, Netherlands

## BACKGROUND

Bisphosphonates are utilized for breast cancer patients with bone metastasis to prevent skeletal complications. The role of these agents is less established in the non metastatic setting. In post menopausal patients with Stage II or III breast cancer, the presence of MAF amplification has been associated with shorter invasive disease free survival and overall survival[1]. Conversely, patients lacking MAF amplification in the primary tumor are more likely to benefit from adjuvant bisphosphonates as shown in a retrospective analysis of the AZURE trial[2] and confirmed with a subset of NSABP-B34 specimens[3]. We sought to identify a genomic signature associated with MAF amplification, which could guide patient selection for use of adjuvant bisphosphates. As MAF amplification is associated with high risk of bone metastases, 70-gene risk of distant recurrence signature (MammaPrint/MP) and 80-gene molecular subtyping signature (BluePrint/BP) were used to stratify the patient groups.

## METHODS

A total of 166 breast cancer patients treated at UPMC with genomic information available from the FLEX registry (ClinicalTrials.gov Identifier: NCT03053193) were included in this pilot cohort. Fluorescence in situ hybridization (FISH) was performed on primary breast tumor tissue to detect MAF copy number. Signal-to-nucleus ratio (SNR) of ≥2.5 was used as the MAF-amplified (MAF+) cut-off. Differential gene expression analysis was performed with R limma using whole genome microarray data.

MAF+ and MAF- status (SNR<2.5) was compared within all patients (20 vs 146) and within patients matched by MP/BP to balance high risk groups (20 vs 20). Differentially expressed genes (DEGs) were defined as absolute fold change ≥2 and adjusted p-value <0.05 for all patients and as absolute fold change ≥2 for matched comparison.

Prediction of MAF amplification based on gene expression was performed using a correlation-based metric using:

- 166 patients from the UPMC discovery set
- 1179 patients from the FLEX Study (non UPMC patient samples)

## RESULTS

**Table 1:** Among the 166 UPMC breast tumors:

- 20 (12%) were MAF+ and 146 (88%) were MAF-
- 19 out of 20 (95%) MAF+ patients were MP High Risk, as expected from the association of MAF amplification and bone metastasis, as opposed to 42 (29%) MAF- patients.
- There were more BP Basal and HER2 subtypes within MAF+ compared to MAF-

**Figure 1 and Figure 2**: Comparing whole transcriptome of all MAF+ and MAF- samples, 48 DEGs were found. Genes with top fold changes were labelled in Figure 1. From the MP/BP matched comparisons, there were no genes with adjusted p-value <0.05 due to statistical power (20 vs 20 comparison), therefore 9 genes ≥2-fold change were included in the final set of 57. C-X-C motif chemokine ligand and S100 calcium binding protein encoding genes were enriched in the final set.

**Table 2A:** The 57-gene classifier of MAF status yielded 92% accuracy, 94% specificity, and 75% sensitivity on the training set. Columns are FISH results and rows are classifier results.

**Table 2B**: When the classifier was applied on the independent FLEX cohort, 11.5% MAF+ cases were identified, similar proportion observed in the training set (12%).

## TABLES and FIGURES

**Table 1. Characteristics of the patients from the UPMC discovery set.**

| | MAF + | MAF- | *p*-value |
|---|---|---|---|
| **Age** | | | |
| *Mean (yrs)* | 58.7 | 56.8 | 0.45 |
| **MammaPrint (MP)** | | | |
| *High Risk* | 19 | 42 | 1.01E-08 |
| *Low Risk* | 1 | 104 | |
| **BluePrint (BP)** | | | |
| *Luminal* | 12 | 140 | 1.74E-05 |
| *Basal* | 2 | 3 | |
| *HER2* | 6 | 3 | |

**Table 2. Contingency table and concordance metrics from MAF status predictions**

**A. UPMC discovery set (n = 166)**

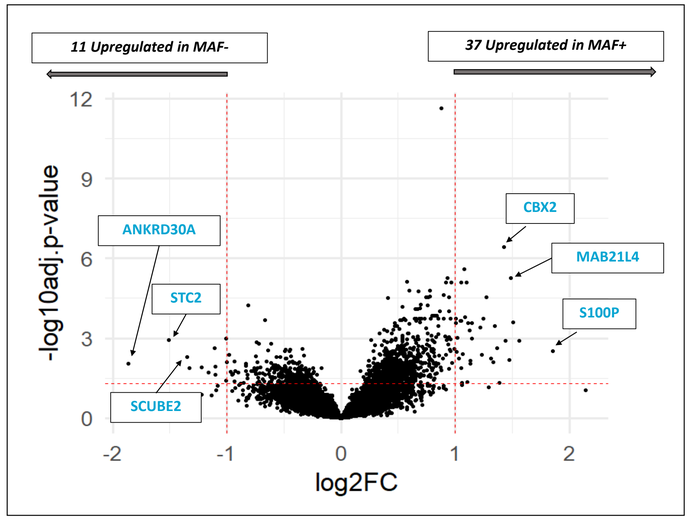
| 57-gene classifier | FISH MAF+ | FISH MAF- |
|---|---|---|
| MAF+ | 15 | 9 |
| MAF- | 5 | 137 |

| Metric | Value |
|---|---|
| Accuracy | 91.6% |
| Sensitivity | 75.0% |
| Specificity | 93.8% |

**B. Independent FLEX cohort (n = 1179)**

| | # of samples | % of samples |
|---|---|---|
| MAF+ | 136 | 11.5 |
| MAF- | 1043 | 88.5 |

**Figure 1. Volcano plot comparing MAF + vs MAF- cohorts**



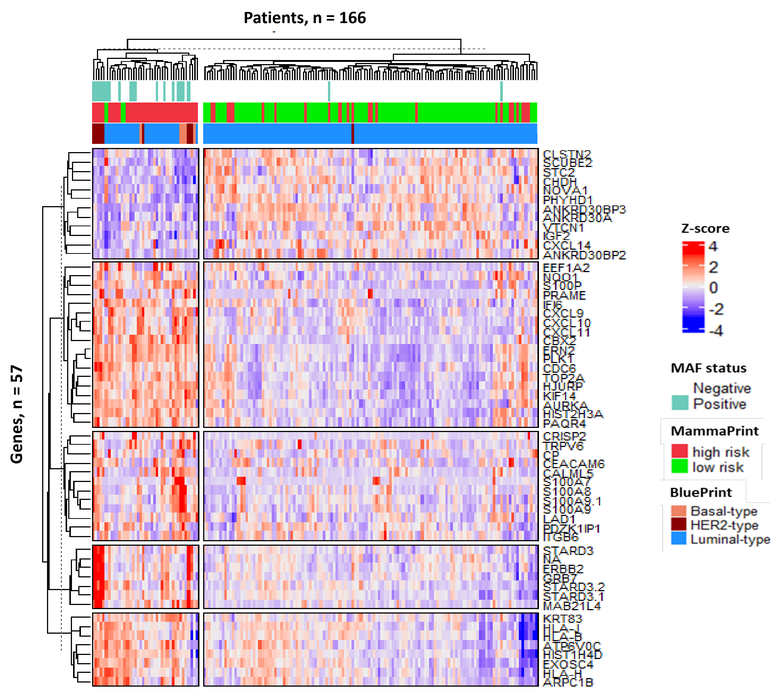

**CORRESPONDENCE TO**:
Adam Brufsky, MD PhD brufskyam@upmc.edu

REFERENCES

1. Pavlovic et al 2015 *J Natl Cancer Inst*
2. Coleman et al 2017 *Lancet Oncol*
3. Paterson et al 2021 *JNCI Cancer Spectr*

## TABLES and FIGURES continued

**Figure 2. Heatmap of selected genes from DEGs**




## CONCLUSION

**Whole transcriptome analysis shows that breast cancers with MAF amplification are transcriptionally different than those without. A set of 57 genes could potentially predict MAF amplification status, which could guide patient selection for bisphosphonate utilization in the adjuvant setting.**

## FUTURE DIRECTIONS

- We plan to confirm MAF amplification status utilizing a validation set and further explore the predictive value of the genomic signature in response to bisphosphonate treatment and consider its utility to guide patient care