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To: Dr. Francesco Marincola
Editor in Chief, Journal of Transitional Medicine

Dear Dr. Marincola,

On behalf of my co-authors, I am submitting the manuscript titled **"The tumor immune microenvironment of primary and metastatic HER2- positive breast cancers utilizing gene expression and spatial proteomic profiling"** for consideration for publication in The Journal of Transitional Medicine. The abstract titled "Digital spatial profiling in HER2 positive breast cancer: The road to precision medicine" was presented in the AACR annual meeting (tracking number 21-A-1959-AACR).

The rationale for this study is that even though the outcomes for patients with HER2-positive breast cancer are improving, there is still an unmet need to prevent recurrences in patients with early stage breast cancer and to achieve more durable responses in those with metastatic disease. There is going interest in understanding if there is a role for immune checkpoint inhibitors in the HER2-positive breast cancer subtype. A better understanding of the tumor biology may help us elucidate which patients may benefit from these therapies and the optimal combinations, line of treatment and, stage. We selected 15 samples of eight patients with primary and metastatic HER2-positive breast cancers and performed gene expression profiling and GeoMx Digital spatial profiling (DSP). We found that primary tumors had higher numbers of immune cells compared to metastases. Primary breast tumor samples also had higher expression of immune activation and checkpoint markers when compared with metastatic samples. Our findings suggest that there may be a role for immune checkpoint inhibitors in early stage HER2-positive breast cancer.

To our knowledge, this is the first study specifically utilizing digital spatial profiling in HER2-positive breast cancer primary and metastatic breast cancer and although it is a limited sample size, we performed extensive analyses of the samples which allowed us to see not only the characteristics or the tumor immune microenvironment, but also protein expression and PAM50 intrinsic subtypes. We also were able to see differences in paired tumors of patients with HER2 positive disease.

All this material is original and has not previously been published in another journal. The manuscript was read and approved by the authors. Thank you in advance for your consideration of our manuscript. We believe the readership of The Journal of Transitional Medicine will find it of high interest.

Best regards,

Sandra M. Swain, MD, FASCO, FACP
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