Progress Report (March. 30, 2015)

Title: Identification and Targeting of Kinases Involved in Breast Cancer Brain Metastasis

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Our goal in this project is to identify and target kinases involved in breast cancer brain metastasis, and we have made significant progress thus far in the identification of kinase driver genes. As reported in Sept. 2014, we observed that 30 kinase sequences were enriched an average of at least 30% in the *in vivo* brain metastasis samples, and termed these "candidate driver kinases." Under the support from METAvivor, over the past two quarters, we have sharpened our focus in our search for druggable breast cancer brain metastasis targets. After developing the list of 30 candidate kinases from our *in vivo* screen, we sought to prioritize the list into those most likely to be true driver proteins responsible for metastatic growth.

First, we collaborated with Drs. Sunil Lakhani and Jodi Saunus at the University of Queensland to analyze the expression of the candidate kinases in brain metastasis. They have a precious collection of patient brain metastasis specimens, and they tested all of those specimens for kinase transcript expression. They confirmed that all 30 kinases were indeed expressed in the brain metastasis specimens and ranked our "candidate driver kinases" according to their expression.

Next, in order to further prioritize which kinases to test in mice, we used the method described in Guo et al (1). In short, they showed that growth of breast cancer cells in a hard agar assay *in vitro* is predictive of ability to form brain metastasis. By this method, we have been able to further prioritize our list of "candidate driver kinases", and by combining the hard agar assay data with the data of kinase expression in patients brain metastasis (see above), we have further prioritized the list of candidate kinases for validation in mice.

As we discussed in our previous progress report, since our initial candidate kinase list was generated in a pooled screen, it is necessary to validate all candidates individually before considering them as potential targets. Previously, we attempted to validate the top eight candidates (based only on fold enrichment in our *in vivo* screen, without the patient or hard agar data). We overexpressed eight kinase sequences respectively in luciferase-labeled MDA-MB-231 triple negative breast cancer cells and injected the cells into nude mice via the carotid artery, using empty vector-expressing cells as controls. Unfortunately, we discovered at the termination of the experiment that no brain metastasis had formed, even in our control mice. When we examined the brain tissues of the mice, we discovered CD3-positive T cells in the brains, which should not have been present in nude mice, suggesting the mice we received had problems. After performing troubleshooting, we are now repeating these mouse experiments with mice from an alternative supplier to prevent the immune rejection of tumor cells and avoid similar delays in the future.

At the same time, we have assayed a panel of breast cancer cell lines in order to determine the endogenous expression of our top candidate kinases. We have selected two cell lines derived in our lab from experimental brain metastasis *in vivo* (231.Br, and BT-474.Br3) as they represent triple-negative and HER2+ breast cancer, respectively. We have knocked down

the expression of the top candidate kinase identified in the screen in these cell lines and will perform experimental brain metastasis assays to determine whether reduction of the level of this kinase can slow or stop brain metastasis growth. If so, it would indicate that this kinase is not only a driver of brain metastasis growth, but that its inhibition may serve as an effective therapy. Once we have performed the overexpression validation on the other top candidates, we will perform knockdown studies as well to determine whether they may also serve as potential therapeutic targets.

To **summarize** the progress in the past two quarters, our collaboration empowered us to acquire kinase expression data from patients' breast cancer brain metastases, which ensure that the kinases we are pursuing are indeed clinically relevant targets. We have combined this expression data with kinase-driven hard agar tumor cell growth *in vitro* data and with our *in vivo* kinase screen enrichment data to rank and prioritize the kinases that will be further validated and targeted *in vivo*. While we experienced unexpected setbacks in the form of immune cell infiltration in the brains of the used nude mice, we have since obtained mice from a different vendor and begun repeating the in vivo validation, and the top kinase candidate-overexpressing cells have been injected into mice and are being monitored for growth and survival differences compared with controls. We have generated knockdown cell lines for the top candidate kinase and will expand both the overexpression and knockdown experiments based on candidate kinase ranking.

References:

Guo L, Fan D, Zhang F, Price JE, Lee JS, Marchetti D, Fidler IJ, and Langley RR. Selection of brain metastasis-initiating breast cancer cells determined by growth on hard agar. Am J Pathol. 2011 178(5):2357-66.