Memorial Sloan Kettering
Cancer Center

November 16, 2021

Dear Andrew and Friends:

The steady, generous support of the Cure Breast Cancer Foundation has enabled our team to make major strides in advancing our knowledge of the science of breast cancer, all directed at better diagnosis, therapy, and eventually prevention of this and related diseases. The work started with the discovery that cancer is not just a disease of abnormal cancer cell division (growth), but also cell mobility (metastases, including cells circulating in the blood and returning to their site of origin). When these cancer cells move through the blood system, they bring white blood cells (leukocytes) with them as they lodge in tissues as seeds for tumor formation. This discovery led us to study the nature of the leukocytes, which we then discovered to have mutations (DNA changes), called *clonal hematopoiesis* (CH). We also became interested in a molecule called *p21*, which helps cancer cells move through the system. This past year has seen considerable progress in these investigations.

The MSK CH Program has led studies investigating the frequency and clinical relevance of CH in MSK patients with advanced solid tumors including breast cancer. This includes studies of the frequency and clinical relevance of CH in breast cancer. The MSK patient population represents an ideal resource for detailed investigation into CH, its influence on the biology of solid tumors, the risk for progression of CH cells to leukemia, and implications of CH for other diseases of aging that occur within the cancer population.

In 2018 we launched at MSK the first comprehensive clinic for cancer patients with CH, and we continue to lead the field in investigating the relevance of CH to patients with breast cancer and other common malignancies. We have assembled a team of leaders in the fields of CH and hematologic malignancies, single cell sequencing, and breast cancer biology. Our team of laboratory, computational, and clinical investigators is ideally suited for collaborative, translational studies into CH aimed to functionally investigate how CH alters breast cancer biology and therapeutic response and aim to identify novel therapeutic targets in CH in solid tumor patients. In this regard we have organized our research into six specific aims.

> Specific Aim 1: We will perform state-of-the-art single cell genomic/cell surface profiling of CH clones, wild-type (*i.e.* normal) hematopoietic cells, and solid tumor cells within the tumor's micro-environment. We will use these studies to delineate the potential role of key mutant hematopoietic subsets in cancer-immune cell interactions

Specific Aim 2: We will develop and use genetically accurate preclinical models to mechanistically investigate the interplay between CH and solid tumor cells including in the context of metastatic progression.

Specific Aim 3: To define the impact of CH, including those that infiltrate the tumor (mutant TILs), on the response of triple-negative breast cancers to chemotherapy and immunotherapy.

Specific Aim 4: To perform *in vitro* screening and *in vivo* testing of epigenetic and immunomodulatory drugs with known activity in hematologic malignancies in order to identify therapeutic targets which attenuate CH and which enhance the response of the solid tumor/immune microenvironment to tumor-directed therapies.

Specific Aim 5: To identify the clinical and CH molecular features associated with the development of secondary leukemia in breast cancer patients in order to develop a clinically tractable model to predict secondary hematopoietic malignancies.
Specific Aim 6: To explore the reasons why mutations on leukocytes and in other non-malignant tissues, including ductal carcinoma in situ (DCIS) of the breast, do not always progress to frank malignancy.

Single cell sequencing platforms, including the MissionBio Tapestri and the 10x single cell sequencing platforms are available in the laboratories of Drs Ross Levine and Jorge Reis-Filho laboratories and the protocols for both liquid samples and solid tumor DNA and RNA sequencing have been fully established. The methods to be employed for Specific Aims 2 and 3, includingC5TBL16J CH mouse models with SCL:CreERT2 deletion of Dmnt3a, Tet2 and Asxll with a TdTomato reporter, are fully established in the Levine laboratory. Samples for the studies proposed in Specific Aims 1 and 4 are being collected under MSKCC IRB approved protocols and available in the Precision Pathology Biobanking Center (Department of Pathology) and the Reis-Filho laboratory. The MSK Clonal Hematopoiesis Program (Drs Levine, Larry Norton, Elizabeth Comen, and Reis-Filho) provides a unique opportunity to follow solid tumor patients with CH, to bank serial samples, and to support discovery science and cutting-edge trials aimed to prevent secondary malignancies.

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As described in our last report, Dr. Rachel Hazan at the Albert Einstein College of Medicine has found that the cellular molecule p21 plays an unusual role in breast cancer metastasis by promoting cell migration while inhibiting cell division. She discovered that p21 levels go up during chemotherapy, thereby encouraging tumor cells to become seeds and metastasize to distant organs. Furthermore, she found that p21 promotes seeding by increasing the production of a molecule called TCFI that in turns activates a molecule called Wnt and thence cyclin Dl.

Over the last year, she made a leap in our understanding of TCF1 function in cancer metastasis. Using mouse and human breast cancer models, she showed that TCF1 promotes cancer stemness—the most primitive state of a cell—through striking regulation of the epithelial to mesenchymal transition (EMT) process. The E stands for epithelial, the state in which cells do not more but are capable of mitosis. The M stands for mesenchymal, the mitosis-free state of cell mobility. But challenging previous views of EMT she has shown that TCF1 shifts tumor cells towards a hybrid epithelial/mesenchymal (E/M) state rather than a pure mesenchymal state. This E/M population was shown to carry stemness and metastatic properties and to be interestingly represented by a small "stem-like" subpopulation in triple negative breast cancer (TNBC) cells.

Based on characteristic cell surface markers, she isolated E/M cells from TNBC cells and show that both TCF1 and another gene called Snail were highly enriched in this population. Consistent with posttranslational regulation of Snail by Glycogen Synthase 3 (GSK3), she also found that the E/M subcellular fraction of TNBC contained high levels of phosphorylated GSK3. On this basis we are now hypothesizing that the TCF1 potentiation of Wnt signaling by p21 results in GSK3 phosphorylation (which inactivates GSK3), thereby inhibiting Snail phosphorylation and ubiquitination, resulting in Snail protein stabilization and upregulation. This is a very important discovery in that it means that inhibitors of GSK3 may be used as medicines to suppress Snail and E/M cells, which in turn may stop them from metastasizing. This work will proceed following three specific aims:

> Specific Aim 1: Determine whether E/M cells promotes cancer stemness and metastasis in the laboratory and the clinic.

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Specific Aim 2: Determine whether GSK3 inactivation by the activity of TCF1 results in Snail upregulation. If so, this will provide further novel therapeutic targets.

Specific Aim 3: Determine whether therapeutic inhibition of GSK3 kinase attenuates stemness and metastasis in TNBC models and, eventually, in clinical trials.

Hence, in summary, CBCF support is enabling us to better understand both the relationship between cancer cells and white blood cells and the regulatory mechanisms within the cancer cells that underly cell mobility. Stopping cancer cell mobility could stop tumor growth (via interrupting self-seeding) and metastases (distant seeding), making tumors shrink and preventing them from forming potentially lethal metastases. These groundbreaking experiments are moving us closer to our goal: the eradication of breast (and other) cancers as a cause of human suffering and death. For the physicians and scientists whom you support I extend our gratitude and our appreciation for your unswerving dedication to this noble mission.

Sincerely,

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