**Lay Progress Report**

**Year 1 (07/01/2020 – 06/30/2021)**

We wish to determine how BrCa cells that have spread from primary tumors are able to gain a foothold in distant tissues to which they are poorly adapted at the moment of their arrival in such tissues. We believe that such lack of adaptation forces these metastasized cells to enter into a non-proliferating (dormant) state in such tissues from which they can only emerge when and if they have developed adaptations that allow them to thrive in such tissues. On rare occasion, some of these cells contrive a solution to adaptation and, as a consequence, begin to proliferate and ultimately generate clinical relapses driven by life-threatening macroscopic metastases. We propose that in order to do so, disseminated BrCa cells must undertake a period of proliferation during which they diversify the spectrum of genes that each of these cells expresses (that is, their “transcriptomes”), yielding variant cells, some of which are now capable of autonomous proliferation, yielding large metastases. Wishing to test the predictions of this proposed mechanism, we have successfully generated experimental tools for forcing in vivo proliferation and transcriptional diversification of initially non-proliferating breast cancer cell lines and we are on the verge of developing an experimental system that facilitates the tissue-adaptive evolution and metastatic colonization by previously non- proliferating BrCa cells. This experimental setup will offer a versatile platform for understanding the molecular mechanisms underlying metastatic “colonization” (that is, the formation of actively proliferating metastatic BrCa cell colonies), the steps of which are currently not very well understood. More importantly, success here will provide a versatile tool for other researchers studying metastatic colonization. It will allow, for the first time, a systematic study of a spectrum of tissue-adaptive ‘solutions’ that cells from various subtypes of BrCa devise in order to colonize distant organs. Most importantly, elucidation of these altered gene expression (that is, “transcriptional”) programs may reveal specific therapeutic targets whose pharmacologic inhibition will prevent the outgrowth of disseminated BrCa cells in distant tissues, as well as potentially halt further growth of disseminated but dormant BrCa cells and possibly also reverse the growth of already-established metastases by attrition of such cancer cells.

**Year 2 (7/1/2021-6/30/2022)**

A relatively high proportion of breast cancer-associated mortality is associated with patients whose primary tumors are deemed cured with no obvious residue of cancer cells in their bodies but who confront metastatic relapses erupting months and years after their initial treatment. Such recurring tumors are due to micrometastatic deposits in various tissues around the body that were dispatched by their primary tumors prior to the removal of the latter. These micrometastatic deposits, composed of single or small clumps of breast cancer cells, are not detectable by current methods and are unlikely to be detectable by newer methods to be developed over the coming decade. These disseminated breast cancer cells vastly exceed the number of metastatic outgrowths that occur upon metastatic relapse, indicating that the great majority of the initially disseminated breast cancer cells have entered into non-proliferating states, thereby representing the phenomenon of “metastatic dormancy”. Such dormant breast cancer cells can remain so for months and even years while retaining viability, that is, the ability under the proper conditions to re-initiate active proliferation and generate new tumors, notably metastases. There has been little information available to reveal how and why dormant disseminated breast cancer cells are “awoken” and thereby placed in a state where they can launch active proliferation and the outgrowth of readily detectable, life-threatening metastases. The presently proposed research investigates a phenomenon solidly demonstrated in the applicant’s laboratory in which the inflammation of the tissue surrounding disseminated dormant breast cancer cells provokes these cells to emerge from dormancy and enter into a cell state inwhich they can actively proliferate for extended periods of time and spawn macroscopic metastases. Insight into this process may one day yield treatments that reduce the likelihood of delayed metastatic relapses.