

## The Gatekeeper Function of Lymph Nodes in Breast Cancer Metastasis

**Hypothesis:** Metastatic breast cancer cells acquire a "migratory phenotype" similar other migratory cells, including lymphocytes, which allows them to transit lymph nodes and enter the blood via the efferent lymph. This phenotype involves distinct and identifiable alterations in gene expression, which can be identified and exploited to trap tumor cells within lymph nodes, similar to the process which sequesters lymphocytes during the immune response.

**Broad Goals:** A lethal component of breast cancer is the ability of tumor cells to separate from the original mass, migrate to distant tissues of the body, and initiate new tumors. While the precise mechanism remains unclear, the principal route whereby most carcinomas leave the original mass is via the lymphatics. In order to enter the systemic circulation, neoplastic cells must first pass from the afferent lymph through a regional lymph node and exit in the efferent lymph. Therapies to restrict this transit are as yet unknown. It is the broad, long term goal of this proposal to identify the molecular regulators of tumor cell transit through lymph nodes, and to target these proteins to restrict tumor cell emigration in the efferent lymph.

**Aim#1:** To define the molecular regulators of tumor cell exit from lymph nodes.

*Hypothesis: Tumor cells which successfully exit lymph nodes will be phenotypically unique from those which are trapped within the lymph node.*

**Aim#2:** To define soluble factors which promote tumor-cell retention within lymph nodes.

*Hypothesis: Cytokines and immunosuppressants capable of restricting the exit of lymphocytes from lymph nodes will similarly restrict the exit of metastatic tumor cells.*

**Aim#3:** To define the molecular basis of cytokine or immunosuppressant-based tumor cell retention.

*Hypothesis: Treated cells will express unique genes and proteins which may be exploited to selectively inhibit tumor cell emigration from lymph nodes.*

**Aim#4:** To analyze the effects of tumor cell retention on the anti-tumor immune response.

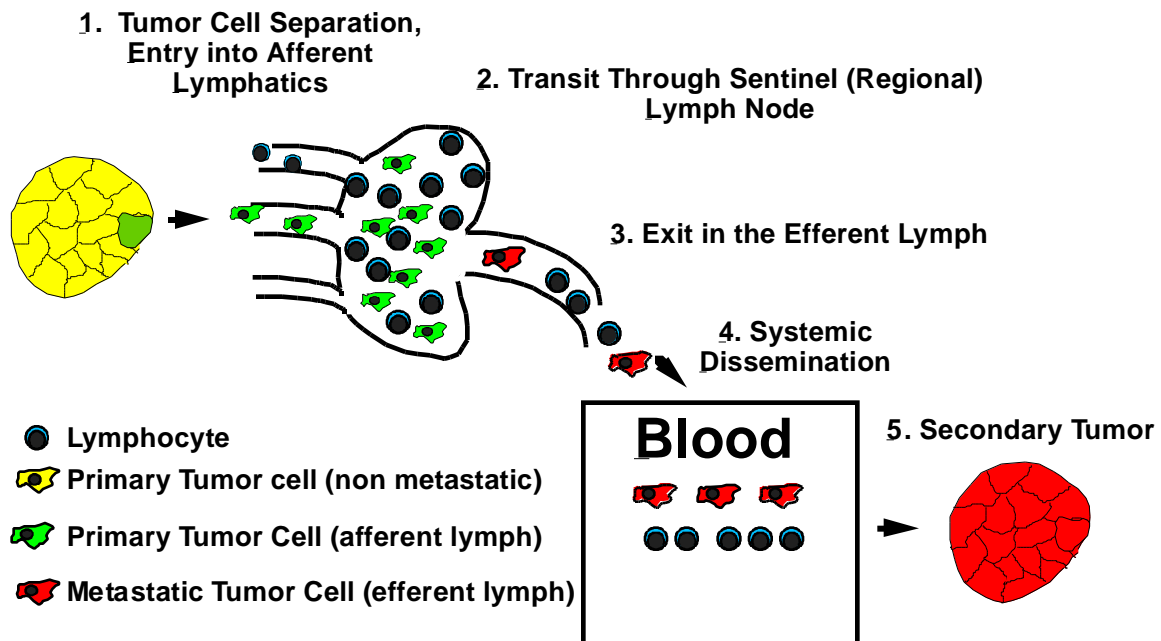
*Hypothesis: Local tumor retention will increase the frequency of tumor-specific lymphocytes in treated lymph nodes.*

## **B. Background**

### ***B.1 Relevance – Why target tumor migration in lymph nodes?***

Despite the major advances which have been made in uncovering the cellular biology of tumor cells, surgical excision remains the most successful treatment of most human cancers. Unfortunately, many tumors are subject to metastatic spread, which severely limits the success of surgical excision. Furthermore, the role of the local immune system in regulating tumor growth is as yet unclear, and it remains in question whether local excision of sentinel lymph nodes provides therapeutic benefit. A great many studies have attempted to describe neoplastic metastasis in terms of the end stage of the process, namely the ability of the cell to leave the blood, enter the distant organ, and establish a secondary tumor. Although lymphatic metastasis remains the major pathway whereby breast cancer cells are disseminated from the original tumor mass, relatively little is known regarding the mechanisms used to effectively navigate through the lymphatic system and enter the blood. Most notably, almost nothing is known regarding the mechanisms used by tumor cells to transit lymph nodes, a necessary gateway in lymphatic metastasis (Figure 1). While sentinel lymph nodes are routinely identified and excised during treatment of breast cancer, the benefit of this procedure remains in question. Aside from the immunological consequences of lymph node excision, the procedure can also markedly affect other systems

within the patient and result in serious complications. In breast cancer, removal of the sentinel lymph nodes induces various severity of lymphedema in 40% of all patients. Therapies designed to preserve the integrity of the lymphoid system in cancer patients would therefore appear to be preferable, if targets could be uncovered which would limit the metastatic spread of the primary tumor. The ability to successfully halt tumor cell metastasis within regional lymph nodes would provide two distinct benefits. Firstly, treatment to inhibit metastatic cell exit from lymph nodes could effectively localize the tumor, increasing the efficiency of later therapeutic interventions. Secondly, restricting tumor cell migration to the local lymph node and effectively providing a natural antigen depot could act as a natural adjuvant, and induce the immune system to promote a strong response to the local tumor. Our overall hypothesis is that metastatic breast cancer cells effectively acquire a migratory phenotype, similar to lymphocytes, stem cells, and other migratory populations. This characteristic can be effectively used to manipulate the metastatic potential of these cells during treatment. The evidence for this hypothesis, and the means which we will use to test it and thereby identify novel therapeutic targets, are discussed below.



**Figure 1: Schematic Representation of Lymphatic Metastasis of Breast Cancer**