# Understanding the molecular mechanisms of brain metastasis with orthotopic patient derived xenograft models and an *in vitro* blood-brain barrier

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## *Introduction:*

The improved management of early stage cancer has led to prolonged patient survival but has been accompanied by an unexpected increased incidence of brain metastasis [1, 2]. This phenomenon results from the fact that a fraction of disseminated cancer cells can survive undetected subsequent to successful treatment of a primary tumour, allowing these cells to penetrate the blood-brain barrier and colonize the highly protected brain microenvironment many months or years later [3, 4]. Brain metastasis occurs in 10-20% of cancer patients and is a virtual death sentence, with median survival of treated brain metastasis patients being less than 1 year [5]. In addition, there are no therapies specifically designed to treat brain metastasis, with the standard of care remaining whole brain radiation, stereotactic radiosurgery, surgical resection and chemotherapy [1]. Due to the associated morbidity and mortality, as well as the increasing prevalence, preventing or treating brain metastasis is a new bottleneck in improving cancer patient outcomes. In order to begin developing novel treatment strategies, there is critical need to better understand the molecular mechanisms that are engaged by cancer cells during the formation of brain metastases. While several studies have proposed targets thought to be worthwhile in treating brain metastasis, many will ultimately fail pre-clinical and early clinical trials due to the lack of reproducibility caused by the use of highly passaged cell lines and non-ideal animal models [6]. The overall aim of this project is to define the molecular mechanisms by which cancer cells colonize the brain, with the goal of identifying molecular targets to treat brain metastasis.

### *Hypothesis:*

We hypothesize that brain metastasis xenografts implanted directly into the brains of mice will closely resemble the metastatic lesions from which they are derived by gene expression analysis and immunohistochemical markers. Next generation sequencing approaches and cDNA-based screens will be used to identify candidates that promote the formation of brain metastases. Genetic and pharmacological inhibition of targets will be assessed using an *in vitro* blood-brain barrier model and growth of brain metastasis patient derived xenografts *in vivo*.

## *Results & Ongoing Work for Summer #2:*

***Establishing patient-derived xenograft models of brain metastasis:*** With my supervisor and our collaborator Dr. Morag Park, I have established a pipeline to access brain metastases from Dr. Kevin Petrecca (Neurosurgeon, Montreal Neurological Institute). This process has allowed us to generate a biobank of brain metastasis tissue from 33 brain metastasis patients from diverse primary sites (18 lung, 6 breast, 3 melanoma, 3 colorectal, 1 ovarian, 1 renal, 1 gastric). Cancer cells derived from 11 of these brain metastases have thus far been implanted into the brains of immune compromised mice (Table 1). In addition, three of these tumours have been established as subcutaneous xenografts to be expanded before intracranial injection due to small tumour fragment received (1 melanoma, 1 colorectal cancer, 1 ovarian cancer). We have also established cell lines grown as either tumourspheres or in conditional reprogramming cultures from a number of these xenografts, allowing for high-throughput studies [7, 8].

As this work is still ongoing, I will ultimately establish n=5 PDX models of brain metastasis from each of breast cancer, lung cancer and melanoma, representing a number of different histological and molecular subtypes seen in each of these cancers. At this point, we will perform whole transcriptome sequencing and immunohistochemistry for a number of cancer-type specific markers on both the patient tumour and the passaged xenografts. This will allow us to compare expression profiles of both, validating the models that are highly representative of the patient's tumour. Furthermore, genetic and pharmacological inhibition of specific targets will be performed in the PDX models and derived cell lines to understand the function of individual genes and signaling pathways in brain metastatic progression. This will allow us to establish improved or novel treatment paradigms that can be used in patients. This is particularly relevant as many of the patients from whom the xenografts are established will still be alive and eligible for experimental

treatments due to the late stage of their disease. The fact that these patients were candidates for surgical resection implies their prolonged life expectancy compared to other brain metastasis patients, making treating these patients with insights gained from the PDX's a distinct possibility.

***Identifying drivers of brain metastasis with an in vitro blood-brain barrier:*** Brain metastases often form after a longer latency period than other sites of cancer metastasis [9]. This phenomenon is believed to be caused by the impermeability of the blood-brain barrier, a system comprised of the brain's blood supply surrounded by a number of stromal components such as brain microvascular endothelial cells, astrocytes and pericytes [10]. To discover molecules that drive circulating tumour cell infiltration into the brain, we have established an *in vitro* blood-brain barrier model consisting of murine primary brain microvascular endothelial cells and primary astrocytes cultured on opposing sides of a Transwell cell culture insert. We have already established a barrier with a transendothelial electrical resistance (TEER) of 100 $\Omega \bullet cm^2$, which approaches the standard commonly accepted in the literature [10].

We will further develop this system to include human cells, using the hCMEC/D3 immortalized human BMEC cell line with human primary astrocytes. After final optimization of the barrier function (TEER of 150 $\Omega \bullet cm^2$), we will use a pooled barcoded cDNA library expressed in breast cancer cell lines with a low potential for trans-endothelial migration across the BBB (MDA-MB 231 and 4T1 cells) to identify targets that increase a cancer cell's ability to cross the blood-brain barrier. These are the targets that will be explored within the *in vitro* BBB and in our PDX models, using CRISPR/Cas9 and shRNA techniques already developed in our laboratory, as well as through pharmacological inhibition using both approved and experimental agents.

***Follow up of patient from xenograft bank: Melanoma with BRAF597 mutation:*** In the early stages of this project, we have already identified a clinical case of interest that will be explored further using these model systems. In melanoma, over 50% of patients harbor a mutation in the V600 amino acid of the BRAF protein, activating the protein to drive tumour growth, while making these patients sensitive to the drug combination of dabrafenib and trametinib [11]. A tumour from a melanoma patient in our PDX bank harbours a mutation at position L597, three amino acids away from the canonical V600 mutation. This is also known to be an activating mutation that may respond to targeted therapy [12-14]. This patient is still alive with a high performance status. However, they have experienced significant adverse effects from immunotherapy, the current first line therapy to treat metastatic melanoma. For this reason, demonstrating that targeted therapy with BRAF and/or MEK inhibitors can work in a PDX model of their tumour, we can influence care of this patient to receive targeted therapy. We have established intracranial xenografts from this patients tumour, as well as a cell line growing on plastic. We will treat mice engrafted with the patient's brain metastasis as well as the established cell line with a variety of therapeutics targeting MAPK signaling. We hope that our work can directly impact the clinical management of this one patient to improve their quality of life and overall survival, as well as that of many other patients with this rare, but present mutation in melanoma.

*Conclusion:*
We have been successful thus far in generating a bank of patient derived xenografts of brain metastasis. As we obtain more patient samples, this bank will continue to grow and allow us to perform functional and pharmacological experiments exploring particular factors that we hypothesize to be pertinent in driving brain metastasis, as indicated by our future sequencing experiments as well as the *in vitro* blood-brain barrier screen. We have already begun exploring one of the patients from our bank in greater depth, and are excited by the potential outcome of these studies influencing the patient's treatment. I strongly believe in the work outlined herein, and am certain that it will develop into a project that will impact both the research field, as well as patients suffering from brain metastasis.

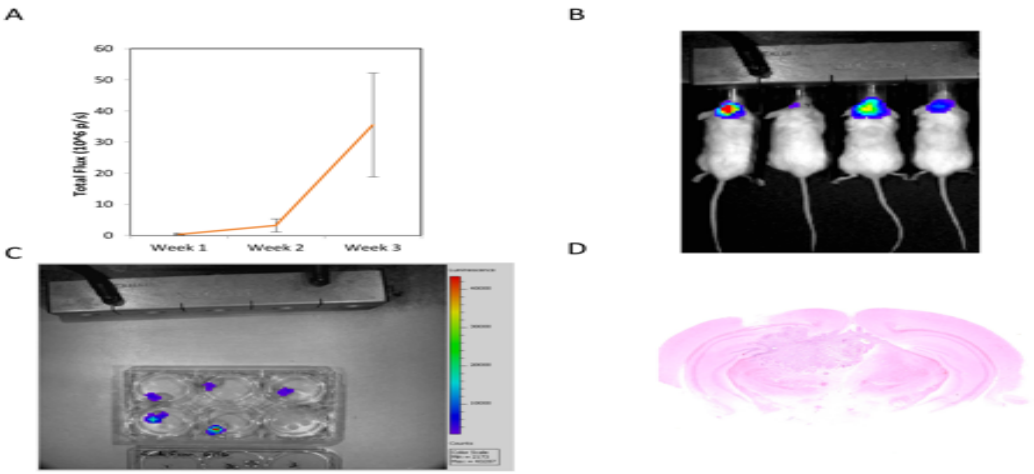


Figure 1: Proficiency of Intra-Cranial Injections

**Figure 1: Proficiency of intra-cranial injections:** A) Growth curve measured by IVIS100 of mice injected intra-cranially with 200,000 luciferase-tagged MDA-MB-231 BrM2 cells. B) I*n vivo* IVIS image of mice harboring MDA-MB-231 BrM2 cells in the brain. C) *Ex vivo* IVIS image of brains of mice injected intra-cranially with the same cells as A and B. D) Hematoxylin and eosin stained histological section of mouse brain injected with 500,000 cancer cells derived from a mammary fat pad patient derived xenograft of a triple-negative breast cancer patient's brain.

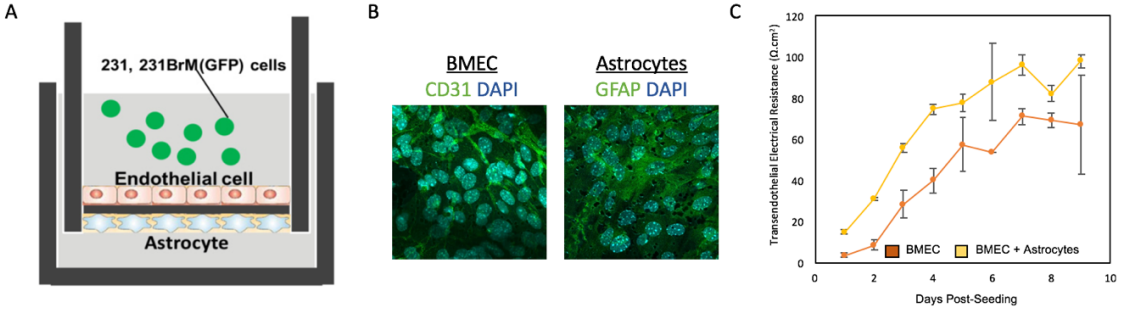


Figure 2: Establishment of an *in vitro* blood-brain barrier

**Figure 2: Establishment of an *in vitro* blood-brain barrier**. A) Diagrammatic set-up of the *in vitro* blood-brain barrier with astrocytes, brain microvascular endothelial cells (BMEC) and MDA-MB-231 breast cancer cells. B) Immunofluorescence imaging of isolated BMEC and astrocytes C) Transendothelial electrical resistance (TEER) measurement of *in vitro* blood-brain barriers.

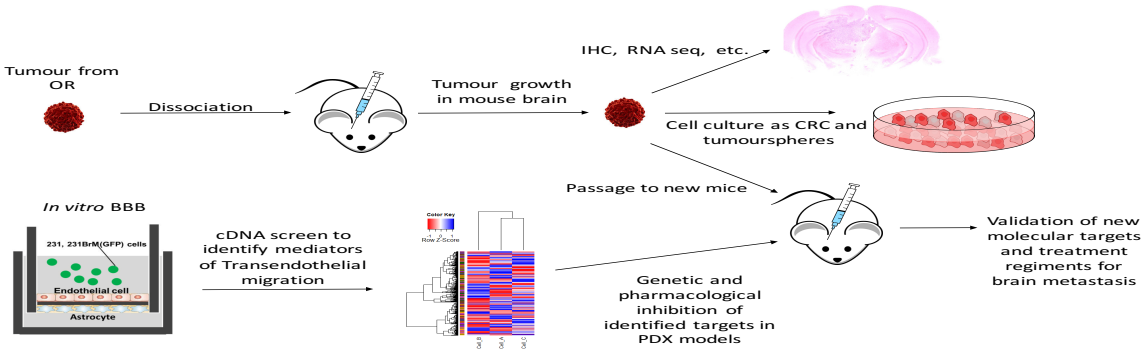


Fig 3. Experimental pipeline of models for brain metastasis target development

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| Tumour # | Primary Site | SubQ/MFP Xenograft Attempted | SubQ /MFP Xenograft Grew | IC Xenograft Attempted | IC Xenograft Grew |
|---|---|---|---|---|---|
| 1872 | Lung | Yes | Yes | Yes | Yes |
| 1888 | Lung | Yes | Yes | Yes | Yes |
| 1927 | Lung | Yes | Yes | Yes | Yes |
| 2045 | Lung | Yes | Not yet | Yes | Not yet |
| 1887 | Breast | Yes | Yes | Yes | Yes |
| 1944 | Breast | Yes | Yes | Yes | Not yet |
| 1945 | Breast | Yes | Yes | Yes | Yes |
| 2051 | Breast | Yes | Not yet | Yes | Not yet |
| 2015 | Melanoma | Yes | Yes | Yes | Not yet |
| 2042 | Melanoma | Yes | Not yet | Yes | Not yet |
| 1987 | Melanoma | Yes | Not yet | Not yet | N/A |
| 2046 | Gastric | Yes | Not yet | Yes | Yes |
| 1862 | Colorectal | Yes | Not yet | Not yet | N/A |
| 1858 | Ovarian | Yes | Not yet | Not yet | N/A |

**Table 1: Summary of brain metastasis PDX bank.**


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