**Rare Cancer Dependency Map Initiative**
**at the Broad Institute of MIT and Harvard**
Year One – Research Project Update

Together with the SDHB Pheo-Para Coalition and the Paradifference Foundation, the Pheo Para Alliance has funded a first-of-its-kind research effort at the Broad Institute of MIT and Harvard to lay the foundation for the creation of a pheochromocytoma and paraganglioma tumor dependency map. Launched in 2019, this three-year project aims to establish a fresh tissue acquisition pipeline for any patient, whether nationwide or across borders, to donate living tissue for the derivation of patient models, and develop a leading translational research platform focused on systematically identifying drug repurposing hypotheses and genetic dependencies of pheochromocytoma and paraganglioma tumors.

During the first year of this initiative, efforts focused on establishing and fine-tuning pipelines for fresh tissue acquisition from patients, submitting these tumor tissues for sequencing to confirm expected genomic characteristics, and devising an initiation strategy that might increase the likelihood of successful long-term cultures. Due to the Covid-19 pandemic, which forced the Broad Institute to temporarily close the majority of its lab operations (with the exception of work on Covid-19), tissue acquisition was paused from March through June. Lab operations resumed in early July and, since, the team has collected 4 fresh tissue samples, yielding a total of 13 Pheo/Para tumor samples from 13 different institutions from across the United States. Twelve of these samples were acquired through the online consent webportal Pattern.org. Excitingly, this now enables the launch of the next phase of the project's laboratory work, as several samples were required in order to have a robust cohort for its pilot plans.

During the inaugural year of the project, the Broad Institute was also able to establish deep scientific collaborations with organizations with expertise in pheochromocytoma and paraganglioma tumors. As a result, leading researchers in the field are now donating more than 50 living pheochromocytoma and paraganglioma samples collected over a 30-year period to the project, emphasizing the enormous traction that this effort is gaining.

Given the historical challenges in deriving new cell lines from samples of rare tumors, the Broad is taking a multi-pronged approach to culturing this collection of pheochromocytoma and paraganglioma tumor samples in the coming months. First, they will initiate each sample across 64 combinatorial conditions in the hopes of enriching tumor

purity. They will also monitor the genomics of every culture using a multiplexed sequencing strategy that involves next-generation sequencing. Then, they will perform genome-wide CRISPR/Cas-9 screens on the fresh tissue to look for genes that when knocked out increase the growth of these tumors in the lab. If successful, these efforts will help reveal why these tumors do not grow well in the laboratory and point the way to the development of media supplements that emulate these effects.

Finally, the Broad has also begun the computational arm of the project by analyzing data from its Cancer Dependency Map initiative to look for information from existing drug and CRISPR screens that could prove useful for pheochromocytoma and paraganglioma tumors. Working closely with its partnering foundations, the Broad is using a computational tool they developed, called CellLigner, to overlay pheochromocytoma and paraganglioma data from the Cancer Genome Atlas onto their collection of more than 1,000 cell-line models to find those that most closely match these tumors in transcriptional space. They have found several candidate models, including from neuroblastoma, and are using their findings to re-analyze existing drug repurposing and CRISPR data to generate hypotheses over the next 6 months.

In summary, the Broad Institute is making excellent progress, despite the shutdown caused by Covid-19, towards its ultimate goal of creating a pheochromocytoma and paraganglioma tumor dependency map. They have established a solid foundation for the project in Year 1 and look forward to a substantial scale up in laboratory work during Year 2.