NYU Langone Health

New York, June 12th 2023

Dear Kenneth Warren Fellowship Committee,

I would like to express my gratitude to the IHPBA Research Committee for awarding me the 2022-2023 Kenneth Warren Fellowship. This grant supports my study on the Phenotypic and Transcriptomic characterization of Circulating Tumor Cells as Biomarkers for Near "Real-time" Assessment of Treatment Response in Pancreatic Cancer patients, under the supervision of Professor C.L. Wolfgang and Doctor A.A. Javed.

I hereby report on my first 6-month activity at New York Langone Health. Since the start of my experience in December 2022, we faced some difficulties regarding the laboratory set up and the approval from the local institutional review board committee. During this time, we decided to expand the initial project design. My initial proposal was aimed at temporal assessment of CTC characteristics to validate their role as a biomarker for assessment of treatment response. Additionally, we aimed to identify unique single-cell RNA sequencing based CTCs subtypes and evaluate the ability to use it as a biomarker for treatment response. If successful, findings of the study will help develop tools to guide personalized therapy in pancreatic cancer. Initially our goal was that beginning in July we would start collecting blood from 50 pancreatic cancer patients before initiation of systemic therapy and monthly following during neoadjuvant therapy until surgical resection. CTCs were to be isolated and immunofluorescent staining would be performed for enumeration and phenotypic subtyping. Additionally, single-cell RNA sequencing would be performed to identify unique CTC subtypes at the RNA expression level.

Taking into consideration the well-known dismal prognosis of patients with pancreatic cancer and increasing evidence of its association with circulating tumor cells (CTCs), coupled with the low yield observed in prior experiments we shifted our focus to culture them. The low yield hampered single-cell RNA sequencing. The aim of this was to increase the CTC population to a point where phenotypic and transcriptomic assessment could be performed effectively. Additionally, with a

larger population of CTCs we could potentially perform pharmacotyping on them to assess drug susceptibility of circulating disease. We focused on determining the best technique to culture them, that will allow for optimal expansion of the population of isolated CTCs, resulting in the ability to further characterize them and understand their biology, thus identifying novel therapies that can specifically target them in a personalized-based approach. Specifically, we identified numerous emerging techniques that have shown promise in other cancer types. I have drafted a review titled “The Current State and Future of Circulating Tumor Cell Culturing for Precision Oncology” which is currently being finalized and will be sent out for publication shortly.

Based on the findings we planned an experiment where we would compare the well-established 3D organoid culture method with the Chicken Chorioallantoic Membrane (CAM) cultures assay, which has already proven to be a reliable alternative to the murine model for starting from primary tumor specimen, but culture conditions for CTCs- derived *in ovo* xenograft have still to be defined. Since then we have established the lab to be well equipped to perform these experiments and we have initiated the experiments on working with the CAM model and fine tune our technique. In the initial experiments we have tested the CAM model through the direct implantation of human pancreatic cancer cells line in the CAM in different concentration. We are harvesting the first culture, which will be stained using specific cell-surface antibodies to prove the growth of these cell lines. It is expected that within a month we will start receiving human samples in our lab to move ahead with the human experiments. Our plan is to isolate and culture CTCs from 40 patients to demonstrate the success rate of culturing them and performing single-cell RNA sequencing and pharmacotyping on these cultures. Following this we will isolate and culture CTCs from 20 patients with metastatic pancreatic cancer, perform pharmacotyping and determine correlation with clinical response observed to therapy. We expect that this work will be completed within the next six months.

Moreover, I have helped to build the data base on the pancreatic cancer patients who underwent surgical resection at NYU Langone Health, through which I am working on clinical analyses focused on the response to therapy. This work is also focused on biomarker assessment including CA19-9, CEA, CBCs with differential, and CRP. Our hope is that by finding promising biomarkers we can integrate them with CTC data obtained from our study to come up with a robust multianalyte marker of treatment response.

This terrific experience is enriching me professionally and personally. I am sincerely grateful for this great opportunity that is helping me build my future career as a surgeon scientist.

I look forward to presenting the results of my research in Cape Town, in 2024.

With my distinguished regards,
Elisabetta Sereni, MD, PhD student