Study plan stipend summer 2021 (dnr GU 2021/1261)

To thrive and proliferate, cancer cells must counteract the oxidative stress they are constantly subjected to. In order to do so, they must either obtain antioxidants from their surrounding environment (exogenous) or upregulate their synthesis (endogenous). KEAP1 is a negative regulator of NRF2, the master transcriptional regulator of the endogenous antioxidant response.

Loss of KEAP1 function hyper-activates NRF2 and accelerate KRAS-driven p53 mutant Lung adenocarcinoma (LUAD). Despite extensive studies conducted by several groups regarding the role of glutamine in KRAS-driven LUAD, inhibiting glutaminase has led to limited responses in pre-clinical models and in ongoing clinical trials. This indicates a need for identification of cancer subtypes that may have a greater dependency on glutamine.

We previously showed that hyperactivation of the NRF2 antioxidant pathway, due to KEAP1 mutations in KRAS-driven p53 mutant LUAD, causes a metabolic imbalance and a dependence on glutamine metabolism. This dependence could be targeted therapeutically through glutaminase (GLS1) inhibition. However, all of these studies were performed in a p53 mutant condition. More than half of LUADs remain wild type (wt) for p53. More importantly, 78% of Kras-Keap1 mutant LUAD have wt p53, which is a significantly large group of lung cancer patients where the impact of glutaminase inhibitors is yet to be defined, as p53 is a well-established factor in rewiring of glucose and glutamine metabolism to support cancer cell proliferation.

This research project will focus on determining whether p53 status modulates lung cancer sensitivity to glutaminase inhibitors. To answer this question, through CRISPR/Cas mediated genome engineering, we will establish and validate cellular models that are Kras mutant, Keap1 wt/mutant and p53 wt/mutant. We will define the sensitivity of these sets of cells to glutaminase inhibition. We will also validate the growth and signaling properties of these cells including some metabolic profiling, antioxidant and ROS levels. These investigations will be performed by a variety of methods including compound screening, cytotoxicity assays, effects on gene and protein expression by qPCR and western blot, and others.

At the end of the study period the student should know how to work with different *in vitro* models of lung cancer, as well as to assess viability (by luminescent and colorimetric methods), proliferation, invasion, The student should also be able to set up simple western blot and genetic expression assays.

At the end of the study period the student should know how to plan and set up simple experiments (number of replicates, controls, biological vs technical replicates, etc.), how to report and present results (image quantification, graphical interpretation, simple statistical analysis), and how to analyze and interpret his/her own results.

Weeks 1-8

1. Generation of murine and human cell lines knockouts for Keap1 or p53 or double knockouts
2. Validation of Keap1, Nrf2 and P53 status in the set of cell lines generated above

Weeks 9-16

1. Study the effects of glutaminase inhibitors on different cell lines through viability and proliferation assays
2. Investigate the metabolic profile of glutaminase inhibitors-treated cells, and determine the amount of intracellular and extracellular glutamate, glutamine, cysteine.
3. Evaluate the levels of ROS and intracellular GSH.

Weeks 17-18

1. Evaluation of results