Masters project description

Project 1:

Masters project Description at CANSEARCH Research Platform in Pediatric Oncology and Hematology, Department of Pediatrics, Gynecology and Obstetrics, University of Geneva, Geneva, Switzerland. Link

Title: ***In vitro*** **evaluation on the role of each specific glutathione s transferase isoforms in the metabolism of electrophilic compounds used in pediatric HSCT setting (e.g. Busulfan) using recombinant GST isoforms.**

Pediatric conditioning regimen prior to allogeneic haematopoietic stem-cell transplantation includes commonly alkylating agents (electrophilic) such as Busulfan, treosulfan, malphalan. Some of the metabolites of the compounds such as nitrogen mustard and acrolein from cyclophosphamide are also electrophilic in nature. Glutathione S transferase (GST) enzyme system comprising several isoforms is predominantly involved in elimination of the electrophiles upon conjugation with glutathione. However, it is unclear which isoform is predominantly involved in catalyzing this conjugation process, except for Busulfan where we have some evidence showing greater contribution from alpha1 isoform, but role of alpha 2 isoform still remains unknown. However, enzyme kinetic parameters from recombinant pure proteins is not precisely known, that could be incorporated into physiological modelling tools such as simcyp. In this current project, we aim to estimate enzyme kinetic parameters of specific isoform of GSTs towards elimination of busulfan in independent systems, and in combination with other isoforms mimicking the tissue specific expression and also in the presence of other electrophilic compounds that may be administered simultaneously or present in the physiological system along with busulfan. This would result in understanding role of GST system in drug-drug interactions of busulfan with other electrophiles and can aid in understanding tissue related toxicities that may be related to differential expression of GST isoforms and whose function/expression is predicted by certain pharmacogenetic markers.

Our laboratory has extensive collaborations with several research groups within University of Geneva, especially with Clinical pharmacology division and across the world which will allow the student to establish a vast scientific network.

The student is needed to assist in one of the projects mentioned below. A strong undergraduate (or master's degree) background in biology is required, as is some prior research experience. Some prior training in statistics, population genetics, molecular biology, and a working knowledge of a computer programming language are desirable.

The student will gain experience in LC-MS/MS analytical method for drug levels measurement in collaboration with clinical pharmacology division of HUG, setting up and optimization of enzyme kinetics experiments, calculation of the enzyme kinetic parameters, drug-drug interaction analyses, and theoretical knowledge in the area of metabolism and pharmacodynamics functions of the drug/s studied at our platform.

Duration: 8-12 months, Biology, Pharmacy students may apply for this project.

Relevant References:

1. Zenodo; 2017; https://doi.org/10.5281/zenodo.1005592
2. Rapid Commun Mass Spectrom . 2012 Jun 30;26(12):1437-46.doi: 10.1002/rcm.6241.
3. J. Vis. Exp 2020; (164), e61347, doi:10.3791/61347.
4. Mol Biol Res Commun. 2014 Mar; 3(1): 21–32.

Masters project description

Project 2:

Masters project Description at CANSEARCH Research Platform in Pediatric Oncology and Hematology, Department of Pediatrics, Gynecology and Obstetrics, University of Geneva, Geneva, Switzerland. Link

Title: Functional investigations on Pharmacogenetic markers to predict clinical outcomes post-transplant in pediatric allogeneic hematopoietic stem-cell transplantation setting

We are seeking to support a student interested in Master's project on functional evaluation of the pharmacogenetic markers. Our laboratory has extensive collaborations with several research groups within University of Geneva and across the world which will allow the student to establish a vast scientific network.

The student will participate in the research work aimed at evaluating the functional impact of the associated genetic markers form clinical association studies (see bibliography below). The student is needed to assist in optimizing and evaluating the cytotoxicity screening of the electrophilic compounds (e.g. busulfan), using spheroid cell cultures in collaboration with ACCESS platform of UNIGE. He / She will participate in functional investigation of DNA damage, DNA-Protein cross linking as a pharmacodynamic function alkylating agents (e.g. busulfan, melphalan) and compare between the different cell models that were developed in our lab with the presence or absence of the candidates of our interest (see bibliography below). The thesis work will be related to answering one specific biological question related to one of the candidates from our clinical association studies.

Thus, the student will acquire skills in cell culture, cytotoxicity screening, working knowledge on safety conditions while working with chemotherapeutic compounds, imaging techniques, comet assays, western blotting, and statistical analyses.

Requirements: A strong undergraduate (or master's degree) background in biology is required, as is some prior research experience or theoretical knowledge with cell culture is desirable.

Duration: 8-12 months. Biology student OR Pharmacy student

Relevant References:

1. Pharmacogenomics J. 2022 Feb;22(1):9-18. doi: 10.1038/s41397-021-00251-7.
2. J Cancer Res Clin Oncol. 2022 Jan;148(1):71-86. doi: 10.1007/s00432-021-03769-2
3. Biol Blood Marrow Transplant. 2020 May;26(5):920-927. doi: 10.1016/j.bbmt.2019.11.026

Masters project description

Project 3:

Masters project Description at CANSEARCH Research Platform in Pediatric Oncology and Hematology, Department of Pediatrics, Gynecology and Obstetrics, University of Geneva, Geneva, Switzerland. Link

Title: Hepatotoxicity potential of supportive care therapy used in pediatric hematopoietic stem-cell transplantation setting in *vitro* using 3D hepatocyte cultures

We are seeking to support a student interested in Master's project on functional evaluation of the ppharmacogenetic markers. Our group has recently identified *UGT2B10* genetic variant as an important pharmacogenetic marker of sinusoidal obstruction syndrome in pediatric hematopoietic stem-cell transplantation setting. In this regard, our group also identified supportive care therapy that is likely to inhibit UGT2B10 or possible substrates of UGT2B10, and with a hepatotoxic potential. However, there is no clear evidence on the hepatotoxic potential of these compounds is demonstrated using 3D hepatic cell cultures.

The student will participate in the research work aimed at evaluating the hepatotoxic potential of these putative supportive care therapy or their metabolites *in vitro*. The student is needed to assist in optimizing and evaluating the cytotoxicity screening of the electrophilic compounds (e.g. busulfan), using spheroid cell cultures in collaboration with ACCESS platform of UNIGE. Thus, the student will acquire skills in cell culture, cytotoxicity screening, ELISA, imaging techniques, western blotting, and statistical analyses.

Requirements: A strong undergraduate (or master's degree) background in biology is required, as is some prior research experience or theoretical knowledge with cell culture is desirable.

Duration: 3-4 months. Biology student OR Pharmacy student OR PREM student

Relevant References:

1. BMC Mol Cell Biol. 2022 Jan 21;23(1):5. doi: 10.1186/s12860-021-00402-5.
2. Toxicol Sci. 2013 May;133(1):67-78. doi: 10.1093/toxsci/kft021
3. Hepatology. 2011 Mar; 53(3): 974–982. doi: 10.1002/hep.24132

Masters project description

Project -4

Masters project Description at CANSEARCH Research Platform in Pediatric Oncology and Hematology, Department of Pediatrics, Gynecology and Obstetrics, University of Geneva, Geneva, Switzerland. Link

Title: Evaluating the concentration dependent effect of alkylating agents used in pediatric hematopoietic stem-cell transplantation setting on activation of NRF-2 and consequent impact on glutathione S transferase system

Our research group has been working on personalizing busulfan dosing in children receiving HSCT, to avoid unwanted effects or lack of efficacy when the levels of busulfan are not in the optimal window. Our group and several other research groups have investigated on the optimal window for busulfan levels in pediatric HSCT setting. Though there is exposure -response relationship is established, there are still overlapping discrepancies on the relationship between the busulfan levels with clinical outcomes. Especially lower event-free survival when the busulfan levels are lower, unwanted effects the levels are lower, and more pronounced toxicities when combined with that of another alkylating agent. These observations indicate need of further investigations on the exposure-response relationship in relation to that of the cellular protective mechanisms that are activated upon electrophilic stress (e.g. busulfan, melphalan possibly by alkylating the partner protein i.e. Keap1 in cytosol allowing nrf2 to translocate to nucleus) such as NRF2 system. There is also evidence showing that nrf2 can induce the cell protective mechanisms such as induction of GSTs mainly via acting at anti-oxidant responsive elements in the promoter region of these genes, GSH synthesis that are directly related to the unwanted effects of alkylating agents used in pediatric HSCT setting. In our earlier studies we also noticed that GST inhibitors such as curcumin, that is also known to activate Nrf2 counteract the consequences of GST inhibition upon prolonged exposures alone, but not in combination with busulfan.

The student will participate in quantifying this clinically observed exposure -response relationship of alkylating agent/s in relation to that of the NRF-2 activation and consequent GSTs expression, GSH synthesis and cytotoxicity using hepatic cell models. Correlation of the observed effects in relation to that of other markers of pharmacodynamic function of alkylating agents such as DNA damage, DNA-protein cross linking, and cytotoxicity will be assessed.

Duration: 8-12 months. Biology student OR Pharmacy student

1. J. Vis. Exp 2020; (164), e61347, doi:10.3791/61347.
2. Cell Metab 2021 Jan 5;33(1):174-189.e7. doi: 10.1016/j.cmet.2020.12.007

Masters project description

Project 5

Masters project Description at CANSEARCH Research Platform in Pediatric Oncology and Hematology, Department of Pediatrics, Gynecology and Obstetrics, University of Geneva, Geneva, Switzerland. Link

Supervisor: Marc Ansari, MD, PhD

Co-supervisor: Vid Mlakar, PhD

TITLE: Evaluation of GSTA1 and GSTM1 cross-talk and their influence on cellular response to chemotherapeutics used in conditioning regiments of HSCT.

Our research group has been working on personalizing conditioning regimens prior to Hematopoietic stem cell transplantation. The conditioning regimen is associated with treatment outcomes (relapse of tumor) and treatment-related toxicities (aGvHD, SOS, etc.). Busulfan and closely related treosulfan are central to the treatment. Their function is myeloablation with two main purposes, (1) to make space for transplant, and (2) to decrease tumor burden. Because busulfan causes toxicities (in particular VOD) the main effort up to now was focused on the pharmacokinetics of busulfan and its appropriate dosing. Busulfan is detoxified by the Glutathione-S transferases group of enzymes, mainly GSTA1 and GSTM1. We demonstrated that the metabolism of busulfan is associated with SNPs found in the promoter region of the GSTA1 gene.

To further understand the role of GSTA1 and GSTM1 in the metabolism of drugs used in a conditioning regimen prior to HSCT we established several hepatic cell lines (HepaRG, HepG2) with GSTA1 and GSTM1 knock-outs or knock-ins. The cell lines will serve to investigate changes in cellular sensitivity to the above-described agents. They will also serve as models to study their influence on cellular physiology and how they interact with each other.

The student will participate in:

1. EC50 experiments on established cell lines to understand sensitivity due to GSTA1 and GSTM1
2. Analysis of intracellular glutathione levels in cells.
3. RNAseq analysis to investigate global transcriptomic changes due to GSTA1, GSTM1.
4. Western blot analysis to explore how GSTs interact with each other and other genes.
5. Reporter assay analysis to investigate the association of GSTM1 to GSTA1 expression.

Methods used: Cell culture (HepaRG, HepG2), IC50, western blot, expression, RNAseq (analysis), gene editing (CRISPR/Cas9, recombinant expression)

Duration: 6 – 9 months. Biology student

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