



RESEARCH SCHOLAR PROGRAM – 2018

SUPERVISOR & PROJECT INFORMATION FORM

Please complete and return, via email only (crems.programs@utoronto.ca) by **November 3rd 2017** (*forms received after this date will not be posted*).

Supervisor Information

Name: David Spaner

Email: david.spaner@sunnybrook.ca

Degree: MD, PhD, FRCP(C)

SGS Appointment (IMS, IHPME, LMP etc.): MBP, IMM

Academic Rank: Associate professor

Field of Research: Cancer Immunology, Leukemia biology

Research Institution Affiliation (if applicable): Sunnybrook Research Institute

Allocation of student contact time (number of hours per week YOU are available to the student for any concerns or to review progress): 20 h

Project Information

MANIPULATING TYPE 1 INTERFERON-SIGNALING IN LEUKEMIA IMMUNOTHERAPY

Immunotherapy with genetically modified (CAR)-T cells or checkpoint inhibitors that awaken tumor-reactive cytotoxic T cells (CTLs) can potentially cure cancers that are otherwise fatal. Unfortunately, the promise of immunotherapy is thwarted by the intrinsic immunosuppressive properties and cytotoxic resistance of cancer cells. *Strategies to make cancer cells immunogenic, or able to stimulate and be killed by T cells, may overcome resistance to immunotherapy.* Our laboratory uses Chronic Lymphocytic Leukemia (CLL) as a uniquely informative clinical model to optimize cancer immunotherapy. Drug-resistant CLL is a high-fatality cancer that can potentially be cured by CAR-T cells. Importantly, CLL facilitates detailed studies by providing essentially unlimited access to primary cancer cells.

Type 1 interferons (IFNs) are ubiquitous components of cancer microenvironments where they can contribute to disease control by activating dendritic cells and CTLs. High doses of synthetic IFN α can sometimes be an effective form of immunotherapy for some cancers (Cancer/2006). However, prolonged exposure to low-doses of IFN is immunosuppressive and mediates resistance to checkpoint inhibitors in solid tumors and cell-lines. A better understanding of how IFNs affect cancer cells and novel reagents to manipulate IFN-signaling are needed to realize the potential of IFN in cancer immunotherapy.

Upon binding of IFNs to the interferon- α/β receptor (IFNAR), signaling processes are initiated that lead to phosphorylation and activation of signal transducer and activator of transcription 1 (STAT1), via the Janus kinase (JAK), JAK1, and a more transient phosphorylation and activation of STAT3, mainly via tyrosine kinase 2 (TYK2). STAT1 is a transcription factor with properties of a tumor suppressor, whereas STAT3 is oncogenic and promotes cell growth and resistance to apoptosis. IFN-signaling is terminated following endocytosis of the IFN-IFNAR complex, which is affected by cellular cholesterol content. We have found that IFN-signaling pathways become corrupted in aggressive CLL cells, leading to prolonged STAT3 phosphorylation that can be blocked by tool TYK2-inhibitor compounds (Blood/2011) and novel genetically modified type 1 IFNs. In addition, we have found that cholesterol content is elevated in aggressive leukemia cells, also causing enhanced STAT3-phosphorylation in response to IFN (Ebioscience/2016; Leukemia/2017). Combinations of cholesterol synthesis inhibitors and cholesterol efflux agonists switch the outcome of IFN-signaling to an immunogenic response.

The purpose of this project is to characterize in more detail the effects of clinically relevant TYK2 inhibitors, recombinant IFNAR agonists, and cholesterol modulators on the outcome of IFN-signaling in CLL cells. The student will work mainly with primary leukemia cells from CLL patients *in vitro* and measure effects on: 1. signaling using immunoblotting techniques, 2. cell phenotype using flow cytometry, 3. gene and protein expression using ELISAs and RT-PCR assays, 4. ability of leukemia cells to stimulate and be killed by CTLs in cell cultures. Experimental results will be correlated with clinical parameters of the leukemia cells to determine effects on CLL subtypes. By the end of the project, it is expected that some of the approaches will be tested in CLL mouse models to inform the design of clinical trials.

If human subjects are involved, have Ethics been obtained?

XYES

NO

Application Submitted

N/A

Do you expect this work will be published within the 20 months?

XYES

NO

Uncertain

Student's roles and responsibilities (please be specific)

Please indicate who will serve as the student's direct report (PI, PhD student, technician etc...)

1. Acquire knowledge about the biology of type 1 IFN in cancer and autoimmunity that is beyond the scope of the medical school curriculum.
2. Meet with supervisor (Dr. Spaner) to discuss progress in the context of formal lab meetings or informally on a weekly basis that may include weekends.
3. Work cooperatively with other members of the lab.
4. Help purify CLL cells obtained from patients who attend the specialized CLL clinic at Sunnybrook.
5. Master techniques required to perform the experiments, including cell-culture, flow cytometry, immunoblotting, and RT-PCR.
6. Organize results in figures that can be used for publication.