

Project information

Project title	Precision CHaRM (Child Health and Regenerative Medicine)
Applicant institution	University of Alberta
Collaborating institutions	None
Team leader(s)	Voronova, Anastassia

Project funding

Total project cost	\$10,900,121
Amount requested from the CFI	\$4,360,048
Percentage of the total project cost requested from the CFI (maximum 40%):	40%

Keywords

Research or technology development	hiPSC, stem cell, embryonic, cell differentiation, single cell, organoid, sequencing, microscopy, live imaging, genophenotyping, chronic disorder, rare disorder
Specific infrastructure	Automated stem cell maintenance, cryogenic storage, long read sequencer

Canadian Research and Development Classification

Type of activity | Basic research

Fields of research

Primary

Subclass Cell differentiation, proliferation and death

Secondary

Subclass Molecular genetics

Tertiary

Subclass Cellular neuroscience

Socio-economic objectives

Primary

Group Human health procedures and pharmaceutical treatments

Secondary

Group Health, n.e.c. - Advancement of chronic disorders

Project summary

Should the project be funded, it may be used in the CFI's communications products and website.

Regenerative medicine therapeutics are poised to revolutionize the medical field by targeting the underlying causes of diseases, halting disease progression, and promoting cellular repair of organs. Traditional therapeutics focus on symptom management and take a "one size fits all" approach that assumes that all patients with a given disease have the same underlying pathobiological disease mechanisms. This approach ignores human genetic diversity that stems from ancestry, sex and importantly, individual genetic mutations ("typos" in DNA) that cause disease or modify disease progression and/or response to treatments. Development of next generation targeted regenerative therapies requires a more precise understanding of how genetic mutations cause and influence disease than we have now. Advanced diagnostic and predictive disease modelling, using stem cells with disease-associated genetic mutations, provides a means to determine the pathogenicity of thousands of patient-specific mutations in a single experiment, transforming the way we study genetics. Establishing an integrated human stem cell facility will support innovative, high-throughput screening of mutations, allow modelling of disease mechanisms and progression, and identify novel pro-regenerative treatments that enhance tissue regeneration. This will enable development of personalized therapies and scientific breakthroughs that will have a transformational impact on the health research sector in Alberta and Canada.

Rationale. Neurological disorders affect more than 1 in 3 people, causing significant illness and disability worldwide. Beyond leading to morbidity and mortality, these disorders cost the healthcare system \$61 billion per year in Canada alone. Currently, there are no effective regenerative therapies capable of halting progression of, or reversing, neurological diseases. Drug discovery programs for these disorders have high risk profiles, and ***most drugs*** that make it to clinical trials ***fail for two major reasons:***

First, most drug candidates are tested in pre-clinical animal models (e.g. rodent, pig, dog). These do not fully recapitulate uniquely human physiology and disease pathology. This has become such a widely acknowledged shortcoming that the FDA has recently announced that drugs showing efficacy in relevant human cells may be approved for clinical trials without additional animal testing. *Thus, there is a pressing need for complementary methods that can model human cellular mechanisms to accelerate clinical translation.*

Second, genetic variants (inherited or spontaneous genetic mutations) are linked to many different neurological disorders by either directly *causing* the disorders or *modifying* disorder progression and/or response to treatment. While researchers have largely mastered the study of fundamental roles of genes in cellular differentiation and function, each gene can harbour thousands of individual mutations. Many of these specific mutations have unknown or poorly understood effects on cellular function (proliferation, differentiation, migration and/or death). As a result, development of therapeutics has traditionally relied on a “one size fits all” approach, and patients with a given disease are assumed to all have the same cellular dysfunction. *To overcome these shortcomings, a more personalized approach must be taken, where the biological processes perturbed by each mutation are targeted directly to fully “restore” cellular dysfunction.*

Research and technology development program. Recent advances and innovation using human induced pluripotent stem cell (hiPSC) technology have transformed this field. Although not yet implemented, it is now possible to simultaneously test hundreds to thousands of mutations in disease-relevant primary human cells that can be subsequently screened against therapeutic compounds. With the requested infrastructure and existing expertise, the University of Alberta (**UofA**) and Canada will be in a unique position to lead this transformation. hiPSCs can be generated from virtually any human cell (e.g. skin, blood) at any point in the lifespan. Reprogramming into hiPSCs effectively ‘turns back the biological clock’ to embryonic-like cells. These can then be used to generate different specialized tissue cells to study development, disease mechanisms and tissue regeneration. Moreover, given that hiPSCs propagate indefinitely, this provides an inexhaustible supply of ‘raw material’, enabling a flexible, accelerated and innovative experimental platform. By either deriving hiPSCs from humans with genetic mutations potentially linked to a disorder, or leveraging gene editing methods to introduce mutations into hiPSCs, this model provides unmatched capacity to probe the role of genetic variants and human genetic diversity in disease. *hiPSCs are the way forward.*

Our Approach: This CFI IF will equip an integrated hiPSC service research lab (SRL) that will function as i) a ***diagnostic platform*** to systematically test pathogenicity of hundreds of genetic mutations on human stem cell differentiation; ii) a ***prognostic platform*** to understand cellular dysfunctions caused by patient-associated genetic mutations, and iii) an ***innovation platform*** for high-throughput testing of pharmacological regenerative therapies for neurological disorders. Notably, development and degeneration are mediated by many reciprocal cellular processes, and adult-onset chronic disorders often have early-developmental origins. Thus, adult tissue regeneration and early embryonic development are inextricably linked and study of one can inform

the other. Leveraging our team's expertise, our initial focus will be on genetic mutations affecting cells of the human brain along with non-neuronal comorbid tissues (e.g. heart, kidneys, pancreas and placenta) affected in patients with neurological disorders. Merging local expertise in genetics, child development, adult chronic disorders, stem cell biology, and drug screening, the SRL will facilitate the launch of the **precision Child Health and Regenerative Medicine (CHaRM) hub**.

Equitable participation of SRL team members: Our hiPSC SRL is a young and collaborative facility that is already up and running on a small scale, enabled by a strategic partnership with Women and Children's Health Research Institute at the UofA. The team is comprised of ten faculty members who are globally recognized for their expertise in neurological, placenta, cardiovascular, renal and pancreatic disorders as well as drug screening. Together with local and national collaborators and HQP, this diverse team has the necessary expertise in stem cells, genetics, human development, neurodegeneration and drug discovery to ensure success of our proposed projects.

Enhancing and optimizing research capacity: The SRL, while housed at the UofA, will complement an existing research ecosystem by capitalizing on previous CFI investments and bringing together and supporting local, national and international leaders in regenerative medicine. To this end, we have recruited an external advisory board consisting of hiPSC experts Drs. Tom Durcan (McGill/QC), Julien Muffat (SickKids, ON), Justin Chun (UCalgary, AB) and Lisa Julian (SFU/BC). This will promote multi-institution exchange of protocols and resources to fast-track development of reproducible and robust hiPSC methodology. Beyond clinical and fundamental researchers, the CHaRM hub will benefit from the perspectives of other multidisciplinary experts (e.g. commercialization) and persons with lived experience, a capacity we have built into the governance structure. CHaRM will act as a *pan-Canadian catalyst for multidisciplinary innovation*, bringing together stem cell biology experts, neuroscientists, clinicians, and persons with lived experience positioning UofA, Alberta, and Canada as a global leader in stem cell biology and regenerative medicine. These goals will be realized through three research themes.

Theme 1. Innovation platform to study brain cell-cell and brain-body communication. Differentiation of hiPSCs into brain cells (neurons and glia) recapitulates the molecular mechanisms of both brain development early in life and brain regeneration in adulthood. This makes them an excellent platform to advance understanding of neurological disease with a direct path for regenerative therapeutics. **Technology development.** We will establish high-throughput, automated methods for 2D (monolayer) and 3D (organoid) neuronal and glial hiPSC differentiation to facilitate large-scale phenotypic analysis. This will also allow derivation of other organ-specific cells, such as placenta, kidney, pancreas and heart including investigation of inter-organ communication (e.g. brain-heart, brain-placenta organoids, etc.) to inform comorbidities commonly found in neurological disorders.

Theme 2. Diagnostic platform to study genetic diversity of rare diseases affecting the brain. Our integrated stem cell platform will enable better understanding of the biological impacts of *disease-causing* genetic variants. This will facilitate early diagnosis (known to improve patient outcomes) and mechanism-informed precision therapy design. **Technology development.** We will reintroduce wildtype (WT) or mutated genes-of-interest into corresponding gene knockout (KO) hiPSC lines to assay phenotype "rescue". We will leverage massive single-cell RNA sequencing to identify and confirm effects of each variant on whole-cell changes in gene expression as a proxy for function. The expectation is that WT and benign mutant constructs will *rescue* defective function, while pathogenic mutants will *fail* to rescue function. For KOs that do not perturb gene expression, we will use high throughput imaging assays and neuronal activity measurements. This unique, integrated approach means that *a single experiment can re-classify hundreds or*

thousands of mutations according to their cellular role. This is a transformative improvement over current approaches, which are usually years-long efforts to test mutations individually.

Theme 3. Prognostic platform to study genetic diversity of rare and common diseases. Better understanding of how variants modify disease progression and alter response to pro-regenerative drugs will facilitate development of more effective therapeutics and inform clinical trial design (inclusion/exclusion criteria) and interpretation (responders and non-responders). **Technology Development.** We will use ***high-throughput drug and pro-regenerative molecule screening coupled with AI algorithms*** and integrated phenotyping consisting of imaging, neuronal activity recordings and metabolic state testing ***to discover and develop drugs that enhance regeneration of neurons and glia differentiated from hiPSCs with and without disease modifying variants.***

Description of the main infrastructure items. An ***hiPSC suite***, primarily consisting of an automated hiPSC maintenance and organoid-seeding robot, will deliver large-scale collections of cell or organoids with multiple mutations into a ***drug screening suite*** to facilitate pro-regenerative drug discovery and development. ***Cell analysis*** and ***imaging suites*** will enable hiPSC differentiation analysis at single cell and organoid/tissue level, respectively. To facilitate integrated analysis of patient-linked data across multiple assays along with their medical records, this will be coupled with on-site digital ***data storage***. This integrated facility will be overseen by a dedicated ***project manager*** to coordinate custom infrastructure integration and installation. ***Training*** is required for facility HQP. ***Renovations*** are required to accommodate the equipment.

Global context. This SRL will be the ***first of its kind in Canada*** to analyze the impact of ***genetic diversity*** on specialized cell function *en masse*. Subsequent to our initial focus on neurological disorders, our assays will be expanded to any disorder of interest that can be modeled in hiPSCs. The proposed program is aligned with UofA and Government of Alberta strategic priorities, which prioritize *Health and Disease Prevention* via research and commercialization. Alberta is uniquely positioned to stratify results obtained in the hiPSC SRL with patient-specific clinical data (via ConnectCare), allowing rapid transition from diagnoses to regenerative medicine breakthroughs. In line with the World Health Organization's recent key priority of "accelerating access to genomics for global health", the goal for CHaRM - linking genetic diversity to chronic health conditions via our large-scale SRL platform - will position Canada as a leader in these efforts.

Benefits to Canadians. By improving diagnostic and prognostic capacity, identifying key targets for therapeutic development, and offering unprecedented insights into stem cell based therapies, the requested infrastructure will transform research discoveries into therapeutic applications to improve the health of ***genetically diverse*** Canadians with debilitating neurological illnesses. Ultimately, we aim to establish a pipeline that can be applied to ***any disease*** for which regenerative medicine and Precision Health approaches would be beneficial. The hiPSC SRL's advanced research infrastructure will also have a profound impact on economic growth in Canada by attracting top talent to Alberta, creating jobs that stimulate the economy, and producing innovative research outputs of broad interest to academia, industry and technology companies. This SRL will further enable research projects conducted at the UofA to leverage support from tri-council and other large scale national funding agencies, enhancing the provincial and national economies.

Anticipated outcomes. Our proposal combines hiPSCs technology and novel disease modeling in a high-throughput diagnostic, prognostic and drug discovery platform that uniquely accounts for ***human genetic diversity***. Knowledge of the biological impacts of genetic variants, and systems they perturbate, will enable early diagnosis and medical management and inform the development and clinical testing of precision therapies.

Key participants

Name	Institution	Department
Voronova, Anastassia	University of Alberta	Department of Medical Genetics
Alexander, Robert Todd	University of Alberta	Paediatrics/Nephrology
Eitzen, Gary	University of Alberta	Cell Biology
Ioannou, Maria	University of Alberta	Physiology
Korbutt, Gregory	University of Alberta	Surgery
MacPherson, Melissa	University of Alberta	Medical Genetics
Riddell, Meghan	University of Alberta	Department of Obstetrics and Gynecology
Simmonds, Andrew	University of Alberta	Cell Biology
Sipione, Simonetta	University of Alberta	Pharmacology
Zochodne, Douglas	University of Alberta	Department of Medicine

CRITERION 1: RESEARCH OR TECHNOLOGY DEVELOPMENT

Rationale. Neurological conditions affect over [1 in 3 people](#), making these a leading cause of illness and disability worldwide (PMID [38493795](#)). Genetic variants (both inherited and *de novo* [spontaneous] mutations) play a significant role in these conditions by **causing** a disease or **modifying** disease progression and/or response to treatment. Better understanding of how genetic variants affect normal brain cellular function is needed to improve diagnosis and prognosis, and ultimately enable **regenerative medicine** approaches to halt disease progression and restore function. Human induced pluripotent stem cells (hiPSCs) are a powerful and innovative way to study genetic variants by modeling genophenotypes in human primary neural cells differentiated from these stem cells. This approach is poised to overcome the limited capacity of animal models to recapitulate human conditions and accelerate therapeutic development.

Vision. Enabled by a unique world-class integrated human stem cell Service Research Laboratory (SRL), our University of Alberta (UofA) based team will develop **innovation, diagnostic, and prognostic platforms that incorporate human genetic diversity into research design** (Fig. 1). This SRL will facilitate high-throughput testing and leverage computational analysis (i.e. artificial intelligence [AI]) to determine the impact of genetic variants on stem cell function, enabling the development of “personalized” pharmacological regenerative therapies. Regenerative medicine is an emerging strength in Canada, supported by discoveries born from human genetics and stem cell biology. Acknowledging that *embryonic* development and *adult* tissue regeneration are often mediated by analogous cellular processes, and that many adult-onset disorders have developmental origins, this SRL will be integrated with the **Child Health and Regenerative Medicine (CHaRM) hub**, a pan-Canadian catalyst for multidisciplinary innovation comprising stem cell, drug screening and development experts, clinicians, and persons with lived experience. Together these initiatives will position UofA, Alberta and Canada as global leaders in stem cell biology and regenerative medicine. Leveraging our team’s expertise, our initial focus will be genetic variants affecting the human brain along with non-neural comorbid tissues in patients with neurological disorders via three research themes.

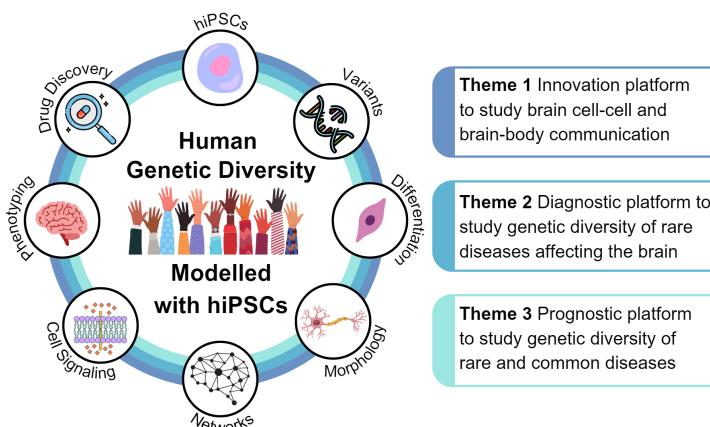


Figure 1: Probing human diversity and disease with human stem cell innovations

Figure 1: Probing human diversity and disease with human stem cell innovations

Theme 1 Innovation platform to study brain cell-cell and brain-body communication

Theme 2 Diagnostic platform to study genetic diversity of rare diseases affecting the brain

Theme 3 Prognostic platform to study genetic diversity of rare and common diseases

Theme 1. Innovation platform to study brain cell-cell and brain-body communication.

Knowledge and technology gap: Human disorders are traditionally modeled in animals (e.g. rodents), despite differences in physiology and composition making translation back to humans challenging. Indeed, >90% of drugs tested in pre-clinical mammalian models (e.g. rodent, pig, dog) fail in human clinical trials (PMID [18548043](#)). Thus, most current practice lacks sufficient insight into human cellular mechanisms for rapid clinical translation. To address this, the FDA recently announced that drug candidates showing success in relevant human cells may be approved for clinical trials without animal testing (PMID [37909337](#)). *Human primary cells derived from hiPSCs are the way forward.*

Rationale: hiPSCs can be reprogrammed from virtually any human cell (e.g. skin or blood),

enabling scientists to rewind the “biological clock” to a state similar to that of the embryonic cells that generate all human body tissues. The cells used to generate hiPSCs can come from healthy donors or patients with genetic diseases, at any point in their lifespan. Alternatively, patient analogous mutations or even ‘gene knockouts’ can be engineered in these stem cells. hiPSCs can propagate indefinitely, providing an ***inexhaustible supply*** of ‘raw material’ from which we can generate (differentiate) specialized cells to study human development, disease mechanisms and tissue regeneration for any organ. The scope of this new, integrated research program will be bolstered by our **pan-Canadian effort**, working with leading hiPSC experts including our advisory board: Drs. Durcan (McGill/QC), Muffat (SickKids/ON), Chun (UCalgary/AB) and Julian (SFU, BC). Working together with the SRL governance board (see Criterion 4), our pan-Canadian partners will promote free exchange of unique protocols, resources, and training materials, and accelerate our impact across Canada and worldwide.

Background: To ‘build’ the brain, neural stem cells (**NSCs**) proliferate, then differentiate into neurons (responsible for cognition, memory and behaviour) and macroglia (astrocytes and oligodendrocytes that support neurons). The brain is also populated by microglia (brain immune cells) that shape neuronal networks, myelination, and phagocytose (engulf) excess cells. In many neurodevelopmental disorders, abnormal brain structure and function arises from aberrant NSCs that take on erroneous fates resulting in abnormally formed neurons, macroglia, and neural networks. Although microglia do not arise from NSCs, diseased microglia nonetheless contribute to abnormal **neurodevelopment** by releasing pro-inflammatory factors that in turn affect NSCs, neurons, axons, and macroglia (PMID [28539882](#); **Voronova** PMIDs: [36937181](#), [38616269](#), [25556659](#), [27760310](#), [24247009](#); **MacPherson** PMID [34107259](#); **Simmonds** PMIDs: [37294080](#), [32191843](#)). ‘Maintenance’ of adult brain function (e.g. motor skill learning or memory formation) and response to injury requires regeneration of neurons and macroglia via adult NSCs and/or re-growth of axons (**Voronova** PMID [31629772](#); **Zochodne** PMID [26174154](#)). Defects in this process lead to permanent damage or loss of neurons and macroglia (**neurodegeneration**). hiPSC-derived NSCs and microglia recapitulate molecular mechanisms of human brain development and regeneration, providing a robust model to study the perturbations to these processes observed in human neurological disorders (PMIDs [32142662](#), [34883115](#)).

1.1. Modelling cell-cell communication in the human brain (*Voronova, Ioannou, Sipione, Simmonds*). Brain development and regeneration are regulated by interactions (i.e. cross-talk) between different cell types (glia, neurons and NSCs) that occur through soluble factors, extracellular vesicles and/or lipid particles (PMID [39251030](#); **Ioannou** PMID [37036445](#); **Voronova** PMIDs [36937181](#), [39775528](#)). For example, neurons secrete molecules that instruct NSCs to form oligodendrocytes (**Voronova** PMIDs: [28472653](#), [31629772](#)). Thus, changes in one cell type can have a ‘domino effect’ on other cell types, underscoring the importance of studying cellular communication. We will employ 2D mono-cultures to identify lineage-specific disease mechanisms, and 3D multilineage, multicellular organoids to interrogate cellular development and function in a cellular milieu similar to the human brain (PMID [32142662](#)).

Technology development. The team (**Voronova, Ioannou, Simmonds, Sipione**) will establish 2D methodology of neuronal, macroglial and microglial-hiPSC differentiation, as well as multicellular organoid assays to facilitate large-scale phenotypic and molecular analysis. Brain organoids (containing NSCs with differentiated neurons and macroglia as well as exogenously introduced microglia [PMID: [38976205](#)]) are imperative to accurately recapitulate the cell-to-cell interactions (PMID [31879153](#)), and have remarkable utility for modelling brain development and degeneration (PMIDs: [35610212](#), [38397069](#)). Capitalizing on existing expertise in cell-cell

communication (**Voronova, Ioannou, Simmonds**), and enabled by our *pan-Canadian advisory board*, cerebral and midbrain organoids will be used as a discovery platform to uncover new cell-cell communication cues (PMIDs [38976205](#), [34314827](#), [34632384](#)). An initial focus will be on the function of genes known to be implicated in neurological disorders (e.g. *ANKRD11* and *CX3CR1* [**Voronova, MacPherson**], *CLN3* and *GBA1* [**Ioannou**], *PEXs* [**Simmonds**], as well as *HTT*, *B4GALNT1* and *ST3GAL5* [**Sipione**]; also see themes 2-3).

The hiPSC platform will also provide a unique opportunity to incorporate sex and patient ancestral genetic diversity into research study design. While monogenic disorders (e.g. rare KBG syndrome) are usually highly penetrant, the prevalence and/or progression of many diseases differs by sex- and/or ancestry. For example, Parkinson's disease (**PD**) has a more severe disease progression and higher mortality in women. Moreover, *GBA1* variants confer a higher risk for developing PD in people of African than European ancestry (PMIDs [31282427](#), [37633302](#)). Despite this known complexity, normal human genetic variation is poorly captured in most disease models, population studies and clinical trials. Our team will model brain development using (or creating) hiPSCs from people with different ancestral backgrounds (e.g. available from New York Stem Cell Foundation "[Global Stem Cell Array](#)" and Broad Institute's [Stem Cell Bank](#)). Current practice is to use 2D cultures and organoids derived from one donor hiPSC line. We will instead adopt multi-donor "cell village" and "brain chimeroid" approaches (PMIDs [36796362](#), [38926573](#)), where cells from different sexes and genetic backgrounds are co-cultured to increase scalability and reduce costs. In these models, the function of each genotype (donor) is inferred from an integrated analysis of single-cell RNA sequencing (**scRNASeq**) of multi-donor cultures with whole genome sequencing of each donor line (PMIDs [36796362](#), [38926573](#)).

Team members will also use scRNASeq to predict ligand-receptor interactions and cell-cell communication. Ligand-receptor pairs will be prioritized based on novelty in brain development, and intellectual property (**IP**) potential as therapeutic treatments for neurological disorders. Functional analyses will include immunocytochemistry, live-cell imaging, confocal microscopy and neuronal activity measurements. Dr. **Simmonds** is the academic lead of the Faculty of Medicine & Dentistry Cell Imaging Core (**CIC**), and will coordinate development of hiPSC and organoid imaging. Transcriptomic, metabolomic and proteomic analyses will be used to profile wildtype (**WT**) and gene-of-interest knockout (**KO**) organoids to identify disease-specific genes and pathways that could be targeted for therapy. We expect to uncover new cell-cell communication molecules and novel disease associations with ancestry or sex.

1.2. Modelling human brain-organ communication (*Riddell, Alexander, Korbutt, Voronova, Zochodne*). The brain is intrinsically connected to other organ systems through direct innervation, as well as through less well understood paracrine signalling. For example, neuronal innervation of the pancreas and heart controls pancreatic beta cell formation and function as well as cardiomyocyte proliferation and regeneration, respectively (PMIDs [27207534](#), [26256209](#)). The brain also secretes a paracrine signalling molecule Sonic Hedgehog to regulate frontonasal (facial) bone tissue formation (PMID [22275045](#)). In a reciprocal fashion, other early organs, such as placenta, produce critical neurotransmitters for fetal brain development (PMID [31711155](#)). Although it is clear that this crosstalk influences tissue morphogenesis and function (PMID [29497473](#)), little is known about the factors and cell types that direct these processes.

Technology development: This theme will allow for the derivation of organ-specific cells (e.g. cardiomyocytes) and multicellular organoids (e.g. placenta, kidney, pancreas and heart), to enable investigation of inter-organ communication. The team (**Voronova [brain & heart expert**

PMIDs: [28472653](#), [34270934](#), [36608690](#), [22199256](#), [27484899](#), [38951500](#)], **Zochodne** [peripheral nervous system expert PMIDs [38904854](#), [38697733](#), [37821230](#), [22548616](#)], **Riddell** [placenta expert [37468163](#), [35124330](#)], **Alexander** [kidney and epithelial biology expert [34810264](#), [32197346](#), [32149733](#), [34699268](#), [37575484](#), [37563989](#)], **Korbutt** [pancreas expert [10911004](#), [35697798](#), [31581152](#), [35335450](#), [36748727](#), [8621802](#)]) will utilize published protocols (PMIDs [31813827](#), [35232775](#), [39024165](#), [31813827](#), [37676312](#)) or develop new 2D and 3D protocols for each organ of interest. Brain-heart (**Voronova**), brain-placenta (**Riddell**), brain-kidney (**Alexander**) and brain-pancreas (**Korbutt**) communication will be studied by co-culturing different organoid types using microfluidic devices to study paracrine brain-organ cellular communication (with collaborator Dr. Amy Tsai, Dept. of Mechanical Engineering, UofA) and assembloids (integrated structures formed by fusing different organoid subtypes) to study brain-organ communication enabled by neuronal innervation (PMIDs: [39454582](#), [31879153](#), [38562819](#), [35338360](#), [28265064](#)). Assembloid generation may require fusing spinal cord and/or peripheral nervous system organoids with non-neural organoids to mimic physiological conditions. In testament to their institutional commitment, the UofA has recently recruited Dr. Sandeep Gupta (start date Jan/2025, SRL user), a world expert in differentiating sensory neurons in human spinal cord organoids, who will be an asset to this project (PMIDs [34730293](#), [29337120](#), [33659900](#), [35858555](#), [38804879](#)). We will initially focus on modelling paracrine signalling between brain and non-neural organoids before subsequently applying established pipelines to the development of specific assembloid assays. As in theme 1.1., scRNAseq profiling will be used to i) identify cell types, ii) perform interactome analyses to identify secreted factors and iii) examine how cellular heterogeneity is altered by the exposure to factors released from distant organs. The structural and functional analyses enabled by this technology will yield new discoveries in the brain-body communication axis.

1.3. Identifying novel pro-regenerative molecules (Voronova, Sipione, Ioannou). Beyond modelling human diseases in hiPSCs, the SRL will enable discovery and development of pro-regenerative drugs, which this team has extensive experience with. For example, Dr. **Voronova** has shown that neuronally secreted molecules like fractalkine (CX3CL1) promote oligodendrocyte formation from NSCs (PMIDs: [34270934](#), [36608690](#)), which led to the development of fractalkine receptor agonist (USPPA #63/561,005). Likewise, Dr. **Sipione** has shown that a specific glycolipid known as ganglioside GM1, produced abundantly by neurons, reduces neurodegeneration by modulating microglia/neuroinflammation (PMIDs: [37996924](#), [34991625](#), [33117120](#), [28993428](#)) leading to two patents [US 9,023,812](#), [PCT/CA2022/051803](#). In addition, growth factors secreted by cells in other organs (e.g. liver) promote brain regeneration (PMID [25537484](#); **Voronova** PMID [35293825](#)). Therefore, modelling cell-cell and inter-organ communication is a powerful platform for pro-regenerative molecule discovery.

Research plan: **Voronova, Sipione, Ioannou** will test if known or novel cell-cell communication cues identified in themes 1.1-1.2 can enhance human NSC differentiation into neurons and oligodendrocytes, to replace those that are dysfunctional or lost in neurodevelopmental and neurodegenerative disorders (PMIDs: [12214070](#), [34107793](#)). The researchers will also identify cell-cell communication cues that can modulate human microglia reactivity to prevent chronic neuroinflammation increase production of neuroprotective factors and enhance phagocytosis (vital for clearing cellular debris and promoting regeneration; PMID [37452201](#)). Cell fates will be assessed using high throughput immunocytochemistry, neuronal activity measurements, live imaging and confocal microscopy. We anticipate discovering novel neuroprotective and/or pro-regenerative molecules that will form the basis for drug development.

Theme 2. Diagnostic platform to study genetic diversity of rare diseases affecting the brain.

Knowledge and technology gap: Although individual rare disorders are infrequent, collectively these >7000 disorders affect one in 12 Canadians. Diagnosis of rare genetic disorders has improved due to advances in genome sequencing technologies, which detect specific patient variants. If these variants are known to be pathogenic and are found in established **disease causing genes**, genetic testing facilitates definitive diagnosis and informs clinical management. However, 40% of currently identified variants (including those in known disease-causing genes) have unknown pathogenicity, and are thus labeled “**variants of uncertain significance**” (VUSs) (PMIDs [33853652](#), [37878314](#)). Classification as a VUS does not aid in diagnosis, thus precluding access to therapies or inclusion in clinical trials. Moreover, disease-causing genes typically harbour hundreds of VUSs, while current methods assess one variant at a time and are therefore prohibitively time consuming and expensive. Current practice also typically uses null gene variants, transmitted in a germline manner and therefore integrated into the majority of cells in the body. By conducting functional variant testing in hiPSC-derived clinically relevant *specialized* cell types, we will address these shortcomings and improve patient outcomes.

Rationale: Our initial focus will be on the brain, which is affected in more than half of all rare disorders (PMID [33519696](#)). We will create an integrated stem cell platform focused on understanding the biological impacts of genetic variants, including VUSs, to enable early diagnosis (known to improve health care costs and patient outcomes [PMID [37283971](#)]), inform clinical trials (e.g. design of inclusion/exclusion criteria; interpretation of responders and non-responders) and promote molecularly-informed design of precision therapies.

2.1. High-throughput hiPSC genophenotyping as a diagnostic platform for rare mutations and VUSs (*Voronova, Ioannou, Sipione, Simmonds, MacPherson*). Many genes associated with neurological disorders control NSC proliferation and/or differentiation, leading to dysfunctional neurons, macroglia and/or neuronal networks (PMIDs: [36253345](#), [32675289](#), [37590144](#); **Voronova** PMIDs: [38616269](#), [24247009](#), [34270934](#)). Although informative, it is difficult to extrapolate these findings to hundreds or thousands of different variants in each gene, given that each variant may have unique impact(s) on distinct cellular functions. Thus, an innovative aspect of our proposal is to extend the ‘standard’ hiPSC workflows optimized to study the role of genes to a high-throughput systematic analysis of multiple genetic variants.

Research plan. Along with other groups, members of our team have identified that knockout, knockdown or loss-of-function (**LOF** i.e., reduced gene expression or protein activity) of *ANKRD11*, *PEX5*, *PEX7*, *CLN3*, *GBA1*, *ST3GAL5* or *B4GALNT1* results in measurable defects in proliferation, neurogenesis, survival, neuronal activity or organelle formation and/or function in neurons or glia. Specifically, **Voronova** has shown that NSCs lacking *ANKRD11*, the most frequently *de novo* mutated gene in monogenic neurodevelopmental disorders including KBG syndrome (PMID [31981491](#)), have reduced proliferation and neurogenesis due to aberrant global gene expression (PMIDs: [38616269](#), [25556659](#)). **Simmonds** has shown that fruit flies lacking functional *Pex5* or *Pex7*, which cause rare peroxisome biogenesis disorders, have abnormal gliogenesis due to aberrant peroxisomes (PMIDs [29669255](#), [30389805](#)). **Ioannou** is studying *CLN3* and *GBA1* (lysosomal proteins that impact neuronal function), which cause neurodegenerative Batten and Gaucher diseases, respectively (PMIDs [37400440](#), [31685979](#), [37063480](#)). Finally, **Sipione** studies the ganglioside biosynthetic genes *ST3GAL5* and *B4GALNT1*, which cause rare GM3 synthase deficiency and Salt and Pepper syndrome as well as Hereditary spastic paraparesis type 26 (PMIDs [30185102](#), [24103911](#)). In addition, *B4GALNT1*

variants impair neuron activity and survival (PMID [34595861](#)). While these discoveries are significant, 130-900 VUSs remain, with unknown links to disease phenotype, in each of these genes-of-interest (ClinVar database, accessed in Oct/2024; PMID [24234437](#)).

To address this gap, we will use an overexpression screen to reintroduce individual WT, known pathogenic, or VUS constructs into NSCs with gene-of-interest KOs in each well of a 96-well plate. This approach is patterned after similar high-throughput screens in *Drosophila*, where overexpression of WT human cDNA is used to rescue cellular phenotypes of orthologue KOs (e.g. PMID [31227826](#)). This approach is also supported by recent publications, which annotated cancer VUSs in the engineered human pluripotent line with a haploid genome and non-coding VUSs in human NSCs (PMIDs [37488236](#), [39848247](#)). We will adapt these assays to clarify the effects of known pathogenic variants in clinically relevant *human* cells and to rapidly re-classify hundreds to thousands of VUSs. We will prioritize VUSs that have a low frequency in normal population but are prevalent in patients with monogenic neurological disorders with well-characterized cellular “signatures” in hiPSCs (examples given in theme 1.1). Each variant expression will be driven by an NSC suitable promoter (e.g. PMID [34075124](#); Voronova PMID [28472653](#)), contain a puromycin resistance cassette and a barcode (shortDNA sequence identifier; PMIDs [30448000](#), [37852259](#)). Transduced NSCs that survive puromycin will be differentiated in 2D, then pooled by combining different wells (variants) and subjected to scRNAseq to identify and quantify the level of each variant expression in each cell. Differential changes in transcriptome will serve as an initial proxy for function. We expect WT and benign variants to rescue defective functions (gene expression more similar to WT), while pathogenic variants will not. For KOs that do not affect gene expression, we will use assays as described for LOF studies cited above, such as high-throughput imaging to identify restoration of cell morphology or organelles, and neuronal activity measurements to identify changes in neuronal firing, analyzing each well separately. To correlate the level of variant expression with specific cellular rescue, we will use laser dissection microscopy followed by qPCR in individual cells (PMID: [27387371](#)) or subject an entire well to qPCR. Pathogenicity of the most interesting individual VUSs will be validated by engineering specific mutations via CRISPR into endogenous genes. We expect to successfully reclassify the majority of VUSs into pathogenic or benign. We will integrate this knowledge into existing clinical platforms (e.g. ClinVar) with Drs. **MacPherson** and **Alexander**, physician scientists specializing in rare disorder phenotyping.

2.2. hiPSC genophenotyping as a prognostic platform to predict co-morbidities in neurological disorders (*Riddell, Alexander, Korbutt, Simmonds, Voronova, Ioannou, MacPherson*). Patients with neurological disorders have non-neural co-morbidities, including anomalies in orofacial and musculoskeletal systems, the heart, pancreas and kidneys (PMIDs: [38745008](#), [39304265](#)). Our team members have demonstrated direct links between disease causing variants and comorbidities in these organ systems, e.g., *Ankrd11* LOF in neural crest cells leads to defective skull and heart formation (Voronova PMIDs: [38951500](#), [33996804](#)) while *PEX5* and *PEX7* variants affect muscle development (Simmonds PMID [30389805](#)). Since the majority of patients with genetic disorders are diagnosed after birth, the prevalence of fetal congenital anomalies *in utero* is not known (Voronova PMID: [37226940](#), MacPherson PMID [36360262](#)). This includes a poor understanding of abnormal formation of the placenta, which can have deleterious effects on fetal brain development (PMIDs [38420785](#), [33898362](#)). The development of this integrated high-throughput stem cell assay will identify biological impacts of variants on neuronal and non-neuronal cells derived from the same hiPSC sources.

Research Plan: Researchers (**Voronova, Ioannou, Simmonds, Alexander, Korbutt, Riddell**)

will use hiPSCs with gene-of-interest KO (e.g. *ANKRD11*, *PEX5*, *PEX7*, *CLN3*, *GBA1*, *ST3GAL5* or *B4GALNT1*). As in theme 2.1., for each KO model that results in a significant phenotype (i.e. that models a comorbidity) in cells of the heart (e.g. cardiac and smooth muscle), kidney (e.g. renal tubular or glomerular epithelial), pancreas (e.g. beta) or placenta (e.g. epithelial trophoblast), researchers will introduce WT, pathogenic and VUS-expressing constructs back into KO hiPSCs to determine whether organ-specific cell formation and function can be rescued. This will re-classify VUSs into harmless or pathogenic with respect to each organ/comorbidity, providing insights that can then be integrated into clinical datasets.

2.3. Drug discovery and screening platform for rare genetic neurological disorders. Current pharmacological therapies for neurological disorders primarily manage symptoms or slow down disease progression; drug therapies aimed at rescuing cellular dysfunctions to stop or reverse the disease have seen less success. This is likely due to lack of in-depth clear genophenotypes that demonstrate *how* pathogenic variants affect cellular function, and failure to include relevant variants in human multicellular models. Establishing an integrated hiPSC assay with high-content analysis will allow our team to capitalize on i) the development of deeply phenotyped human cell models; ii) reclassification of VUSs in disease-associated genes; and iii) an in-depth understanding of their impact on both neuronal and non-neuronal cells and organ systems (Themes 1.1, 1.2 and 2.1, 2.2). Together with national and international collaborators, this integrated approach will accelerate the development of novel pharmacological treatments.

Research Plan: Team members (**Voronova, Ioannou, Simmonds, Alexander, Korbutt, Riddell, Zochodne, Eitzen**) will screen pharmacologic compounds in hiPSC-derived cells (2D differentiation) containing *pathogenic* variants (via CRISPR or derived from patients) identified by collaborators and in Theme 2.1. Assay readouts will be carefully curated using cellular deficiencies identified in Themes 1 and 2.1-2.2. Dr. **Eitzen** is an expert in the design of cell- and target-based assays for screening large drug libraries (PMIDs: [38565852](#), [35940876](#), [34434934](#), [34350186](#), [31601145](#)), while both Drs. **Eitzen** and **Zochodne** are experienced in AI-driven drug screening for nerve regeneration (PMID [38565852](#); USPPA #63/481,506). Dr. **Eitzen** will design high-throughput experiments that measure rescue of cellular defects or protein function, initially focusing on rescue of deficits that *drive* primary neurological pathology, and later on non-neuronal cells that *contribute* to disease comorbidities. Compounds that show rescue in 2D cultures will be further validated in 3D organoids, in collaboration with our *pan-Canadian advisory board*. We will first focus on pro-regenerative molecules previously identified by **Voronova** (fractalkine, hepatoma-derived growth factor PMIDs: [34270934](#), [35293825](#), [36608690](#)) and **Sipione** (gangliosides PMIDs [34991625](#), [31447771](#)), followed by novel factors identified via cellular interaction studies in Theme 1.3. To ensure widest utility of our SRL infrastructure, BBB-penetrant drug libraries (Chembio CNS-MPO) will be screened, with selection driven by affected signalling pathways and cellular communication knowledge generated in Theme 1. Our workflow will use a multiparameter AI model to increase the likelihood of selecting true-positive “hit” compounds (post high-throughput screen) that can be developed into leads. For example, PAINS (Pan-Assay Interference Compounds) filters will be used to eliminate false-positive leads due to drug interference with assays or reagents, while SAR (Structure-Activity Relationship) filters will facilitate positive selection via computational modeling of hits with the target and/or prediction of biological activity. In collaboration with Drs. Khaled Barakat (UofA) and Raimar Loebenberg (UofA [Drug Development and Innovation Centre](#) [DDIC] director), further SAR computational modeling of lead compound analogues may be explored while reviewing patent space (e.g. **Barakat** PMID [37676596](#)). Analogues with IP

potential that pass computational modelling will then be synthesized and re-tested.

Theme 3. Prognostic platform to study genetic diversity of rare and common diseases.

Knowledge and technology gap: While studies in laboratory animals and cells have yielded considerable mechanistic insights into neurological disorders, the vast majority of these models do not incorporate variants in *disease modifying genes* that impact age of onset, speed of disability progression and response to treatments. Investigating these relationships through traditional patient-based studies requires massive numbers of patients with all variants, lacks mechanistic insight, and is often inconclusive. A high-throughput integrated hiPSC facility will allow functional studies of disease modifying genetic variants observed in human patient populations, thus fast-tracking pro-regenerative therapy development for *all* patients.

Rationale: Understanding how disease modifying variants alter cellular response to treatments is essential to develop therapies that repair and restore tissue function in carriers of disease modifying variants. This critical knowledge will inform clinical trial design (i.e. stratification by genetic variants predicted to differentiate responders from non-responders).

3.1. A prognostic platform to study the effect of disease-modifying variants on neurodegeneration (*Voronova, Sipione, Zochodne*). People with severe neurodegeneration often harbour variants in genes affecting formation and/or function of neurons, oligodendrocytes, and microglia. For example, *CX3CR1* (fractalkine receptor) variants are associated with severe neurodegeneration or increased mortality (PMIDs: [22916723](#), [24806473](#), [28343297](#)). In agreement, a mouse model that carries pathogenic variant in *CX3CR1* displays worse brain degeneration and regeneration relative to WT *CX3CR1* carriers, due to aberrant NSC and microglia fates (PMIDs: [35560167](#), [30386211](#), **Voronova** unpublished data). As another example, Huntington's disease (HD) age of onset and progression is largely determined by variants in disease modifying genes and/or the length of the inherited CAG repeats in *HTT* (PMIDs [3981710](#), [26232222](#), [28642124](#), [8401589](#)), due to aberrant NSC and neuron fates (PMIDs [30811996](#), [38749429](#)). These studies provide a causative link between *disease modifying* variants and cellular dysfunctions that impact health outcomes. Moreover, **Sipione** and others have observed ganglioside perturbations in several neurodegenerative disorders (PMIDs: [36207292](#), [29902255](#), [26056266](#), [30797170](#), **Sipione** PMID [20237277](#), manuscript in preparation). Prediction of disease onset, progression and severity could improve quality of life, underscoring the need to understand the biological impact of disease modifying variants.

Research Plan. Using existing or newly generated hiPSC lines from 'as diverse as possible' ancestral backgrounds that harbour patient-specific variants in *CX3CR1* (already generated by Voronova), *B4GALNT1*, *ST3GAL5* and *HTT*, researchers (**Voronova, Sipione, Zochodne**) will determine the fates and function of differentiated cells in 2D cultures (e.g. cell types and organelles, neuronal activity, transcriptomic and metabolomic signatures). This will generate a comprehensive understanding of how disease modifying variants affect neural cells based on gene expression, organelle formation and function, neuronal activity and other parameters. Analogous high-content analysis of 3D organoids will extend understanding of how risk variants alter cellular interactions. Once workflows have been established using the variants listed above, we will collaborate with national and international experts to characterize severity risk variants for other neurological diseases (PMIDs [34749609](#), [34099642](#), [37380766](#), [38895438](#)).

3.2. hiPSC SRL as an integrated innovation platform for developing therapies for disease modifying variants (*Voronova, Sipione, Zochodne, Eitzen*). Current approaches for the development of pro-regenerative therapies focus on i) reducing neuroinflammation to allow

endogenous tissue regeneration, ii) offering neuroprotection to preserve neurons or their axons, or iii) facilitating replacement of lost or damaged cells and re-growth of degenerated axons. Despite many attempts, **there are currently no approved regenerative therapies for neurodegenerative disorders**. Drug discovery programs for these disorders have high risk profiles, and most drugs that do progress to clinical trials fail due to heterogeneous response (i.e., statistical power is lost due to mixing of “responders” and “non-responders”). While there are several disease modifying therapies that slow down (but do not stop) disease progression (e.g. PMID [39376160](#)), the efficacy of these drugs depends on individual genetics (PMID [39264442](#)). Our proposed integrated SRL will provide a platform for functional genotyping that identifies the cellular response of disease modifying variants to drug therapies, thus facilitating effective, targeted treatments and strategically designed clinical trials.

Research Plan: Team members (**Sipione, Voronova**) have developed novel pro-regenerative therapies via drug discovery and medicinal chemistry programs, including a novel pro-regenerative small molecule CX3CR1 agonist that facilitates regeneration of oligodendrocytes from NSCs (**Voronova**, USPPA #63/561,005), and a ganglioside-based therapeutics to treat HD via clearance of mutant huntingtin protein (**Sipione**, patent US 9,023,812). The proposed hiPSC suite will expedite screening of novel drug analogues and libraries, **as well as promising drug candidates that failed in late-stage clinical trials**, on WT hiPSCs and hiPSCs that carry disease modifying variants. This theme also lends itself to testing drugs on hiPSCs from different ancestral backgrounds, an area that has been unjustly neglected in pharmaceutical research despite strong evidence showing that pharmacogenomic properties of drugs depend on human genetic background (PMIDs: [29528249](#), [32115000](#), [34536772](#)). hiPSCs represent a powerful tool to rapidly expand our understanding of how genetic diversity impacts response to drug therapies. Researchers (**Voronova, Sipione, Zochodne, Eitzen**) will use high-throughput screening with integrated phenotyping consisting of scRNAseq, imaging, neuronal activity recordings and metabolic state testing to determine which pro-regenerative molecules or drugs enhance regeneration of genetically diverse hiPSC-derived neurons and/or their axons and oligodendrocytes. Readout assays will be carefully designed using information obtained from Themes 1 and 3.1. For example, *CX3CR1* variants affect NSC proliferation and cytokine production via microglia (**Voronova** unpublished data). Readouts for these variants will be high throughput imaging for proliferative markers in NSC cultures and analysis of cytokine levels in microglia cultures. “Hits” will be prioritized for secondary assessment via AI as in theme 2.3 and further validated in several relevant hiPSC-derived cells. Multimodal assessment of cellular parameters (cytokine production, proliferation, differentiation, survival, neuronal activity, phagocytosis) will provide uniquely robust drug discovery data that increases likelihood of progression to clinical trials. Together, **this will identify variants that impede drug efficacy, enable discovery of drugs that can overcome variant-induced block in cellular regeneration, and advance pharmacogenomic testing by enriching the dataset of known genetic variant-driven differences in drug response**.

EDI in Research Design: >90% of hiPSC lines and genome-wide association (GWAS) studies available via public or private repositories are generated from people of European ancestry (PMIDs [36190496](#), [38260595](#), [37114803](#)). Although these platforms have made transformative changes in the study of the genetic drivers of health, stratification based on underrepresented ancestries often reveals unique genetic influences on various disorders (PMIDs: [35861770](#), [37749244](#)), including variable penetrance of disease modifying variants in specific populations (e.g. prevalence of the APOE-ε4 allele and the degree of AD risk it confers differs by ancestry

[PMID [33155766](#)]). This suggests that poor representation of the global population in research leads to significant gaps in our understanding of basic human cell biology and the genetic basis of disease (PMIDs: [36435871](#), [39362853](#)). Furthermore, the majority of variants identified via GWAS have unknown significance, due at least in part to lack of appropriate methods to study their function. Therefore, we need **relevant human cell models** that accurately represent **global genetic diversity** in order to accelerate discoveries. Finally, **sex and gender** play important roles in neurological disorders, as demonstrated by different prevalences of neurodevelopmental disorders in boys and girls (PMID [37611041](#)), and sex-linked differences in onset and progression of neurodegenerative disorders (e.g. PMID [34683148](#)). Nonetheless, the majority of pre-clinical studies have used only male or only female mice/human cells to model diseases, limiting mechanistic understanding and precluding the development of sex-specific diagnostic/prognostic tools or treatments (PMIDs [20620164](#), [33656929](#)). This is further exacerbated by **historical exclusion and ongoing underrepresentation of women in clinical trials** (PMID: [35687931](#)), limiting our ability to understand sex and gender differences in response to treatment. Our program will strive to develop hiPSC lines from individuals of both sexes and diverse ancestries in order to overcome these limitations. Our clinical team members (**MacPherson, Alexander, Zochodne**) will guide basic researchers in selecting appropriate hiPSC lines that best represent the population affected in each disorder of interest. Our approach will reduce publication bias, enable meta-analysis, support the identification of confounding variables, and advance our understanding of the role of sex and genetics in the field of neurological disorders. Notably, we cannot address social constructs like socioeconomic status, gender, race and ethnicity; however, precision medicine can help to reduce **health inequities** faced by these populations by providing more objective diagnostic and prognostic measures. We recognize the broader challenges that intersect with these efforts, including racism in medicine and historical exclusion of marginalized populations that has led to biased characterization of the clinical populations that will inform our hiPSC development. These issues will be continually discussed among team members, collaborators, trainees and the wider scientific community in order to overcome these barriers and improve efforts to understand disease and wellness in all populations. Informed by emerging resources in this field (e.g. [Jackson Lab Diversity in a Dish training](#)), we will also make every effort to interpret our findings appropriately and not allow their use to fuel discrimination narratives. Collectively, these efforts will yield scientific advances that will benefit both people with lived experience and health research as a whole.

Feasibility: The UofA Women and Children's Health Research Institute ([WCHRI](#)) recently funded a small scale human stem cell lab that is already up and running, and the operating costs of many of the proposed themes have already been funded. Thus, **this SRL is positioned for immediate success**. For example, themes 1.1-1.3 and 2.1 are partially funded by NSERC Discovery grants (**Riddell** and **Sipione**), One Child Every Child CFREF team and catalyst grants (**Riddell** and **Voronova**) and CIHR project grants (**Sipione** and **Simmonds**). Theme 3.1 is funded by the MS Canada discovery grant (**Voronova**), and theme 3.2 is partially funded by a CIHR project grant (**Sipione**). Drug libraries are funded by CFI #42736 "Alberta High Containment Research Infrastructure" (Drs. Hobman and **Eitzen**, UofA). Establishment of hiPSC SRL at UofA will accelerate the success of these research projects, improve translation of the findings as novel diagnostic, prognostic and drug development platforms and support national collaborations facilitated by our **pan-Canadian external Advisory Board**.

Potential for breakthroughs on a global scale: Drugs with mechanisms of action that target biologically significant genetic variants are 2.6 times more likely to succeed in clinical trials,

thus lowering the cost of drug discovery and development (PMIDs [38632401](#), [33748968](#)). Our integrated SRL will be the **first of its kind in Canada** to analyze the impact of genetic diversity on specialized cell function *en masse*. To support these efforts, our SRL will be integrated with WCHRI's [Translational Genomics Hub](#) (TGH; co-led by Dr. **Alexander**), which pairs clinicians and basic researchers studying genetic causes of rare disorders. With WCHRI's support, we have already established a small-scale basic stem cell support laboratory and recruited hiPSC and neurodevelopment expert Dr. Sandeep Gupta, who will join the local team of experts as well as Albertan, Canadian and international partners. Acceleration of pre-clinical drug discovery will be enabled via partnership with UofA's Applied Pharmaceutical Innovation ([API](#)), Canada's largest not-for-profit life sciences commercialization organization, and the Drug Development and Innovation Centre ([DDIC](#)) (please see "Knowledge Mobilization" in Criterion 5). Cell-based therapy development will be enabled through partnership with the Alberta Cell Therapy Manufacturing ([ACTM](#)) facility (led by team member **Korbutt**), which is the only GMP compliant facility in western Canada capable of producing therapeutic-grade cells (e.g. mesenchymal stem and cancer T-cells) for transplantation. Clinical translation will be aided by UofA's Northern Alberta Clinical Trials and Research Centre ([NACTRC](#)) and Multiple Sclerosis Experimental Therapeutics Program (led by collaborator Dr. Fabrizio Giuliani), which provide clinical trials project and data management as well as navigation of the regulatory landscape. Together, we will leverage this infrastructure and existing programs to obtain functional information about the effects of variant sequences, their pathogenicity as well as development and implementation of pro-regenerative treatments.

Alberta is uniquely positioned (relative to other Canadian provinces) to directly correlate results obtained in the hiPSC SRL with patient-specific clinical data, due to the existence of a single province-wide electronic medical record [ConnectCare](#), which provides central access to patient records for almost 5 million people. ConnectCare will fast-track diagnoses and regenerative medicine breakthroughs, supporting significant immediate and long-term impacts on precision health research. This is in line with the United States National Research Council Committee's consensus statement on precision medicine, which recommends in-depth understanding of intrinsic pathobiological mechanisms of disease progression to inform effective individualised treatments (PMID [22536618](#)). Our proposed SRL and research will directly address the need for functional assessment of human genetic diversity to enable precision therapies.

Future directions. While our proposal is focused on neurological disorders, our pipelines can be applied to any disorder of interest that can be modeled in hiPSCs. For example, the [Stollery Children's Hospital](#), which serves several populations with unique founder mutations and genetic conditions. Team member **Alexander** is studying founder mutations that cause renal phosphate wasting, kidney stones and bone disease in Hutterite populations. Likewise, **MacPherson** and colleagues have identified many unique founder variants and candidate genes in Indigenous populations in Alberta, many of which are still unpublished or in preparation, including Lethal Congenital Contractures Syndrome type 3 (PIP5K1C), Short-rib polydactyly (Majewskiy type, TEK1), Central Congenital Hypoventilation Syndrome (due to novel mutations in MYO1H), Juvenile Hemochromatosis, Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay, Autosomal Recessive Polycystic Kidney Disease, among others. Once operational, the hiPSC SRL will facilitate identification/understanding of the mechanism of these disease-causing mutations, enabling novel treatment options and enabling clinical trial participation for these unique populations. We expect numerous additional avenues for innovation and discovery through the work of interested users and collaborators (described in Teams, below).

Alignment with Institutional, Provincial and Global Priorities.

UofA: This proposal is directly aligned with the UofA's Strategic Research Plan (*Forward with Purpose*), which aims to “*develop streamlined and coordinated processes for integrating and supporting the operation and expansion of shared institutional infrastructure*” and to “*broaden, deepen and sustain existing areas of global excellence and growth for addressing grand challenges, while building areas of emerging strength*”. This SRL builds on existing UofA strengths in regenerative medicine to tackle the grand challenges of chronic disorders and the unknown role of genetic diversity in health and disease, facilitated by the strategic positioning of this SRL within existing resources and cores to accelerate its success. Key partners supporting our SRL include: UofA's [WCHRI](#), Neuroscience and Mental Health Institute ([NMHI](#); led by Dr. **Zochodne** [team member]), [Alberta Diabetes Institute](#), Alberta Glycomics Centre ([AGC](#); co-led by Dr. **Sipione** [team member]), Alberta Cell Therapy Manufacturing ([ACTM](#); led by Dr. **Korbutt** [team member]), [Cardiovascular Research Institute](#) and [MS Centre](#). Within "Health and Well-being" *Forward with Purpose* specifically aims to: 1) “*expand and translate knowledge...to advance the health and well-being of all, including underserved populations*”, which we will achieve through a focus on genetic diversity to expand diagnostic and prognostic capacity for underserved groups; 2) “*discover and develop new...cell-based therapies and small molecule therapeutics*”, which our SRL is specifically designed to tackle within the context of regenerative medicine; and 3) “*accelerate cutting-edge research in digital health and applications of artificial intelligence*” which we will advance by generating rich multimodal data that can be used for future training of machine learning models (AI). We will integrate AI in hiPSC maintenance and differentiation (please see Criterion 3: Infrastructure) and when mining for novel drug hits (please see themes 2.3 and 3.2).

Canada: While hosted at the UofA, the goal for the integrated SRL is to improve fundamental understanding of brain development, brain-organ communication and the cellular role of human genetic variants for scientists across the country. To this end, Dr. **Voronova** has assembled an **Advisory Board** to bring the discoveries and technical advancements enabled by this facility to other institutions in Canada. Our team's integration of the **pan-Canadian advisory board** will be supported by lab exchanges between UofA, UofC, SFU, McGill and SickKids to create a coordinated national training effort for future Canadian leaders in regenerative medicine.

World: The World Health Organization has recently recognized “[accelerating access to genomics for global health](#)” as a key priority. A key novel aspect of our integrated SRL is the ability to directly probe links between genetic diversity and human health. While there are multiple efforts underway using organoids to understand the role of genes implicated in human disorders and to screen for potential therapies, to our knowledge, our team would be the first to combine both with systematic high-throughput assessment of individual genetic variants, including reclassification of VUSs into harmless or pathogenic. Our efforts will **provide novel diagnostic tools that accelerate personalized disease prognosis and treatment modelling** for patients worldwide.

CRITERION 2. TEAM

The proposed research will be conducted by a multidisciplinary team of scientists and clinicians with expertise in development, genetics and rare disorders, stem cell biology and culture, cellular communication, and regenerative medicine. Each investigator has a strong track record of research excellence, demonstrated by a combined total of 746 peer reviewed articles and 52,481 citations. Our team members are active in the national and international stem cell, neuroscience

and developmental biology communities and are well-equipped to complete the proposed research and promote use of the requested infrastructure.

Table 1. Competency matrix of team members for proposed research activities.

Team member	Expertise assessment							Scopus, Lens PatCite & Overton statistics (Statistics extracted in January/2025)							
	Stem cells/Regen. Medicine	Genetics	Organ Development	Cell-cell communication	Neurodegeneration	Drug screening	Institutional management or Leadership experience	EDI/Leadership	# clinical trials involved in	h-index	# peer-reviewed articles since 2019	# life-time citations	# patent citations	# policy citations	Current funding (\$M)
Notable DORA (Declaration on Research Assessment) activities															
Voronova	x	x	x	x	x	x		x	-	17	15	1023	16	3	4.7
Alexander	x	x	x			x	x	2	38	55	4760	33	11	7.1	
Eitzen			x	x	x	x		-	24	15	2417	8	2	0.5	
Ioannou	x	x	x	x	x			-	16	15	1555	8	1	4.1	
Korbutt	x					x		2	52	21	18220	746	24	17.9	
MacPherson	x	x	x					2	4	6	159	1	-	-	
Riddell	x	x	x					-	15	9	219	-	-	2.4	
Simmonds	x	x	x			x		-	17	11	1066	26	2	1.2	
Sipione	x		x	x		x	x	-	31	12	4749	121	2	2.4	
Zochodne	x		x	x	x	x	x	12	70	37	18313	160	78	1.0	
Total	6/ 10	5/ 10	6/ 10	8/ 10	5/ 10	2/ 10	6/ 10	4/ 10	na	196	>52k	>1k	123	41.2	

Dr. Voronova, PhD (lead) is an Associate Professor, *Canada Research Chair in Neural Stem Cell Biology and Sloan Research Fellow in Neuroscience* with expertise in pluripotent stem cells, NSCs, developmental biology and regenerative medicine. Since her recruitment to the Department of Medical Genetics in 2018, she has become an internationally recognized expert in brain development and regeneration, as evidenced by four international Young Investigator Awards (including 2023 Young Scientist Lectureship Award from the International Society of Neurochemistry and 2022 outstanding young investigator Jordi-Folch Pi award from the American Society for Neurochemistry), >\$6M competitive funding (including a European Team grant), 15 publications as a PI and >55 invited talks. She has 34 peer-reviewed publications in top ranked journals such as Nature Communications, Neuron, Developmental Cell, and BMC Medicine. She is an avid global collaborator with co-authors representing 57 institutions and 16 countries. Her research aims to understand the cellular mechanisms of neurodevelopmental disorders (PMIDs: [25556659](#), [33996804](#), [38616269](#), [38951500](#)) and the regenerative power of NSCs for brain repair (PMIDs [28472653](#), [34270934](#), [35293825](#), [36608690](#)). By modelling rare disorders and cell-cell communication in stem cells, her team has identified new clinical phenotypes (PMIDs: [38616269](#)), established an NSC drug discovery platform (partnership with AdMare BioInnovations) and submitted a US patent application to treat neurodegeneration. She was recently identified as a “stem cell science starter” by Nature Methods (PMID [39143380](#)). Her exemplary mentorship abilities have been recognized with the 2021 Award for Excellence in

Mentoring Graduate Students and Postdoctoral fellows, while her leadership skills and dedication to outreach are evident by her active roles as advisory board member for the KBG Syndrome Foundation, UofA Lead for the Prairies Regenerative Medicine Hub, and speaker for MS Canada. Her commitment to EDI is evidenced by her role in the American Society for Neurochemistry (ASN) Inter-American Co-operation committee, which funds South American neurochemists to attend ASN scientific meetings. Together, these efforts and achievements underscore the impact of her research and leadership on community and patient groups as well as its relevance for commercialization and clinical translation. *See competency matrix for publications, grants, clinical trials and DORA activities. Inventions reported: 1; patent applications submitted: 1; agreements related to translational initiatives: 6 (e.g., MS therapy development in collaboration with NCE).*

Team Members (listed alphabetically)

Dr. Alexander, PhD, MD, FRCP is a Professor and Chair of the Department of Pediatrics, a Pediatric Nephrologist at the Stollery Children's Hospital and 1 of 7 Distinguished Researchers within the Stollery Science Lab. He conducts both basic and clinical research, from cell-to-cell dysfunction in pediatric kidney diseases to deep phenotyping of clinical populations with rare genetic renal tubular disorders. Drs. Alexander and Caluseriu (hiPSC SRL user) co-created the Translational Genomics Hub (WCHRI, UofA), a funding platform that connects clinicians of patients who have (ultra) rare genetic disorders with basic scientists who can develop assays to understand the genetic basis of their symptoms. His dedication to excellence in health research and training the next generation of scientists is further demonstrated by his previous work as the Associate Director of WCHRI and National Director of the Kidney Research Scientist Core Education and Training Program, where he also altered the program, leadership, and application procedures to better foster EDI, and initiated a black and indigenous summer studentship. *CRC in Epithelial Transport Physiology; Dossetor Research Award (Kidney Foundation of Canada); inventions reported: 1; patent applications submitted: 1; agreements related to translational initiatives: 2 (e.g., intestinal therapeutic discovery with US biopharma).*

Dr. Eitzen, PhD is a Professor in the Department of Cell Biology. He has held leadership positions in education as the director of the undergraduate Cell Biology program and the High School Youth Research summer position coordinator. He is a faculty advisor for the Advanced Cell Exploration (ACE) core, and was instrumental in expansion of its capacity to provide drug screen services to the broader community, including affiliated companies such as the API. His research program is dedicated to understanding pro-inflammatory signaling mechanisms in human immunity, allergy and chronic inflammation. International collaborations have led to contributions to the design of multiple high-throughput drugs screens, resulting in lead drugs for nerve regeneration (USPPA #63/481,506). He has trained over 60 HQP, with projects integrated into clinical units such as the Alberta Respiratory Centre studying lung inflammation and chemical biology with the investigators in Cell Biology and Biochemistry. *AHFMR scholar (2002-2007); Inventions reported: 2; patent applications submitted: 2; agreements related to translational initiatives: 4 (e.g., collaborative drug screening initiatives with industry partners).*

Dr. Ioannou, PhD is an early career researcher (ECR; based on tri-council funding definition of 84 months since first appointment for those who held this status 2020-2022), Assistant Professor in the Department of Physiology. She is an expert in pluripotent stem cells, neurodevelopmental disorders, and regenerative medicine. Her research program investigates lipid transport and metabolism in iPSC-derived neurons and links to pathobiological mechanisms of neurological

disorders. Her significant scientific contributions have been recognized with the 2021 Sloan Fellow in Neuroscience, Martha Cook Piper Research Prize, and Heart and Stroke National New Investigator Award. She is strongly committed to graduate student achievement and well being, as demonstrated by her work on the “Reimagining Graduate Education Working Group” hosted by the Faculty of Medicine & Dentistry. She has presented 36 oral talks at national or international conferences in the last 5 years, including an invited talk at the DZNE conference on “Common mechanisms in childhood and adult neurodegenerative disorders” in Germany June 2024 and was recently named as Journal of Cell Science’s “[Cell Scientists to Watch](#)”, a series which features highly talented scientists who just opened their laboratories. *CRC in Brain Lipid Biology; Sloan Research Fellowship in Neuroscience; agreements related to translational initiatives: 2 (e.g., US biotech funded study of new targets for neurodegeneration therapies.)*

Dr. Korbett, PhD is a Professor in the Department of Surgery and the Scientific Director of the Alberta Cell Therapy Manufacturing (ACTM) Facility. As one of the original Edmonton Protocol team members that developed islet cell transplant to treat Type 1 diabetes, and current Director of the Alberta Diabetes Institute Histology Core, he is a leading expert in diabetes and translational human cell biology research. One of the Korbett lab’s most notable recent innovations is the development of localized immune-suppression to prevent islet graft rejection (PMIDs: [37765170](#), [31650674](#)). His research group is GMP-certified, allowing for rapid bench to clinic translation. As part of this initiative, he co-founded Transcyto Inc. *Alberta Cell Therapy Manufacturing CFI IF (2009, #21733) and Filling vials for Pandemic Preparedness CFI BRIF (2024, #500117) recipient; co-creator of Edmonton Protocol. inventions reported: 8; patent applications submitted: 22; patents: 1; agreements related to translational initiatives: 50 (e.g., drug delivery spinoff company recently created).*

Dr. MacPherson, MD, PhD, FRCPC, DABMGG, FACMG, FCCMG is an ECR, clinical geneticist and Assistant professor in the Department of Medical Genetics. Her background in basic science facilitates multidisciplinary collaborations to understand the molecular mechanisms of rare genetic syndromes, including neurodevelopmental disorders. Keenly interested in precision medicine research, Dr. MacPherson has been a part of 2 clinical trials and is a member of the Alberta Precision Labs Prenatal and Perinatal Working Group. She is committed to incorporating EDI principles in rare disease research, as exemplified in her recent talk at the Canadian Association of Genetic Counsellors Annual Education Conference in 2023 - "Unraveling the Genetic Tapestry: Exploring Special Populations and Hidden Stories of Genetic Disorders in Alberta, Canada". She serves on the Trisomy 18 Advisory Working Group to address ethical issues for neonates born with Trisomy 18.

Dr. Riddell, PhD is an ECR and Associate Professor in the Department of Obstetrics and Gynecology. Her research program studies cell-cell interactions to understand placental morphogenesis and pathogenesis of pregnancy complications. Although early in her career, they have made a number of significant advances, including the development of a neoglycolipid-containing liposome for target drug delivery to the placental epithelial cells (USPPA #63/497,540), leading to invitations to speak at the largest conferences for reproductive biology (2022; Society for Reproductive Investigation) and the International Federation of Placenta Associations (2024). She is a strong supporter of trainee development and well-being, as evidenced by her active participation in the Graduate Program Committee in the Department of Obstetrics and Gynecology as well as “Reimagining Graduate Education Working Group” in her Faculty. *CRC in Maternal-Fetal Interface Biology in Health and Disease; Peter Lougheed*

Research Award; inventions reported: 1; patent applications submitted: 2; agreements related to translational initiatives: 5 (e.g., NCE funded development of immunomodulatory compounds.)

Dr. Simmonds, PhD is a Professor and Chair of the Department of Cell Biology and the academic lead of the Faculty of Medicine & Dentistry Cell Imaging Core, an essential partner for a hiPSC SRL. His research program is dedicated to understanding the cellular mechanisms of human development, modelling rare diseases and neurodevelopmental defects in fruit flies, mice and mammalian cells. This focus on highly specific regulatory processes led him to develop TRAP tagging early in his career, a patented technique to isolate proteins associated with RNA molecules (US20060105341A1). He is a strong advocate for research training, as demonstrated by >20 years of undergraduate teaching experience (including directing the Cell Biology undergraduate research program) and direct supervision of 84 undergraduate students in addition to 8 graduate students and 5 PDFs. An trainee working in his lab launched the Edmonton-based biotech [Future Fields](#), using novel technology to produce clinical grade growth factors for stem cell culture. The success of his trainees led to a *2019 Award for Excellence in Mentoring Graduate Students and Postdoctoral Fellows; AHFMR senior scholar (2002 - 2014); patents: 1.*

Dr. Sipione, PhD is a Professor in the Department of Pharmacology and Associate Director of Glycomics Institute of Alberta. She investigates how lipids and glycolipids (gangliosides) affect brain cell physiology in neurodegeneration, neuroinflammation, and neuroprotection. She has pioneered studies on the role of gangliosides in HD pathogenesis and treatment, and identified novel functions for gangliosides in microglia responses to their microenvironment and cell-to-cell communication. Her work has led to 1 patent (now licensed to a biotech company), with 2 additional patents pending, demonstrating her commitment to commercialization. Her outstanding research community involvement is exemplified by her roles as the associate director of the Glycomics Institute of Alberta, the Canadian delegate for the Sphingolipid Club (international association) and nomination committee member for the Canadian Association for Neuroscience. Her dedication to EDI is evidenced by the various initiatives she has spearheaded as a member of Canadian Glycomics Network EDI Committee, including adoption of the “LOOP” App for anonymous reporting of discrimination, bullying or harassment, as well as participation in various EDI outreach seminars, panels, and presentations. *AHFMR Scholar (2007-2014), CRC in Neurobiology of Huntington’s disease (2006-2016), Alberta’s Top 50 most influential people (2012 Venture Magazine); inventions reported: 4, patent applications submitted: 17; patents: 2; agreements related to translational initiatives: 23 (e.g., Ganglioside therapy for HD; issued patents in US and Canada licensed to US biotech.)*

Dr. Zochodne, MD, FRCPC is a neuroscientist, clinical neurologist and Director of the UofA Neuroscience and Mental Health Institute (NMH 2015-2025) and has led basic neurobiology laboratory since 1988 with active participation in clinical trials. His seminal work has identified several strategies to promote peripheral neuron regeneration and reverse experimental diabetic polyneuropathy. These include the identification of novel targets PTEN, Rb1, APC and Mad1 (e.g. a provisional patent for small molecule Rb1-E2F1 inhibitors) for enhancing axonal regeneration. He has also co-authored an AI-driven approach to therapeutic drug discovery (PMID [38565852](#)). His lab is a core member of a peripheral neuroregeneration group of investigators within NMHI (Chan, Webber, Curran, Kerr, Zochodne). His leadership is evident through his previously held roles of Divisional Director of Neurology (UofA), Director of Clinical Neurophysiology/EMG (Calgary), and Director of the Regeneration Unit in Neurobiology (Calgary). *2019 Gebhart Prize for Excellence in Peripheral Nerve Research by the International Peripheral Nerve Society; inventions reported: 1; patent applications submitted: 2;*

agreements related to translational initiatives: 8 (e.g., industry funded clinical research in diabetic neuropathy).

Collaborators: In addition to this team, the requested infrastructure will serve numerous collaborators in Canada and abroad as well as support several new and established Networks of Centres of Excellence (NCEs) and industry partnerships to translate discoveries into new clinical tools and therapies for chronic disorders. Collaborators include:

hiPSC experts Drs. Valentina Fossati (NYSCF), Steven Kushner (Erasmus MC), Tom Durcan (McGill; advisory board member), Julien Muffat (SickKids; advisory board member), Lisa Wagar (U. California), Igor Adameyko (Medical U. Vienna), Justin Chun (U. Calgary; advisory board member) as well as Fabrizio Giuliani, James Shapiro and Patrick MacDonald (UofA), along with **microfluidics and tissue engineering experts** Alessandro Grattoni (Houston Methodist), Juan Gnecco (Tufts U.), Lisa Julian (SFU; advisory board member), Amy Tsai and Mahdi Hamidi (UofA). These researchers' expertise in hiPSC differentiation and bioengineering will contribute brain-brain and brain-organ cellular communication activities in Theme 1.

Single cell omics experts Drs. JoAnne Stratton (McGill), Sophie Petropoulos (McGill), Scott Yuzwa (U. Toronto) and **organismal developmental and rare disorders experts** Maria Lehtinen (Harvard), Maria Cecilia Angulo (INSERM), Jing Wang (U. Ottawa), Stephen Renaud (U. Western Ontario), Guang Yang and Micheil Innes (U. Calgary). These experts in disease mechanisms will contribute to the team's disease modelling activities in Theme 2.

Regenerative medicine and drug development experts Hubert Tse (U. Kansas), Jeff Biernaskie (U. Calgary), Valerie Verge (U. Saskatchewan), Ayman El-Kadi, Khaled Barakat, and Raimar Loebenberg (UofA) will contribute to the team's prognostic disease modelling activities in Theme 3 and provide a valuable interface to industry accelerators (e.g. API).

Industry and NCE partners Ardelyx Pharmaceuticals, Biogen, Zulia Biotechnology, WinSanTor Inc., Future Fields, Janssen Research & Development, NAEJA Pharmaceuticals, ChemRoutes Corp, Centre for Drug Research and Development, adMare BioInnovations, GlycoNet, Stem Cell Network, and BioCanRx will help the team to develop and evaluate new and existing approaches to treating neurological diseases in Themes 2 and 3.

Alongside the team members and collaborators, 20 **other users** from the UofA Faculties of Medicine & Dentistry, Science as well as Pharmacy & Pharmaceutical Sciences will utilize the requested infrastructure as an integral part of their innovative basic and translational research programs focusing on the mechanisms of human disease and regenerative medicine:

Table 2: Other Users at the UofA

Other User	Department	Program Description
Ted Allison	Biological Sciences	Drug screening and development for dementias and blindness
Matthew Benson	Ophthalmology & Visual Sciences	Cellular and molecular mechanisms of retinal degeneration
Fred Berry	Surgery	Genetic regulation of growth and development of the skeleton
Oana Caluseriu	Medical Genetics	Clinical characterization of rare pediatric genetic disorders
Brittany Carr	Ophthalmology & Visual Sciences	Genetic and molecular basis of inherited and age-related retinal degeneration.
Sandeep Gupta	Cell Biology	Modelling neurological disorders in human spinal cord organoids
Matthew Macauley	Chemistry	Glycobiology of disease relating to immune and brain cells
Rebecca Hull-Meichle	Cell Biology	Microenvironment in pancreatic islet function in Type 2 diabetes
Marek Michalak	Biochemistry	Calcium signalling in ES-derived cardiomyocytes

Evangelos Michelakis	Medicine; CVRI director	Epigenetic mechanisms of cardiac cell fate regulation
Peter Light	Pharmacology	Mechanisms of congenital long QT syndrome mutations
Gavin Oudit	Medicine	Gene editing and cell based therapies for cardiomyopathies
Andrew Pepper	Surgery	Generation of stem cell-derived islets for Type 1 diabetes
Jason Plemel	Medicine	Microglia cell mechanisms of neurodegeneration in MS
Peter Senior	Medicine; ADI director	Treatments for Type 1 diabetes & related complications
Qiumin Tan	Cell Biology	Mechanisms of hippocampal development
Deniz Top	Cell Biology	Molecular mechanisms of behavioural disorders
Sue Tsai	Medical Microbiology & Immunology	Genetic basis of autoimmune diabetes & beta cell dysfunction
John Ussher	Pharmacy & Pharmaceutical Sciences	Obesity mediated alterations in energy metabolism
Toshi Yokota	Medical Genetics	Antisense oligonucleotide treatments for genetic rare disorders

Any UofA researcher with expertise and/or collaborations in hiPSC and/or regenerative medicine will have the opportunity to join the SRL as a user of the facility, which will be advertised widely within the UofA community. Our environmental scan in collaboration with the College of Health Sciences has already identified >100 members of the Faculties of Medicine & Dentistry, Rehabilitation Medicine, Science, Kinesiology and Nursing who are interested in working with the CHaRM hub. We will continue to perform environmental scans in collaboration with the UofA Research Partner Network and College of Health Sciences in order to proactively seek out users who would benefit from the resources and collaboration offered by this SRL.

Systemic barriers. Despite some recent improvement, the Faculty of Medicine & Dentistry still has a striking lack of gender equity among their faculty members, which is exacerbated at advanced career stages. While there is ~60% female representation at the undergraduate level in the medical and life sciences, only 33% of Full Professors in the faculty identify as female. Generally, gender disparity in Canadian academia is worse in leadership positions (PMID [36382515](#)), with only two U15 universities currently led by women. This imbalance in senior academic positions is linked to uneven recruitment, but also retention (aka the ‘leaky pipeline’). Barriers to advancement for women and visible minorities are multifaceted, ranging from [toxic environments](#), use of less assertive language in reference letters emphasizing efforts instead of accomplishments, increased likelihood to take on ‘non-promotable’ work and similar issues that exacerbate disparities in traditional academic productivity metrics (e.g. committee memberships, etc.) (PMIDs [38507318](#), [38974126](#)). Women and visible minorities also face greater barriers to competitive research funding (e.g. female CIHR applicants receive fewer, smaller value awards [PMID [31613898](#)]), and are under-represented in major awards categories, such as Canada Research Chairs and Nobel prizes (PMID [31587874](#)). Together, this results in a lack of role models for underrepresented groups in research that compounds these problems. Beyond gender, race, age, sexual orientation and other factors similarly drive inequities in academia, but a paucity of data on representation in higher education prevents the quantification of these barriers, and highlights the need to better understand this landscape.

Practices to address underrepresentation and build an inclusive environment. The Faculty of Medicine & Dentistry (in which this SRL will be housed) is committed to championing EDI in training, work and service environments in line with the UofA’s strategic research plan, which aims to “attract, support and retain exceptional researchers to build on the research strengths of the university and contribute to an ecosystem that leads with purpose” and “enhance our research ecosystem to support researchers from equity-denied groups, including Indigenous

*Peoples, women, visible minorities, researchers from Black, 2SLGBTQ+, francophone and diasporic communities, and persons with disabilities or who identify as disabled.” Evidence has shown that [female-led teams have been shown to produce more innovative and disruptive ideas](#), and that publications authored by ethnically, geographically and gender diverse teams have higher impact (PMIDs [36037387](#), [31430380](#), [29875493](#)). Thus, team selection was made with careful consideration of both our institutional EDI goals as well as the Declaration on Research Assessment (DORA), which consider traditional and non-traditional metrics of productivity, to counter the focus on high-impact research papers that often excludes ECRs and undervalues alternative methods of knowledge dissemination. We made every effort to ensure equitable participation of researchers at all career stages (four early, one middle, and five senior career researchers), and aimed to increase representation in as many ways as possible, while building a core team of leaders in this field. We take pride in the current **diversity** of our team members, 50% of whom belong to at least one federally designated equity group.*

When hiring professional staff for the SRL, EDI best practices will be applied throughout job posting, hiring, training and retention. Specific strategies will include: listing only the necessary qualifications and skills and using inclusive and unbiased language in job postings, providing salary transparency, assembling a diverse *selection committee* (members of which will receive EDI training in hiring), blinding submitted applications (before shortlist is created), using the same *assessment* process and pre-selected *shared interview questions* for all interviewed candidates, using an *evaluation checklist* to keep deliberations aligned with the job advertisement. Postings will be shared widely through a range of professional networks (LinkedIn, Canadian Association for Neuroscience, Stem Cell Network), as well as on job boards that aim to increase representation in science (e.g. [oSTEM](#), an international nonprofit organization for LGBTQ+ people in the STEM community).

To support career advancement of our faculty members, we will encourage participation in “[helping hands](#)”, an expanded faculty peer review mentoring program aimed at navigating career stage-specific tasks or growth. We will also provide mentorship by pairing CHaRM hub ECRs (along with mid-career researchers from underrepresented groups) with more senior researchers in stem cell and regenerative medicine fields to support their promotion and avoid seniority attrition. Finally, to avoid any excessive service for researchers from underrepresented groups and encourage opportunities for new leadership and perspectives, leadership positions and membership in CHaRM steering and other committees will be limited to two years.

To build an inclusive working environment, all team members and users will receive ongoing EDI and unconscious bias training provided by UofA, and will be required to familiarize themselves with the Faculty’s [anti-racism policy](#). These efforts will aim to ensure that our research team remains cognizant of unconscious, implicit, overt, prejudicial, and any other kinds of bias. Group meetings will encourage open communication and exchange of ideas through ‘round robin’ discussion to ensure all voices are heard. We will also aim to proactively accommodate personal circumstances/needs that may intersect with work activities (e.g., cultural holidays or customs, communication styles and preferences, working hours, family obligations and more). In consultation with the Faculty Affairs Office, we will include open discussions about EDI as a standing agenda item at our facility meetings, and create multiple avenues for disclosure and assessment of EDI related concerns. Following an initiative co-led by team member Dr. **Sipione**, we will partner with the [LOOP App](#) to offer a safe space for anonymous reporting of harassment, discrimination, bullying, microaggression or other unfair treatment in connection with the SRL or CHaRM hub. In addition, all members will be informed of avenues

for reporting through the UofA Office of Safe Disclosure as well as Professionalism and Racism Reporting within the faculty. Collectively these efforts will ensure that we build an environment that celebrates diversity of perspectives and lived experiences, and builds off these strengths.

Please note EDI in HQP training is described in Criterion 5 (“Benefits”).

CRITERION 3: INFRASTRUCTURE (total cost \$10,900,121)

UofA is requesting the proposed infrastructure to enhance institutional capacity to study human health and genetic diversity. **The requested infrastructure (Table 3) is not currently available at the UofA and is essential to position Canada as a global leader in human genetic diversity and regenerative medicine research.** The team expects to use at least 80% of the capacity of the equipment across the five-year funding period. The remaining 20% capacity will be available to the other users (identified in table 2), as well as current and future collaborators.

Table 3. Infrastructure essential for proposed research activities.

	Theme 1			Theme 2			Theme 3		
	1.1. Cell-cell communication	1.2. Brain-organ communication	1.3. Novel pro-regenerative molecules	2.1. Genophenotype rare mutations & VUSs	2.2. Predict comorbidities in neurological disorders	2.3. Rescue specific cellular deficits	3.1. Genophenotype disease modifying variants	3.2. Therapies for disease modifying variants	
hiPSC suite (Item 1)	•	•	•	•	•	•	•	•	•
Drug screening suite (Item 2)	High throughput neuronal network activity		•	•				•	
	Automated Patch Clamp		•			•			•
	High throughput drug screening station		•			•			•
Cell analysis suite (Item 3)	Single cell multiomics system	•	•	•	•	•		•	
	Hi Fidelity long read sequencer	•	•	•	•	•		•	
	Precision DNA shearer	•	•	•	•	•		•	
	Nucleic acid fragment analyzer	•	•	•	•	•		•	
	Genomic Stability Analyzer	•	•	•	•	•	•	•	•
Imaging suite (Item 4)	Upright and inverse light and fluorescent microscope	•	•	•	•	•	•	•	•
	Fast, gentle super-resolution live imaging of organoids	•	•	•				•	
Project manager (Item 5)	Project manager	•	•	•	•	•	•	•	•
Data Storage (Item 6)	Data Server	•	•	•	•	•	•	•	•
Ext. warranties (Items 7-10)	Extended warranties	•	•	•	•	•	•	•	•
Renovations (Item 11)	hiPSC SRL, ACE and CIC cores renovations	•	•	•	•	•	•	•	•
Training (Item 12)	Training of core personnel	•	•	•	•	•	•	•	•

Valuation and In-kind. All costs are based on quotes procured from specialized vendors; any associated purchases will undergo UofA’s competitive bid process. Extended warranties in excess of standard warranties (in total lasting for the duration of this grant) have been incorporated into the main budget for the majority of the requested equipment to ensure optimal and prolonged lifetime. When extended warranties are not noted, standard warranties cover the initial five years of the grant or will be obtained via infrastructure operating fund (IOF).

Location. All equipment will be housed in the Faculty of Medicine & Dentistry at the UofA. The hiPSC SRL will be housed in a new dedicated space (KATZ 3-105 and 3-108A, LKS 3-116), in physical proximity to CHaRM Hub members (e.g. HMRC 3-17 and 3-32). Single cell sequencing and microscopy systems will be housed in KATZ in the ACE and CIC cores. Thus, all equipment will be located in adjoined buildings (LKS, KATZ and HMRC).

hiPSC suite (item 1; \$2,716,950) and associated extended warranties suite (item 7; \$578,575). **Items 1.01:** Cryogenic storage for cells (\$89,403.88); **1.02:** three ultra-low freezers for supplements (\$49,929 x 3; total \$149,786); **1.03:** cold storage unit for storing media (\$5,742.61); **1.04:** Fume hood (\$22,969); **1.05:** Automated hiPSC maintenance, organoid

seeding and live long-term imaging system (\$1,826,402) and **7.01** associated extended warranty (\$562,425); **1.06:** six biosafety level II cabinets (\$22,785 x 6; total \$136,711) and **7.02** associated extended warranty (\$1,037); **1.07:** five tissue culture incubators; (\$17,916 x 5; total \$89,579); **1.08:** two shakers for organoids (\$7,216 x 2; total \$14,433); **1.09:** five centrifuges (\$17,795 on average x 5; total \$88,975); **1.10:** nucleofector (\$102,255) and **7.03** associated extended warranty (\$27,802); **1.11:** cell counter (\$30,409) and **7.04** associated extended warranty (\$9,048); **1.12:** device temperature monitoring system (\$109,299); **1.13:** autoclave (\$94,550).

The requested hiPSC suite will equip an upgraded facility for multi-user hiPSC line expansion and differentiation. This equipment will be housed in rooms that have the appropriate positive pressure. Team members will utilize this SRL to develop models of cell-cell communication (Alexander, Ioannou, Korbutt, Riddell, Simmonds, Sipione, Zochodne, Voronova; Theme 1), re-classify VUSs associated with neurodevelopmental and neurodegenerative disorders (all 10 team members; Theme 2), and test pro-regenerative therapies in iPSCs with and without genetic disease modifiers (Eitzen, Sipione, Voronova, Zochodne; Theme 3). Thus, the requested infrastructure is *critical to support all proposed themes*.

Importantly, we are requesting infrastructure to enable research that will be the first of its kind in Canada and abroad, while capitalizing on existing CFI-funded infrastructure (available locally and nationally) to support partnerships that will accelerate research and improve functionality. Specifically, generation of hiPSC lines will be outsourced to and supported by Drs. Durcan and Chun at McGill/UCalgary and local researchers Giuliani and Shapiro. For example, the initial hiPSC 10-line repository (funded by One Child Every Child awarded to **Voronova**), has been completed by McGill's Early Drug Discovery Unit (Advisory Board member Durcan CFI #42847). Thus, no equipment is requested for generation of hiPSC lines. We will utilize the existing UofA biobanking facility to deposit and store hiPSC lines, established in 2000 and led by Dr. Bruce Ritchie (Fedorak CFI#8810). We are requesting one liquid nitrogen dewar system for temporary storage of hiPSC vials before transporting for long-term storage. This will also serve as a back-up in case of unforeseen circumstances. We will use a hypoxic workstation available in KATZ under the management of the Department of Oncology (Postovit CFI#32351). This workstation (consisting of separate incubators) allows work under precisely controlled low oxygen conditions without exposure of cells to room atmosphere so growth and differentiation capacity of stem cell lines under different O₂ levels can be compared (e.g. placental organoids). Finally, we will use microfluidics equipment available in the Tsai lab (UofA; CFI#34546).

We are requesting both routine and unique equipment for the maintenance and differentiation of hiPSC lines. Please note that given the nature of hiPSC culture, these items cannot be shared with other groups and must not have been used for other experiments. Common equipment required to maintain iPSC lines includes: 1) three -80°C freezers and fridge for storing media, growth factors, tissue culture and temperature sensitive reagents in main rooms associated with hiPSC SRL); 2) six dedicated biosafety cabinets and five tissue culture incubators (normoxic and hypoxic) for hiPSC maintenance and differentiation; this ancillary equipment will enable experiments not possible to perform in an automated hiPSC maintenance organoid seeding and live long-term imaging system (item 1.05, described below) and will facilitate HQP training (training equipment will be housed separately from main SRL to avoid contamination); 3) cell-counter and five table-top centrifuges for various volume tubes to handle hiPSC lines; 4) two shakers for organoid generation and cultures to be housed in normoxic and hypoxic incubators; 5) nucleofector for gene editing workflows; 6) a device temperature monitoring system to ensure constant temperature monitoring in critical equipment (not offered by UofA).

A unique aspect of our requested infrastructure is automated hiPSC maintenance, organoid seeding and a live long-term imaging system. hiPSC cultures are extremely demanding in terms of knowledge, expertise and time required. Variability in hiPSC results is a serious problem contributing to the reproducibility “crisis” in biomedical research results (PMID [28794201](#)), and arises from differences in techniques, experimenters and/or environmental conditions. Automation of hiPSC cultures improves cell manufacturing, genomic stability and differentiation into various cell types by selecting optimal cells (clones) for maintenance and differentiation (PMIDs [32500349](#), [36524054](#)). Not surprisingly, industry hiPSC biobanks are already using automated iPSC techniques (e.g. [New York Stem Cell Foundation](#)); however adoption in academia has been slow due to high cost, time and knowledge required to build custom robots (PMID [36524054](#)). Fortunately, leading microscopy and automation companies have started to release affordable “one stop” automated cell culture systems that uniquely support hiPSC maintenance and differentiation. The requested automated system consists of an incubator that can house up to 44 plates, a confocal plate imaging system with incubator, automated liquid handler and a robot allowing accurate passaging of hiPSC lines, media exchanges, organoid seeding and morphological long-term phenotyping of cell states. This is enabled by an integrated AI based learning module that removes poor quality (differentiated) clones, while maintaining good quality (undifferentiated pluripotent) clones (PMID [32500349](#)). Using AI-based decision-making and high-content imaging in a fully automated and sterile environment, the requested automation system offers 24/7 hands-free operation, reduces human error, removes variability and increases the likelihood of success. Thus, automated hiPSC passaging and differentiation system is *critical for this project* by i) ensuring regular and consistent maintenance of all cell lines; ii) understanding and tracking differentiation paths and morphologies over weeks/months of growth; iii) elevating quality control to industry level standards, thus accelerating opportunities for drug development and commercialization. **These functionalities are not currently available at UofA or in any Canadian iPSC facilities.**

Drug Screening Suite (item 2; \$2,420,325) and associated extended warranties suite (item 8; \$383,504). **Items 2.01:** High throughput neuronal network activity measurement system (\$517,413) and **8.01** associated extended warranty (\$198,744); **2.02:** Automated Patch Clamp for single neurons coupled with drug screening (\$337,933) and **8.02** associated extended warranty (\$114,379); **2.03:** High throughput drug screening station (\$1,564,980) and **8.03** associated extended warranty (\$70,381).

The requested high-throughput neuronal network activity measurement system is required to trace functional outputs of neuronal networks in 2D cultures and brain organoids. Automated patch clamp allows the electrical activity of *individual* neurons to be measured. Both systems significantly increase efficiency and reproducibility over manual systems, and allow unbiased screening of variants and/or drugs impact on neuronal activity. **These functionalities are not currently available at UofA.**

The high throughput drug screening station is *critical for this project* and will enable testing of pro-regenerative molecules identified across all themes. This station consists of two liquid handlers (one for sterile seeding and maintenance of hiPSCs and one non-sterile for fixing and staining resultant hiPSC cultures for automated analysis), one biosafety cabinet, two tissue culture incubators and one high-content high-resolution imaging plate based system. This essential drug-screening infrastructure will complement the existing Echo 650 Series Acoustic Liquid Handler optimized for precise and contact-free acoustic transfer of drug libraries in nL volumes (Advanced Cell Exploration ACE core). A resulting one-stop station will automate

hiPSC seeding and media changes after drug library replication and dilutions (to be housed in a sterile biosafety cabinet), subsequent incubations in tissue culture incubators (x2), followed by automated cell processing (e.g. fixation and immunostaining) and culminating in a high content imaging plate based system. This imaging system will also be integrated with the Seahorse 96XFe housed in the ACE Core, providing morphology and cell normalization data during metabolic phenotyping of various cell states. This station will be used for drug screening on 2D hiPSC cultures (contrasted by item 4.02 for thick organoid imaging). **An integrated drug screening station is not currently available at UofA.**

Cell Analysis Suite (item 3; \$1,909,867) and associated extended warranties suite (item 9; \$782,827). **Items 3.01:** Single cell multi-omics system for capturing all and rare cells (\$103,867) and **9.01** associated extended warranty (\$42,526); **3.02:** High Fidelity long read sequencer (\$1,085,750) and **9.02** associated extended warranty (\$501,696); **3.03:** Precision DNA shearer (\$61,976) and **9.03** associated extended warranty (\$9,214); **3.04:** Nucleic acid fragment analyzer (\$122,513) and **9.04** associated extended warranty (\$68,351); **3.05:** Genomic Stability Analyzer (\$305,761) and **9.05** associated extended warranty (\$131,040).

Single cell RNA sequencing and associated quality control instrumentation are *critical for this project* to enable analysis of i) differentiation in 2D and 3D (including cell villages and brain chimeroids derived from different iPSC donors; PMIDs [36796362](#), [38926573](#)), ii) re-classification of VUSs, and iii) characterization of the role of disease modifying variants on cellular processes. The ACE core currently houses an oil emulsion partitioning system, which is limited by cell shape, size and robustness as the cells are sent through the microfluidic device. A gentler gravity-well based system is required for iPSCs that differentiate into more delicate cell types with complex morphology (e.g. neurons). The single cell multiomics system for capturing all and rare cells with onboard imaging and viability checks pre-transcript capture will increase confidence in the quality of each assay prior to costly and time consuming sequencing and analysis. **This functionality supports all 3 themes and is not available at UofA.** Notably, we will use existing synthetic biology workstation Telesis BioXP 3250 to build barcoded DNA variant libraries (ACE; funded by a Striving for Pandemic Preparedness award to Dr. Noyce).

High Fidelity long read sequencing is used to accurately sequence long stretches of DNA (18kb versus traditional sequencing of 50-300bp reads), including GC rich and multiple repeat regions at scale. Because there is very little modification of DNA before sequencing, the quality of the input DNA is paramount to a quality run. The Precision DNA shearer will ensure meticulous shearing of genomic DNA into appropriate sized strands. High Fidelity sequencing technology can also call various methylation (epigenetic) states of base pairs without additional processing. This unit is *critical for this project* to sequence hiPSC genomes and transcriptomes (and validate gene-edited iPSCs), survey genomic and epigenetic polymorphisms, and identify additional gene candidates and disease-state modifiers. **These functionalities are not available at UofA.**

The nucleic acid fragment analyzer is *critical* to both long read and single cell sequencing workflows by enabling quantification and quality control of input DNA, captured cDNA, intermediate library prep products and final libraries to be loaded onto a sequencer. A pulse field electrophoresis analyzer is required for precisely analyzing large genomic DNA fragments that cannot be resolved well on a non-pulse field system which is critical for accurately loading High Fidelity long read sequencers. **This functionality is not available at UofA.**

A genome stability analyzer is *critical* to detect any genomic aberrations in hiPSC lines that can occur with gene editing and/or extensive culture (PMIDs [21211785](#), [30010673](#)). The requested

genome stability analyzer will complement long read sequencing (requested above) as well as already available qPCR and ddPCR instruments. Specifically, optical genome stability analyzers were recently shown to detect structural variations in hiPSCs not detectable via other methodologies, impacting the conclusions of any research involving hiPSCs (PMID [39425080](#)). Thus, this functionality, which is not available at UofA, is *critical* for this project.

Imaging Suite (item 4; \$595,731) and associated extended warranties suite (item 10; \$189,174). *Items 4.01:* Upright and inverted light and fluorescent microscope (\$115,045) and **10.01** associated extended warranty (\$26,036); **4.02:** Fast, gentle super-resolution live imaging of organoids and tissues (\$480,685) and **10.01** associated extended warranty (\$163,138).

A vital part of this application is added functionality for our existing core facilities to better support hiPSC and organoid imaging. The Cell Imaging Core (CIC) houses confocal, two-photon and laser microdissection microscopes that will support this project, but the addition of upright and inverted light and fluorescent microscopes will enable initial live and fixed hiPSC screening in the SRL. Likewise, an imaging unit suitable for **fast, gentle super-resolution live imaging of organoids** will bolster an existing CFI-funded system housed in the CIC (Riddell CFI#40656). The resulting suite will enable long-term acquisition of hiPSC-derived organoids under atmospherically controlled conditions. This upgrade combines existing widefield with superresolution imaging of large spatial volumes (e.g. an entire organoid) at high temporal and optical resolution. **While some similar functionality exists at UofA, existing microscopes are not housed within a dedicated sterile laboratory required for analysis of stem cells.**

Project Manager (item 5; \$428,111): The launch of this new SRL will require a project manager (0.8FTE TRAS level I with benefits) to coordinate installation of the infrastructure.

Data Storage suite (item 6; \$183,317): In consultation with the UofA Information Services and Technology (IST) staff, a 650 TB storage server is requested to store data generated with requested infrastructure (including training datasets), and provide on-board computing power for analysis. At 80% capacity, we expect to generate 160 TB per year. Because core service, such as ACE core service, is not included as appropriate usage of Digital Research Alliance of Canada resources, but is central to the success of the experiments enabled by the infrastructure proposed in this grant, this hardware will be housed in a secure server room operated by the IST and ACE core staff in compliance with all required physical and cyber security.

Renovations (item 7; \$626,449) are required to house hiPSC, single cell sequencing, high throughput drug screening station, cell imaging equipment and autoclave in KATZ 3-108A, KATZ 3-101, KATZ 6-081(A), HMRC 3-27, HMRC 3-32, HMRC 2-17(A), LKS3-116.

Initial training of infrastructure personnel (item 12; \$49,276): Extensive user training is required prior to operational use of requested equipment. hiPSC SRL, ACE and CIC personnel will receive training from vendors before training HQP in team members and other users' labs.

CRITERION 4: SUSTAINABILITY

The majority of the requested infrastructure (95%) will be housed in multi-user core facilities to coordinate oversight, maximize access, and facilitate long term sustainability. Consequently, there are already significant dedicated financial and personnel resources in place to support this infrastructure. This includes the newly established WCHRI basic human stem cell lab, and the Faculty of Medicine & Dentistry ACE and CIC cores. This removes the operational burden on the project team (e.g. collecting user fees) and allows the instrument operation within a proven cost-recovery framework. Instruments will be incorporated into existing core facility fee

structures and made available to an existing large user base on a centralized booking platform. Fees and usage rules will be governed by standardized core facility operational guidelines that have been refined over the past 15 years to ensure optimal operation of shared instruments.

Governance. Infrastructure placed in the ACE and CIC will be integrated into existing governance structures, including academic user committees that guide strategic direction, user fees and policies with administrative and operational support (including funding technical staff positions) provided by the Faculty's Office of Research. The hiPSC SRL will be governed by a similar academic user committee, chaired by **Voronova** and representatives from WCHRI and the Translational Genomics Hub (**Alexander**), and supported by an external advisory board composed of national hiPSC experts, which include Drs. Durcan (McGill), Muffat (SickKids), Chun (UCalgary) and Julian (SFU). The CHaRM hub will be led by an advisory board led by **Voronova** along with representatives from involved UofA research institutes (WCHRI, NMHI, ATI, ADI, CVRI), two rotating CFI team members, a commercialization expert Dr. Paramita Chaudhuri (lead of UofA's [Health Innovation Hub](#) and Director of Programs and Ecosystem Development at [Applied Pharmaceutical Innovation](#)), and Alex Bar (person with lived experience). This group will oversee HQP training, commercialization, translation, integration of basic and clinical research and other strategic initiatives.

Access. The requested infrastructure will be used exclusively for research purposes, with priority access granted to the research team, who expects to use 80% of the infrastructure capacity over the five-year period. Any remaining capacity will be made available to other researchers including the 20 other users, as well as local, provincial and national collaborators. As is standard for core facilities, potential users will discuss their projects with core staff and provide a project description for review and approval by the academic user committees. The level of infrastructure access (autonomous use, assisted use under the supervision of core staff, or service request only) will be granted based on the discretion and approval of the academic committee and core management staff. Approved users will book infrastructure and services through a project management software suite like that currently used for ACE and CIC. All users accessing the infrastructure autonomously will be trained by ACE, CIC or SRL personnel. Infrastructure and services will be advertised via public-facing websites (e.g. CFI Navigator) and existing internal mechanisms (e.g. UofA core facility technology seminars, WCHRI Services & Programs website) to promote maximum usage by researchers locally, provincially and nationally.

Operations and Management (O&M). A dedicated CHaRM Project Manager will facilitate equipment installation, supported by an experienced (20+ years) SRL research associate (Dr. Dawn Zinyk) and a research technician to oversee equipment use, maintenance, access, and training. SRL staff salaries will be supported by the IOF (\$717K), WCHRI and user fees. Similarly, existing CIC (0.25 FTE) and ACE (0.75 FTE) technical staff will support equipment calibration, routine maintenance, and facilitate user training with salary support from IOF (\$313K), the Faculty, and user fees. Highly trained technical CIC and ACE personnel including managers Drs. Xuejun Sun, Hilmar Strickfaden and Michael Wong will provide day-to-day operational oversight and support researchers with experimental design, method development and infrastructure utilization. Technical personnel in hiPSC SRL, ACE and CIC cores are estimated to cost \$1.5M/5 years supported by existing internal sources. WCHRI and the Faculty will also provide in-kind O&M administration support. Operating funds held by team members will support minor equipment and technical staff for other routine O&M. Supplies required for operation, such as plates for testing and calibration, gases for hiPSC cultures, cleaning supplies, etc., (estimated cost \$83K/5 years) will be supported by IOF (\$46K) and user fee revenue

(\$37K). As a novel approach to reduce operating costs, we have partnered with a local company ([Future Fields](#)) that specializes in developing lower-cost and bespoke reagents for stem cell culture. To maximize major equipment longevity, extended warranties are included in the capital budget request for each covering preventative maintenance, services and repairs for the first five years. The requested autoclave will be maintained and serviced by the Faculty Autoclave Repair Core with the cost (\$2,200/year) covered, for the duration of the project, by the Faculty of Medicine & Dentistry and supported by internal core budget sources.

User Engagement: The requested infrastructure provides functionalities not previously available in Alberta, and in some cases, Western Canada. Thus, while users identified in the application will undoubtedly make good use of the new infrastructure, we also plan for user base growth as hiPSC technology is now part of almost all areas of fundamental medical research. There has already been considerable interest in the hiPSC SRL from other users, particularly as a hub for development of differentiation protocols and compound testing. Our plan is to provide demonstrations and seed grants (e.g. from Translational Genomics Hub) to support new research questions that could be facilitated by the SRL.

User fees: The CHaRM hub will primarily operate on a cost-recovery basis. ACE and CIC equipment will be integrated into existing fee-for-use structures determined by the academic user committees and based on academic rates at other Canadian institutions. The hiPSC SRL will develop a similar fee recovery structure in consultation with the SRL advisory board to ensure all costs associated with establishing and operating a shared use facility are included. User fees will be stratified by type of use (autonomous, assisted or service-based) to ensure they consider the full cost including labour, experimental supplies, and maintenance. User fees will be collected from all researchers, including the project team, and will be examined yearly by the committees and adjusted for inflation to ensure they align with current market values at other academic institutions. We expect that \$274K (~25%) of projected user fee revenues will be used to support direct O&M costs during the project. These predictions were made in consultation with UofA research cores to ensure full financial costing for the establishment and operations of the SRL and ongoing operations of equipment placed in ACE and CIC. Accumulated revenue and future user fee revenue, including external contracts, will be used to sustain the operations of the hiPSC SRL beyond the project term and extend service contracts for critical equipment.

Institutional commitments and contributions: WCHRI is committing \$208K over five years to support O&M costs for the hiPSC SRL. In addition to direct support for O&M costs, all team members lead well-funded research programs (~\$93M in life-long funding and >\$41M in active funding). This project builds on an initial hiPSC repository supported by a One Child Every Child grant ([Voronova](#)). Operating funds for CHaRM researchers will also come from internal funding to uncover the function of new genetic mutations, which will be provided by the Translational Genomics Hub (funded by WCHRI and the Medical Genetics Department). This project is optimally aligned with the [2023-2033 UofA Strategic Research Plan](#) and will support recently hired emerging leaders in neuroscience, stem cell biology and genetics, including co-applicants Drs. Ioannou, Riddell and MacPherson (2019) and hiPSC expert in spinal cord organoids Dr. Gupta (jointly recruited by Cell Biology and WCHRI; start date Jan/2025). Core staff salaries and software packages to monitor use and billing not covered by the IOF, will be provided by existing internal Faculty sources.

Data Management: Our integrated, high throughput facility will generate considerable digital data that must be organized and stored in a manner that is accessible not only to UofA users, but

also to national and international collaborators. The UofA IST and Chief Information Security offices provide policy and guidance for data integrity and security. IST has a dedicated computing support team who will help with hosting, updating and maintaining data storage infrastructure, including our server (item 6), in their secure rooms. IST also provides up to 50TB of secured data storage at no cost to each investigator, which will be leveraged by all SRL users. De-identification of any patient-sample derived data will take place before data storage, and data will be stored in agreement with all commercial, legal and ethical obligations. Files will be securely shared (via the [Borealis](#) repository) with non-commercial third-parties upon request, which will be evaluated, negotiated and approved in coordination with the Research Services and Technology Transfer offices. Data storage after project completion will require long-term digital archiving facilities, such as the UofA Library Data Repository, which stores data in accordance with ISO 14721:2012 Open Archival Information System reference model. All data will be stored in open-source/non-proprietary formats retaining all relevant metadata. For example, the CIC is using Open Microscopy Environment (OME), a consortium of academic research labs, industry and developers producing open-source software and format standards for microscopy data. OME will allow tagging of CIC image datasets with relevant genomic, proteomic and drug-screening data generated by the SRL and ACE cores, which will be done in accordance with FAIR (Findable, Accessible, Interoperable, Reusable) principles and hiPSC donors' consent agreement. This will help accelerate discoveries and collaborations by streamlining and coordinating the format, accessibility and organization of datasets obtained through use of the requested equipment. In all cases, as research articles are published, associated data will also be made publicly available through the public Gene Expression Omnibus (GEO) repository.

Sustainability beyond the five year project term: All requested infrastructure is expected to have >10-year useful life, exceeding manufacturer warranty support. UofA has proven experience supporting advanced research infrastructure as evidenced by past projects that will also support the proposed infrastructure, including those previously CFI-funded - 2023 JELF #43636 \$1.2M (Marchant and Sipione: Imaging flow cytometer for the analysis of vesicle trafficking in acute and chronic disease); 2021 IF #39588 \$9.6M (Westaway and Sipione: Remediation of protein misfolding); 2020 JELF #40616 \$666K (Ioannou: Super-resolution live-cell imaging platform); 2020 JELF #40656 \$372K (Riddell: Integrated controlled oxygen live cell imaging); 2018 JELF #37828 \$368K (Voronova: Dissecting molecular mechanisms regulating brain development and regeneration) and 2017 IF #36077 \$7.6M (Hendzel including Simmonds as co-applicant: Multi-scalar nanoscopy for advanced cell biology). **Simmonds** leads the CIC and has extensive experience managing internal funding and projecting user fees to support long-term infrastructure sustainability. All Faculty cores each have a five-year rolling budget for expenses (staff salaries, supplies, software, and equipment maintenance and repairs) supported by multiple funding sources (endowments, user fees and other internal funding) allowing the effective allocation of resources and response to any unforeseen costs (e.g. emergency repairs). The same model will be employed to support the WCHRI hiPSC SRL to ensure it remains financially sustainable past the project end date. This will include promoting the hiPSC SRL as a national resource to attract external academic and industry users and sustain future operations through external user fees (full cost recovery).

CRITERION 5: BENEFITS

Benefits for Canadians: improving health and maximizing socioeconomic benefit. Beyond advancing our basic scientific knowledge of stem cell biology, this hiPSC research infrastructure will also address key health challenges faced by Canadians with debilitating neurological

illnesses by improving diagnostic and prognostic capacity, identifying key targets for therapeutic development, and offering unprecedented insights into iPSC-based cell therapies. Ultimately, we aim to establish a pipeline that can be applied to *any* disease for which regenerative medicine and Precision Health approaches would be beneficial. The iPSC SRL's advanced research infrastructure will also have a profound impact on economic growth in Canada by attracting top talent to Alberta, creating jobs that stimulate the economy, and producing innovative research outputs of broad interest to academia, industry and technology companies. This SRL will further enable research projects conducted at the UofA to leverage support from national funding agencies, enhancing the provincial and national economies.

Benefits for end users: The SRL will tremendously enhance hiPSC functionally at UofA, building a unique, integrated facility encompassing the spectrum of discovery science to patient translation. This will enable collaborative innovation, providing a resource for clinicians and basic scientists to pursue advanced projects not previously feasible. Our vision is that the hiPSC SRL will service interested users at UofA, across Alberta, Canada and beyond, providing customized services for research projects and training of **highly qualified personnel (HQP)**.

Benefits for HQP: The requested infrastructure will attract trainees at all levels. Currently, our team members are collectively mentoring >50 HQP, and are actively involved in training initiatives through UofA institutes like NMHI and WCHRI. We anticipate that in the first 5 years of establishing this hiPSC SRL, our team members, users and collaborators will supervise ~200 new HQP. Trainees will acquire skills needed to become future leaders by: 1) cultivating passion for new knowledge; 2) learning critical thinking; 3) training in cutting edge hiPSC techniques; 4) becoming effective science communicators; 5) receiving effective career mentorship.

Technical skills development: HQP at all levels will develop strong technical foundations provided through concrete hands-on training and mentorship from PIs, technicians, and senior staff. Undergraduate HQP will learn accessible molecular biology and imaging skills. Graduate and PDF level HQP will learn human stem cell culture, advanced organoid assembly and analysis, including compound screening. In addition, all HQP will be encouraged and financially supported to attend workshops relevant to their projects (e.g. Organoid Symposium & Workshop by the Stem Cell Network) and to attend in-house training provided by our team members, technical staff equipment providers, modelled after existing programs of the CIC and ACE.

Research and Career Development: In addition to gaining skills in cutting-edge iPSC research, trainees will expand their critical thinking and research capacity through opportunities to collaborate with national and international interdisciplinary leaders in healthcare, industry, and academia. Leveraging the connections afforded by our Advisory Board, our HQP will be given opportunities to be trained at Simon Fraser University, SickKids Research Institute, University of Calgary, and McGill University to broaden their skillsets and provide exposure to new research environments and collaborators. Funding for these activities will be sought from Campus Alberta Neuroscience, the Alberta MS Network, American and International Societies for Neurochemistry, and Company of Developmental Biologists. HQP achievement will be recognized through authorship on publications and patents, leading scientific presentations, and participating in outreach activities for knowledge users. The CHaRM Hub will host a student-led bi-weekly “work-in-progress” seminar series to provide opportunities for HQP to improve presentation skills in a supportive environment while encouraging active and fearless exchange of ideas, protocols and knowledge. HQP will also be made aware of and encouraged to participate in UofA-led career development activities as well as Stem Cell Network and

[Regenerative Medicine Training Program](#) opportunities, including industry networking events, workshops on ethics, entrepreneurship, teaching, writing, and more. For postdoctoral fellows interested in faculty positions, we will continue to contribute to the organization of the “The Academic Interview” workshop (est. in the Faculty of Medicine in 2021) that will give fellows an opportunity to receive feedback on their application documents and participate in mock interviews. HQP who desire industry careers will be made aware of successful HQP development programs hosted by [UofA](#), such as Lab2Market and PhD Career Academy, as well as [API](#), such as Pharmacometrics Fellowship, Talent Hub, and internship program. API manages the UofA’s life sciences incubator Health Innovation Hub ([HIH](#)), where HQP will be connected with start-ups and industry for professional engagement and development.

EDI in Capacity Building and Mentorship: Highlighting our commitment to supporting trainees from diverse backgrounds, several of our team members’ former trainees from underrepresented groups now hold prominent positions in academia or government (e.g. Professor at the University of Southern Denmark; Regulatory Scientist at Health Canada). In line with [DORA](#) criteria, our team is committed to taking a multifaceted approach to ***graduate student*** recruitment by using broad criteria to evaluate each applicant’s potential as a researcher and consider the individual factors and circumstances (e.g. academic and career interruptions, financial situations that required them to work throughout their training) that may have created barriers to their success. We will include and promote underrepresented ***undergraduate trainees*** via mentoring programs groups such as [WISEST](#) (Women in Scholarship, Engineering, Science, and Technology), the [IYMP](#) (Indigenous Youth Mentorship Program) and [ELITE](#) (Experiential Learning in Innovation, Technology, and Entrepreneurship Program) for Black Youth that foster the inclusion and promotion of these groups in science. We will post open positions publicly (e.g. on university, lab and SRL websites, on social media and through professional networks) to reach the widest possible audience and attract a diverse pool of applicants. Team members will also increase engagement by participating in outreach activities such as [Let’s Talk Science](#) and [Stem Cell Talks](#), which in turn will lead to attraction of future trainee applicants.

To ensure trainees receive training in writing scientific proposals, PIs and CHaRM hub members will provide students with iterative feedback on their scholarship applications. Collectively, trainees supervised by our team members have received >\$2.7M in competitive scholarships and fellowships, demonstrating the effective mentorship strategies of our team members. Trainees will be encouraged to seek out non-competitive travel fellowships offered through UofA, which will be supplemented by funds from PIs operating grants to reduce socioeconomic barriers and ensure that trainees can participate in international conferences in their field. Trainees will also be encouraged to present their findings locally via research days.

Recognizing the communication barriers for students with varying discomfort in their language skills, trainees will be made aware of relevant UofA support, such as [English Conversation Club](#) offered by the International Student and Visitor Services and [English Language School](#) offered by the Faculty of Education. We will encourage all trainees to attend virtual NIH-funded [SCOARE](#) (Scientific Communication Advances Research Excellence) and [Writing Services](#) offered by UofA that focus on equipping students with research communication skills. To help researchers from underrepresented groups achieve their career goals, we will host annual career events with representatives from academia, industry, not-for-profit, start-ups, patent offices, funding bodies and other organizations that employ M.Sc. and Ph.D. graduates. Dr. **Voronova** has established such an annual career event at the University of Ottawa.

Knowledge and technology transfer: This SRL is committed to embracing open-science initiatives while protecting space for IP. Beyond making genomic, transcriptomic and other datasets available publicly (please see Data Management in Criterion 4), developed protocols will also be made publicly available (e.g., posted to protocols.io) to promote replication studies. Research results will be disseminated via conferences, symposia, freely available pre-prints (e.g. BioRxiv) and open access peer-reviewed publications. Knowledge mobilization will be maximized via SRL, hub and institute websites and social media pages highlighting our facility, researchers, and research progress. Moreover, we will engage with local, national and international community organizations, societies and non-profit foundations (e.g. [KBG Syndrome Foundation](#), [MS Canada](#), [Stem Cell Network](#) and [Canadian Centre for Diversity and Inclusion](#)) to disseminate our findings through multilingual workshops and accessible reports. Our knowledge translation goals are to inform ongoing and future research on the genetic influences on disease mechanisms as well as resulting clinical guidelines and policies.

Our team members have substantial experience in industry collaboration and commercialization, including the creation of new companies. Together we have submitted 21 reports of invention, resulting in 57 patent applications and 4 issued patents. In addition, we have 110 active commercialization and collaboration agreements including regenerative therapies in development for MS, HD, diabetes and intestinal disorders (notably this does not include sponsored research). The pipeline of discovery to translation will be aided by [API](#), which provides drug development services and GMP-compliant biomanufacturing. [API](#), in collaboration with the UofA, leads the UofA's [Drug Development and Innovation Centre](#) and [Health Innovation Hub](#) (HIH), Canada's leading incubator programs for life sciences ventures. API offers several innovation programs, including [Research to Revenue](#) and [Life Sciences Venture Coaching](#), designed for PIs and early stage companies to accelerate their innovations. Moreover, UofA's [Innovation Fund](#) provides funding, mentorship and global access to strategic networks for commercialization initiatives. Finally, the Government of Alberta has recently launched [Innovation Catalyst Grants](#) (\$250,000) for graduates of advanced STEM programs, such as regenerative medicine, to commercialize science-based technologies. Our team will take advantage of these programs to translate their discoveries into products and spin-off companies.

Any new SRL collaboration agreements and all issues related to data management, sharing of data, intellectual properties and exploitation of the results will be negotiated and administered by the UofA [Technology Transfer Office](#) (TTS). We anticipate developing extensive new IP from research enabled by the SRL. IP and commercialization initiatives related to new methods of diagnosing and treating disease, newly identified compounds, and other drug discovery initiatives will be managed in consultation with the UofA TTS, HIH, and other technology incubators (Technology Alberta, Innovate Calgary) and in accordance with applicable law and institutional policy. For multi-institutional and industry collaborations, ownership of new IP will follow inventorship, which will be attributed according to the legal standard for intellectual contribution. Applicants will identify and disclose any pre-existing IP that will be introduced into the project, and a research use license will be granted to each party if necessary to protect background IP. The applicants will disclose resulting project IP to each other and to their respective commercialization offices in a timely manner to allow new IP to be evaluated and protected as appropriate prior to publication. If project IP is suitable for patenting, UofA TTS will manage the process to file patent applications and manage the IP portfolio. Where needed, TTS will approach relevant companies with the intent to secure a partner for collaborative development and licensing of the discovery.

Financial resources for operation and maintenance

These tables outline annual costs and sources of support committed to ensuring effective operation and maintenance of the infrastructure for the first five years after it becomes operational. They do not include costs related to research and/or technology development. When applicable, funding from CFI's Infrastructure Operating fund (IOF) is included in the institutional contributions category.

Operation and maintenance budget summary

Costs	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Personnel	280,438	291,655	303,321	315,454	328,072	1,518,940
Supplies	16,000	16,320	16,646	16,979	17,319	83,264
Maintenance and repairs	2,200	2,200	2,200	2,200	2,200	11,000
Services	0	0	0	0	0	0
Other (specify)	0	0	0	0	0	0
Total	\$298,638	\$310,175	\$322,167	\$334,633	\$347,591	\$1,613,204

Funding sources

Funding sources	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Institutional contributions	278,115	241,632	210,467	209,081	191,872	1,131,167
Other organizations	8,000	50,000	50,000	50,000	50,000	208,000
User fees	12,523	18,543	61,701	75,552	105,719	274,038
Other (specify)	0	0	0	0	0	0
Total	\$298,638	\$310,175	\$322,168	\$334,633	\$347,591	\$1,613,205

Past/current CFI investment (To be completed by the institution)

The Innovation Fund provides investments in infrastructure to help Canada remain at the forefront of exploration and knowledge generation while making meaningful contributions to generating social, health, environmental and economic benefits and addressing global challenges.

The information captured in this section is for statistical purposes only. It will help us better understand the extent to which proposals enhance capacity in fields the CFI has invested in, as well as the extent to which proposals request funds for infrastructure to be used in core facilities or national research facilities funded under the CFI's Major Science Initiatives (MSI) Fund. This information will not be made available to review committees.

Does this proposal enhance research capacity in a field of research in which the CFI has made past investments at your institution?

- Yes
 No

A core facility provides access to the following, which are generally too expensive, complex or specialized for researchers to cost-effectively provide and sustain themselves:

- State-of-the-art research services and analyses
- Instruments and technology
- Expertise
- Training and education.

Also, a core facility:

- Is broadly available to many researchers to conduct their research activities, irrespective of their administrative affiliation and with no requirement for collaboration or co-authorship
- Has dedicated equipment and space serving one or more institutions, research programs or fields
- Is formally recognized as a core facility and supported by the research institution where it is located
- Has a clearly defined governance and management structure and a sound management plan reflective of its mandate, breadth and complexity
- Has dedicated management involving individuals with the technical and subject matter expertise necessary to oversee all aspects of the facility.

The percentage of the total project cost of requested infrastructure to be integrated into a core facility, as defined above.

95%

The percentage of the total project cost of requested infrastructure to be integrated into a national research facility funded under the CFI's Major Science Initiatives Fund.

0%

If applicable, name the research facilities of national importance funded under the CFI's Major Science Initiatives Fund that will house some of the requested infrastructure.

Project funding

This table provides a summary of the total contributions and eligible costs for the project.

	Total
Total eligible costs	\$10,900,121
Contributions from eligible partners	\$6,540,073
Amount requested from the CFI	\$4,360,048
Percentage of the total eligible cost requested from the CFI (may not exceed 40%)	40.00%

Summary of eligible costs

This table provides a summary of the total eligible costs for each type of expenditure. Individual items are listed in the 'Cost of individual items' section.

Code	Expenditure type	Total
13	Purchase of equipment (including shipping, taxes and installation)	7,686,338
14	Lease of equipment	0
15	Personnel (for infrastructure acquisition & development)	428,111
16	Components	0
17	Travel (infrastructure related)	0
18	Software	183,317
19	Extended warranties / Service contracts	1,925,817
20	Construction/renovation costs	626,449
21	Initial training of infrastructure personnel	50,089
22	Other	0
Total eligible costs		\$10,900,121

Cost of individual items

This table provides the details of eligible infrastructure acquisition and development costs. It shows the full cost of each item, including taxes (net of credits received), shipping and installation. For infrastructure that will be used for multiple purposes, the table includes only the pro-rated research or technology development costs.

The institution was instructed to follow its existing policies and procedures for the preparation of budget estimates. The CFI expects that costs included in this budget are close estimates of fair market value.

Item #	Type	Item description	Number of items / FTE*	Eligible costs			Date acquired (YYYY/MM) or to be acquired (YYYY)
				Cash \$	In-kind \$	Total \$	
1	13	hiPSC suite	1	2,154,187	606,228	2,760,415	2027
2	13	Drug screening suite	3	1,969,696	450,629	2,420,325	2027
3	13	Cell analysis suite	1	1,556,345	353,522	1,909,867	2027
4	13	Imaging suite	1	482,202	113,529	595,731	2027
5	15	Project manager (0,8FTE)	1	428,111		428,111	2027
6	18	Data storage	1	147,261	36,056	183,317	2027
7	19	hiPSC suite - extended warranties and service contracts	1	377,099	223,213	600,312	2027
8	19	Drug screening suite-ext. warranties and service contracts	1	303,577	79,927	383,504	2027
9	19	Cell analysis suite - ext. warranties and service contracts	1	614,515	138,312	752,827	2027
10	19	Imaging suite - -extended warranties and service contracts	1	152,794	36,380	189,174	2027
11	20	Construction/renovation costs	1	626,449		626,449	2027
12	21	Training	4	48,039	2,050	50,089	2027
Total eligible costs				\$8,860,275	\$2,039,846	\$10,900,121	

ESTIMATE OVERVIEW

Anastassia Voronova (FOMD)
CFI IF 2025



Estimate at Application	
Category	Amount
Hard Costs	\$ 323,700
Katz Group Centre 6-081, 6-081A	\$ 46,600
Katz Group Centre 3-108A	\$ 26,600
Katz Group Centre 3-101	\$ 55,000
HMRC 2-17, 2-17A	\$ 2,500
HMRC 3-27	\$ 18,000
HMRC 3-32	\$ 160,000
Li Ka Shing Centre 3-116	\$ 15,000
Soft Costs	\$ 224,745
General Contractor Requirements	\$ 64,740
Design & Project Management Services	\$ 116,532
Quality Assurance/Control, Permits, & Trade Support	\$ 10,000
Overhead @ 6.5%	\$ 33,473
Total Hard/Soft Costs	\$ 548,445
Contingency (10%)	\$ 54,845
Subtotal (Excluding GST)	\$ 603,290
GST (1.65%)	\$ 9,954
Programming Costs (Including GST)	\$ 13,205
Total Project Cost (Including GST)	\$ 626,449

Additional Comments

1. Equipment logistics and delivery into room are the responsibility of the PI, including any requirements for rigging.
2. The cost estimate does not include any furniture.

Note: This estimate is only valid for the CFI submission that is indicated in the title. This cannot be reused for a future submission or as part of other applications.

TIMELINE OVERVIEW

Anastassia Voronova
CFI IF2025



Timeline at Application

Task	Year 1 - Month											
	1	2	3	4	5	6	7	8	9	10	11	12
Tender / In-House Project Queue												
Design												
Materials & Equipment Order												
Construction												Year 2 >
Close-Out & Turnover												

Task	Year 2 - Month											
	1	2	3	4	5	6	7	8	9	10	11	12
Tender / In-House Project Queue												
Design												
Materials & Equipment Order												
Construction												
Close-Out & Turnover												

Additional Comments

1. The start of this schedule commences upon the Notice of Decision (NOD) for provincial matching funds and is dependent upon timing of project financial set up, which can take several months. Timeline is also subject to timing of site access and renovations queue.
2. Design and Construction lines include the procurement of these entities.
3. This timeline has included for the expected long-lead delivery of materials/equipment.
4. This submission contains some hard-wired equipment or equipment that requires construction design integration to complete. This requires appropriate planning and time during the design phase of the project.
5. The construction involves installations in sensitive research spaces and requires special construction considerations when completing work within them. This extends the construction delivery window.