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2021-2022 IgNITE Medical Case Competition: Immunity

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Abstract
The IgNITE Medical Case Competition is an annual research case competition organized by students across North America. Our mission is to provide high school and university students the opportunity to gain valuable research experience while networking with industry professionals. Each year, students in teams of 1-4 are paired with an experienced mentor to develop and present a novel research proposal within a specified theme of the competition. Students are taught the fundamental scientific principles underlying three lab techniques that they can then use during the competition in their proposal or their future research career. This year's theme was Immunity, and competitors learned about Tissue Culturing, ELISA, and Flow Cytometry. Furthermore, this year, the IgNITE community grew internationally with 670 high school and university students competing. This booklet presents the Top 40 teams' abstracts, and we invite you to visit our website (www.ignitecompetition.org) to watch the associated elevator pitch videos. We hope you enjoy reading through some of this year's top proposals and hope you will join our growing community.

Keywords: IgNITE Medical Case Competition; immunity; tissue culture; ELISA; flow cytometry; undergraduate; high school; case competition; medicine

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IgNITE Abstracts

Optimization of Treg cell-based therapies by expression of T-bet

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Over recent years, regulatory T cells (Tregs) have shown promising potential as a new class of highly effective adoptive cell therapies (ACT) for the treatment of autoimmune diseases, with over 50 active and completed clinical trials. However, target-tissue specificity, persistence and functional activity remain major limitations. During helper T cell 1 (T_H1) mediated immune responses, Tregs polarize and acquire unique homeostatic and migratory properties optimized for suppression of T_H1 responses *in vivo* by expressing the T_H1 hallmark transcription factor T-bet. Thus, we propose inducing the expression of T-bet in induced Tregs (iTregs) generated *in vitro* — used for Treg-based ACT; with the aim of enhancing T_H1 suppressive ability, trafficking, and persistence in tissue targeted during T_H1 autoimmunity. We will use a colitis mouse model (T_H1-mediated disease) with the commonly used colitis antigen 2,4,6-trinitrophenol (TNP). TNP-specific naive CD4+ T_H cells will be isolated and converted into T-bet+FOXP3+ iTregs under iTreg-polarizing conditions (anti-CD3mAb;TGF-B;IL-2) followed by IFN-γ stimulation. We will then transfer the TNP-specific T-bet+FOXP3+ iTregs (control: T-bet-FOXP3+ iTreg) into mice with colitis and record metrics of colitis severity and monitor cell fates using *in vivo* bioluminescence imaging. It is expected that mice treated with T-bet+ iTregs will perform better on these metrics and demonstrate more

efficacious Treg trafficking and persistence, suggesting enhanced specific immunosuppression. If successful, this would tailor Treg cell-based therapies towards the treatment of T_H1-mediated autoimmune disorders and provide rationale for other similar autoimmune therapies optimized towards a specific Th subset (e.g. Th2 or Th17).

Are celery allergies dependent on the freshness of celery?

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Celery is the only vegetable of the 14 food allergens required to be declared if contained in a meal by food law. Api g 1 is the major allergenic protein of celery and is responsible for 2.8-11.1% of food-induced anaphylactic reactions, a form of immunoglobulin E (IgE)-mediated hypersensitivity. Numerous studies have been conducted to identify the common allergen in celery however, there is a gap in the literature when it comes to identifying if the freshness of celery is directly correlated to the amount of Api g 1 present in it. Particularly, allergic reactions to celery are almost nonexistent in southern European countries and prevail in northern European countries, where fresh celery is uncommon to eat as the cold climate makes its cultivation hard and thus needs to be imported. Using a sandwich ELISA, this study aims to measure the concentration of Api g 1 protein in celery consumed in southern and northern European countries. It is expected that the Api g 1 protein concentration will be higher in celery consumed in northern European countries and very low to nonexistent in celery consumed in southern European countries. The difference in protein concentration would form a correlation between the freshness of celery and the amount of allergen present. This suggests that techniques to decrease the presence of Api g 1 in celery that is not freshly consumed should be developed to reduce celery-induced anaphylactic reactions. This is an important correlation to be made as this scenario is applicable to other allergy-inducing produce of the celery family such as apples.

Analysis of mucin-coated catheterization in biofilm inhibition

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Approximately 75% of catheter-related urinary tract infections (UTIs) are associated with biofilm formation with the main pathogenic species being *Escherichia coli* and *Enterococcus faecalis*. Mucins are a family of several glycoproteins secreted by mucosal cells, forming a natural barrier against bacterial colonization. As such, these proteins are of interest in developing novel anti-bacterial treatment strategies. This study evaluates the efficacy of various mucin types as coatings and determines their impact on bacterial-adhesion biofilm formation and UTI onset. Trans-membrane, secreted, and soluble mucins will be coated on polyvinyl chloride contact plates using an aqueous gel suspension containing core nutrients for bacterial growth. *Escherichia coli* UPEC and *Enterococcus faecalis* U0317 strains will be used to inoculate each $mucin^+$/$mucin^-$ control plate. A bioluminescent assay and imaging will be used to quantify biofilm formation on each sample at regular time intervals. Mice will be probed with coated catheters varying only in mucin gel type and the strain of bacteria introduced to the catheterization site. Confocal microscopy will then be used to visualize the detailed structures within mouse tissue and thereby assess infection. It is expected that $mucin^+$ plates will have reduced biofilm formation. Additionally, we predict that coated catheters in the mice will have a higher efficacy in preventing biofilm formation. We predict trans-membrane mucins, such as MUC1 and MUC4, will most significantly attenuate biofilm formation and bacterial adhesion as they are naturally found in urinary epithelium. These results could prove vital to minimizing hospital-acquired, catheter-related UTIs.

Assessing the adoptive transfer of Tregs into preeclampsia murine models

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Abnormal uterine inflammation is a central feature in the proposed pathogenesis of preeclampsia — a pregnancy complication characterized by hypertension and proteinuria. In normal pregnancy, regulatory T cells (Tregs) mediate feto-maternal immunotolerance, which is integral for robust placentation. Previous studies have identified that patients with PE exhibit perturbed peripheral and decidual Tregs. The adoptive transfer of pregnancy-induced Tregs has been shown to prevent fetal rejection in murine abortion models through decreased TH1 immunity; however, this therapy has not been tested in mitigating PE. Here, we propose that the adoptive transfer of $CD4+CD25+CD127^{lo/-}Foxp3+$ Tregs into reduced uterine

perfusion pressure (RUPP) rat models will restore immunotolerance at the feto-maternal interface, thus ameliorating PE clinical outcomes. Tregs extracted from the peripheral blood of all rats will be analyzed and isolated using flow cytometric cell sorting techniques that target CD4, CD25 & CD127 surface markers. Polyclonal expansion of Tregs will be stimulated using Anti-CD3/CD28 coated beads in the presence of high dose IL-2. Expanded CD4+CD25+CD127$^{lo/-}$Foxp3+ Tregs or saline will be infused via intravenous tail injections into the treatment or control group, respectively. Primarily, we will monitor changes in blood pressure using a tail-cuff system, as well as protein levels in the urine and spontaneous abortion rates. Ultimately, we expect that the infusion of Tregs will shift the decidual immunological environment towards the anti-inflammatory phenotype seen in healthy pregnancies. These findings could potentially lead to the use of adoptive Treg cell transfer as a therapeutic intervention for PE, a leading cause of maternal mortality.

Inhibition of metastatic cancer in mice via Atopaxar

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When cancer becomes metastatic, tumour cells intravasate out of the primary tumour and spread to other organs, causing about 90% of cancer deaths. One way circulating tumour cells (CTCs) metastasize is by interacting with platelets, resulting in tumour cell-induced platelet aggregation (TCIPA) that shields CTCs from immune attack. Previous studies suggest that tumour cells promote metastasis and induce TCIPA by activating protease-activated receptor-1 (PAR-1) on platelets. Therefore, this study aims to investigate whether administering Atopaxar, a PAR-1 antagonist that has not yet been studied in cancer as other PAR-1 antagonists have, can limit metastasis in mouse models. We will assess the effectiveness of Atopaxar and a placebo (or control) on adult C57BL mice inoculated with GFP-transfected Lewis lung carcinoma cells. Flow cytometry of blood samples taken 7, 14, and 21-days post-inoculation will be performed to quantify the number of GFP+ cells and activated CD8+ (cytotoxic) T cells in the samples. We expect that the Atopaxar treated mice will have reduced numbers of CTCs and higher numbers of cytotoxic T cells, suggesting that the inhibition of TCIPA via Atopaxar will correlate with reduced shielding of CTCs and metastasis rates. These results could provide novel insight into the use of PAR-1 antagonists in confining cancer to its primary site in patients and inhibiting CTCs' function as a seed for metastases. Since CTCs will usually be present in the blood even after removal of a secondary tumour, limiting metastasis can significantly improve the prognosis and wellbeing of patients.

Investigating COVID-19 induced myocarditis of down syndrome patients

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The recent outbreak of severe acute respiratory syndrome coronavirus two (SARS-CoV-2) has caused over 250 million cases of COVID-19 globally. Despite a low case-fatality rate, individuals with Down syndrome (DS) experience almost nine-times greater risk of SARS-CoV-2-related death. Recently, researchers revealed that SARS-CoV-2 can infect cardiomyocytes (CM), causing myocarditis. DS is associated with underlying cardiac dysfunction and overexpression of transmembrane serine protease 2 (TMPRSS2; required for priming of the viral spike protein). Therefore, this research seeks to (i) investigate how SARS-CoV-2 infection affects the hearts of individuals with DS, and (ii) elucidate a correlation between TMPRSS2 expression level and SARS-CoV-2-induced myocarditis. I will create engineered heart tissue (EHT) from iPSC-derived CM, fibroblasts, and endothelial cells from DS and non-DS individuals. EHTs will be inoculated with SARS-CoV-2 and clinical cardiac complications of COVID-19 will be observed. Specifically, I will assess viral infection (focus-forming assay), cardiac injury (troponin T immunostaining), and ventricular systolic function (EHT contractility). I will perform single-cell RNA sequencing (scRNA-seq) to identify differentially expressed molecular signatures of inflammation. From scRNA-seq data, I will compare TMPRSS2 and angiotensin-converting enzyme 2 (ACE2) expression between DS vs non-DS. I expect to observe more severe myocarditis phenotype for DS EHT, and a positive correlation between TMPRSS2 expression level and the severity of CM infection. This study will provide insight into COVID-19 myocarditis, allowing the development of a targeted intervention for the medically marginalized population of individuals with DS.

Development of a personalized dendritic cell mRNA vaccine for cancers

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Over the past few decades, dendritic cells (DCs) have proven to be pivotal in initiating adaptive immune responses. Exploiting dendritic cells has shown enhanced antitumor activity during treatment, but current DC vaccines involve mRNA for a few overexpressed tumor antigens, limiting personalized treatment. Thus, it is hypothesized that a DC vaccine can be developed using a tumor's entire transcriptome. This would produce an individualized treatment that would target overexpressed antigens and non-mutated neoantigens. First, dendritic cells will be generated from autologous CD14+ monocytes in stem culture. To become immunogenic, maturation of the DCs will be triggered by exposure to a combination of cytokines, called MCM-mimic. The DCs will then be transfected with the autologous tumor transcriptome via pulsing and mRNA electroporation, which will be cleaved by the DC's endogenous antigen processing machinery, resulting in relevant epitopes that can fit into the patient's HLA repertoire. Finally, the DCs will be administered to the patient via intravenous vaccination. It is expected that the injected DCs will gain access to peripheral lymphatic tissue to induce tumor-specific T-cell responses that attack the tumor and metastasis. Furthermore, when taken in conjunction with chemotherapy, the vaccine should produce enhanced anti-tumor activity and is not expected to elicit serious side effects. Further research regarding mRNA electroporation and combination therapy is required in mice to ensure that optimal results can be obtained for human application. With the development of personalized DC mRNA vaccines, millions of cancer patients worldwide could be treated more effectively to live their lives cancer-free.

IL-35: A potential therapeutic target in hepatocellular carcinoma immunotherapy

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Hepatocellular carcinoma (HCC) is the most common form of liver cancer, accounting for about 90% of cases. HCC development and progression is associated with an increased number of regulatory B lymphocytes (Bregs) in the tumor microenvironment, due to their immunosuppressive functions mediated by cytokine secretion and cognate interactions. IL-35 is a key cytokine produced by Bregs. It is believed to hinder tumor immunosurveillance by increasing regulatory T-cell proliferation (which have immunosuppressive functions), impairing Th17 responses (a $CD4^+$ helper T-cell subset), and even establishing a positive feedback loop by inducing more IL-35-producing Bregs. This study will, therefore, aim to investigate the effect of targeting IL-35 on HCC progression in mouse models. The mice will receive weekly injections of diethylnitrosamine (DEN) at a dose of 35 mg/kg to induce HCC. The mice will be split into four groups, each receiving further weekly saline injections of IL-35-specific antibodies at set concentrations (Control – 0 μg/mL, Group 1 – 0.3 μg/mL, Group 2 – 0.6 μg/mL, Group 3 – 0.9 μg/mL). Sandwich ELISA will be used to quantify serum fucosylated haptoglobin (a biomarker of HCC development and progression) at the start and at 10-week intervals up to 40 weeks. Serum IL-35 will also be quantified via the same method. We expect to find a negative correlation between the dosage of anti-IL-35 antibodies and fucosylated haptoglobin levels, indicating dose-dependent mitigation of HCC. Such findings could provide useful insights into the potential therapeutic benefits of employing IL-35-targeting mechanisms in HCC immunotherapy and the broader context of cancer treatment.

Targeting IL-20R1 receptor to inhibit binding of cytokine IL-19 in mice with collagen-induced arthritis

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Atopic dermatitis (AD) is an autoimmune skin condition diagnosed in 25% of children and 2-8% of adults, resulting in itchy, inflamed patches of skin. Increased Immunoglobulin E antibodies (IgE) appear in patients with autoimmune conditions such as AD. Genetic variations, namely single nucleotide polymorphisms (SNPs), also increase IgE levels, yet aren't present in all AD patients. Elevated concentrations are found in approximately 80% of AD patients, but due to inconclusive studies on IgE autoreactivity, the development and pathogenesis of IgE in early childhood is unknown. We aim to study specific antibody development in children ages 6 to 15 to identify relationships between age of diagnosis, disease severity, and IgE production. For individuals with unusual levels of IgE, we will explore SNP's influence on associated genes credited with childhood atopy. To measure IgE levels in patients, ELISA will be used to detect antibody presence in plasma samples. SNP data is collected with PCR-based analysis of DNA extracted from blood. Since IgE is a contributor to the worsening of AD, we hypothesize positive correlations between severity and concentration of IgE. As IgE levels in healthy controls peak at 10-15

years of age, resulting IgE concentrations should also increase with age. Our results provide age-based context surrounding atopic development that may fill research gaps regarding pathogenesis and development of IgE in autoimmune conditions, such as AD. Future studies about increased IgE concentration in early childhood and its relation to either the comorbidity or the defense against other autoimmune disorders are recommended.

DOCK8 replacement therapy in $CD8^+$ T-cells through mRNA delivery

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DOCK8 deficiency is a devastating genetic disorder that results in impaired lymphocyte function and survival. The disease is caused by a loss of function mutation in the DOCK8 gene and presents clinically as eczema, high levels of the antibody immunoglobulin E (IgE) in the bloodstream and increased susceptibility to a variety of infections. Unfortunately only 33% of DOCK8 deficient people survive beyond the age of 30. While the exact cellular signalling pathways affected by the DOCK8 protein are unknown, it is known to result in markedly impaired T-cell survival. Our experiment will employ a preexisting $CD8^+$ T-cell targeting lipid nanoparticle delivery system to insert mRNA encoding the DOCK8 protein. This mRNA is expected to be translated into healthy DOCK8 protein by host cell machinery, using the same mechanism employed by mRNA SARS-CoV-2 vaccines. The experiment will be conducted by injecting DOCK8 knockout mice with these nanoparticles and comparing serum $CD8^+$ T cell levels using flow cytometry. The expected result is that by increasing functional DOCK8 protein levels in affected cells, their viability will be improved. This could illuminate a potential therapeutic target to improve the lives of those suffering DOCK8 deficiency. Furthermore, our platform could be applied to develop further therapeutics targeting the other lymphocytes affected by DOCK8 deficiency, including the B cells and natural killer cells.

Using interferon inhibitors in potential gene therapy for psoriasis treatment

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Psoriasis is a chronic inflammatory autoimmune skin disorder characterized by elevated red patches, flaking scales, itching, nail abnormalities, and joint pain. The inflammatory response noted in psoriasis has been associated with the production of interferons (IFN), which are proteins involved in cell-mediated innate immunity. This proposed research aims to determine the anti-inflammatory potential of interferon-1 (such as IFN-α and iNF-β) inhibitors in C57BL/6 mice using Moloney murine leukemia virus-derived (MMLV-derived) retroviral vector mediated gene therapy. The genes for the IFN inhibitors nsp1, nsp2, nsp11, and, N, would be isolated, tagged with a green-fluorescent tag to identify successful transformation, and introduced into the vector. The newly recombinant retroviruses modified with one IFN-1 inhibitor each would then be introduced into the lower epidermal layer of each of the treatment groups. The amount of IFN-1 in the psoriasis-affected site will be measured using ELISA. A visual comparison of the severity of redness, flaking, or presence of lesions will be conducted to examine potential therapeutic effects. It is suspected that the introduction of IFN-1 inhibiting genes will decrease IFN-1 production, reducing the number of inflammatory cells recruited to sites of psoriasis, and lowering the severity of inflammation induced in the tissue observed by lessened redness and thickness of scales. Determining the therapeutic potential of IFN inhibiting genes in mice suffering from psoriasis is intended to inform the creation of a gene therapy treatment plan targeted for humans.

Using lactobacillus to stimulate IL-12 secretion for cutaneous T-cell lymphoma

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The gut microbiota refers to the diverse community of more than 100 trillion microorganisms residing in our intestines. One genus in particular which dominates the microbiome is *Lactobacillus.* Lactobacilli begin the activation of natural killer cells and increase the immune response of cytotoxic T cells and the production of interleukin 12 (IL-12). IL-12 plays a pivotal role against cutaneous T-cell lymphoma (CTCL) by inducing antitumor cytotoxic T cell responses and lesion regression. To

understand the role of gut microbiota on immune response to CTCL, we will supplement mice models with two strains of *Lactobacillus*, specifically *Lactobacillus casei* and *Lactobacillus fermentum*, both of which can induce high levels of IL-12 through the use of oral probiotics. To quantify IL-12 levels present in the mice, we will use enzyme-linked immunosorbent assay (ELISA). Sezary syndrome is a form of CTCL, which has cancerous CD4+ cells in the skin, lymph nodes, and peripheral blood. Using flow cytometry, we will assess the CD4+ cells to evaluate the severity of the cancer and monitor any changes. We expect that an increase of both Lactobacilli strains in the gut microbiota will increase IL-12 secretion which will reduce the severity of CTCL through antitumor cytotoxic T cell response. Understanding the relationship between the gut microbiota and the immune system is critical for the development of effective cancer immunotherapies.

Treg cells treat graft vs host disease

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Acute Graft versus Host Disease (aGvHD) commonly occurs within 100 days of an allogeneic haematopoietic cell transplantation (HSCT). In aGvHD, donated bone marrow (BM) used to cure haematological malignancies, produce $T_{helper}1$-cells (T_H1) and $CD8^+$T-cells. These interpret host tissues as foreign and attack organs, especially intestines. Regulatory T ($CD4^+CD25^+T_{reg}$) cells suppress the immune response against self and foreign antigens, inhibiting the activation of T_H1-cells and $CD8^+$T-cells. Thus, supplementation of $CD4^+CD25^+T_{reg}$ -cells (i.e. T_{reg}-cell therapy) may prevent aGvHD. It is hypothesized that injecting BM-cells comprised of elevated $CD4^+CD25^+T_{reg}$ -cells and depleted effector $CD4^+CD25^+$T-cells will protect recipient leukemic *Mus musculus* (mice) against aGvHD by decreasing the level of T_H1-cells and $CD8^+$T-cells. Initially, flow cytometry will be conducted using $CXCR3^+$ and $CD69^+$ protein surface markers to identify and isolate T_H1-cells and $CD8^+$T-cells, respectively, from the intestines. These cells will then be cultured for *in vitro* analysis. RPMI-1640 basal media supplemented with 10%-fetal bovine serum will activate the T_H1-cells and $CD8^+$T-cells. This activation will primarily result in the release of interferon-gamma (IFNγ) cytokines, quantifiable by ELISA. Next, *M. musculus* will be injected with BM cells containing elevated $CD4^+CD25^+T_{reg}$ -cells and depleted effector $CD4^+CD25^+$T-cells. Flow cytometry and ELISA will be repeated after BM injection. Overall, a decrease in IFNγ is expected upon the BM-cell injection due to inhibited T_H1-cells and $CD8^+$T-cells, attributed to an increased $CD4^+CD25^+T_{reg}$ -cell concentration. Therefore, T_{reg} -cell therapy will significantly reduce the mortality of the experimental group relative to the control over a 100-day period, thereby preventing aGvHD.

Investigating Nrf2 regulation of Foxp3+ in regulatory T cells

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Regulatory T cells (Tregs) inhibit excess T cell proliferation and cytokine production, which is essential to prevent autoimmunity. Two common indicator proteins of Tregs are Cystine-Glutamate Antiporter Solute Carrier 7A11 (SLC7A11), and Transcription Factor Forkhead Box Protein p3 (Foxp3+). Previous studies found that activating Nuclear Factor Erythroid 2-related Factor 2 (Nrf2), a transcription factor that combats cellular oxidative stress, regulated the Treg population. However, contradictory evidence of increased SLC7A11 expression and decreased Foxp3+ expression indicated that Nrf2 activation led to both Treg proliferation and suppression, respectively. To better understand Nrf2's role in mediating Treg population, our study investigates the effect of Nrf2 activation on the Treg population using SLC7A11 and Foxp3+ as biomarkers in healthy subjects, immunosuppressed DiGeorge syndrome patients, and autoimmune disorder Lupus patients. The Treg cell tissue culture is cultivated from Treg cells isolated from subjects by Flow cytometry and is treated with Dimethyl Fumarate (DMF) that activates Nrf2. The changes of SLC7A11 and Foxp3+ mRNA and protein levels of Treg tissue cultures are evaluated using Polymerase Chain Reaction techniques and Western Blotting. We expect an upregulated level of SLC7A11 and Foxp3+ in patients compared to healthy subjects indicating Treg proliferation. Understanding the role of Nrf2 in regulating Tregs further elucidates the regulation mechanism of T cell-centered immune response, which facilitates in developing treatments for immunodeficiency and autoimmune diseases. Future studies should aim to investigate the cellular relationship of Nrf2 and Treg in animal models and in immune dysfunctional patients.

FOXP3 upregulation for the treatment of autoimmune inner ear disease

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Autoimmune Inner Ear Disease (AIED) is an immune-mediated form of sensorineural hearing loss that is progressive, bilateral, and often irreversible. The inner ear has previously been considered an immune-privileged site, though more recent literature shows that patients with immune-mediated sensorineural hearing loss have elevated levels of circulating T-cells specific to several inner ear proteins. These T-cells secrete cytokines and contribute to the development of autoantibodies that direct the immune response against specific inner ear antigens. Symptoms present when the unmediated response causes structural damage – most commonly, cochlear vasculitis and atrophy of the organ of Corti. AIED is primarily treated with a course of corticosteroids, though literature suggests that this treatment is ineffective long-term. Because the structures of the inner ear are difficult to access, several studies suggest that reducing systemic inflammation may be the most viable approach to preventing hearing loss. As such, our goal is to upregulate FOXP3 expression in mice models using the CRISPR-Cas9 gene editing system. FOXP3 proteins contribute to the expression of immune-mediating genes by binding to DNA and are crucial for the development of regulatory T-cells (Tregs). Tregs suppress immune responses by inhibiting both T-cell proliferation and cytokine release. Therefore, we expect that the upregulation of FOXP3 will increase levels of Tregs and reduce the inflammation causing tissue damage to the inner ear. Experimental results will be based on laboratory tests for complete blood count (CBC), which detects inflammation and is one of the primary ways AIED is currently diagnosed.

Anti-NMDAR encephalitis: Thymus treatment by genetic manipulation of negative selection

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Autoimmune encephalitis (AE) is an etiologically diverse condition characterized by impaired memory, poor motor control, and impaired levels of consciousness. The thymus, critical to immune function, is responsible for inducing apoptosis in naïve T-cells that attack endogenous systems. This study will investigate whether upregulation of the negative selection of T-cells for anti-NMDAR autoantibodies will prevent the progression of disease and decrease symptom severity. The presence of NMDAR surface proteins is implicated in the development of over 50% of AE cases. *In-vitro* and *in-vivo* models will be used to study the effects of genetic manipulation of the thymus. Epithelial and dendritic thymic cells isolated from juvenile C57BL/6 mice with NMDAR holoprotein immunization induced AE will be cultured for 24 hours prior to RNA gene therapy. An adeno-associated virus vector (AAV) containing the gene encoding the amino-terminal domain of the GluN1 subunit of the NMDAR will be inserted into the cells. Surface protein expression after treatment will be quantified using ELISA and protein quantification assay. The latter detects NMDAR by binding GFP-tagged IgA, IgG, and IgM and will be visualized using fluorescence microscopy. In mice, thymic cells will be transfected with plasmids injected through intra-thymic injections containing the rodent NR1, NR2A, or NR2B subunits of NMDAR. These will then be killed, and NMDAR expression will be quantified in thymic tissue using ELISA and protein quantification assay. The findings of this study will contribute to the development of novel mechanisms of treating AE and possibly other autoimmune diseases including Graves' and Hashimoto's disease.

Genetically engineering T7 bacteriophages to combat biofilm producing *Escherichia coli*

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Although surgical interventions for spina bifida exist, patients with myelomeningocele still have paralysis below the level of the deformity and therefore better treatments are needed. Axonal regeneration in the central nervous system is partly inhibited by the signaling of Nogo-66 receptors (NgR1) when bound to myelin-associated inhibitors (MAI), such as Nogo-A. Tissue exposed to the protein AXER-204 has been proven to deactivate NgR1 signaling by blocking the MAI from binding to the receptor. This promotes axonal growth and mobility recovery from lesions in the corticospinal tracts of the study's subjects. This research aims to investigate if AXER-204 can inhibit MAI-NgR1 binding for spinal cord neuron regeneration and improve mobility in rats with myelomeningocele. Rats with induced myelomeningocele will be divided into a control and an

experimental group. The experimental group will receive an intrathecal injection of AXER-204 at the site of deformation. Post-injection tissue extracts from each group will be subjected to primary and secondary antibodies for the colocalization of the Nogo-A and NgR1 proteins immunohistochemically. Nogo-A will be localized using mouse IgG1 anti-Nogo-A and goat anti-mouse IgG1 (AlexaFluor568). NgR1 will be localized using rabbit anti-NgR1 and goat anti-rabbit (AlexaFluor647). It is expected that the immunofluorescent images of the experimental group will demonstrate that Nogo-A and NgR1 have a decreased interaction compared to the control group. Paired with mobility tests, the data will indicate that AXER-240 blocks Nogo-A binding to NgR1. Understanding of axonal regeneration in myelomeningocele can provide potential treatment improvement for patients and enrich their quality of life.

Effects of 6-gingerol on the expression of cytokines in GBS

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Guillain Barre Syndrome (GBS) is a rare autoimmune disease that occurs when one's immune system mistakenly attacks neurons in the peripheral nervous system, causing muscle weakness, paralysis, and sometimes death. Although there are available treatments, they are often inaccessible due to their costs. Cytokines are signaling molecules that are major factors in the pathogenesis of GBS by playing roles in proliferating and signaling immune cells to attack neurons. Studies have shown that 6-gingerol, a metabolite of *Zingiber officinale* or ginger, has the ability to suppress proinflammatory cytokines like IL-1β, IL-12, TNF-α, and promote suppressant cytokines like TGF-β. We hypothesize that 6-gingerol can modulate cytokines to restore the immune defects in GBS. Utilizing experimental autoimmune neuritis (EAN) as a model for GBS, we will introduce vehicle or extracts of 6-gingerols to the mice, aged 4 months, through feeding. After 7, 14, and 21 days, we will take blood samples and determine serum levels of cytokines using ELISA and WB to determine which cytokines were inhibited or stimulated, and evaluate whether the mice's conditions improve via rotarod tests. We expect, in 6-gingerol administered mice, that the expressions of IL-1 β, IL-12 and TNF-α will be inhibited while the expression of TGF-β will be stimulated. Additionally, these mice will have improved motor functions compared to control mice, suggesting ginger is a promising therapeutic approach for treating symptoms of GBS. Altogether, this study shows that 6-gingerol can be a better, cost-effective, and accessible treatment for GBS.

Treatment of chikungunya virus by suppressing viral replication using withaferin-A

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Chikungunya virus (CHIKV) is a mosquito-borne infection, with 3,000,000 reported cases annually, yet no available curative treatment. Due to the lack of a vaccine, the virus is free to initiate replication through its dimerized cell-receptor "E2-E1" envelope glycoproteins, firstly infecting dermal fibroblast cells. Infection causes symptoms including musculoskeletal inflammatory reactions such as polyarthralgia, myalgia, and fevers. Patients with severe symptoms at onset were found to be at higher risk of developing chronic symptoms (e.g. arthritis), suggesting that hindering initial viral replication can prevent such long-term illnesses. Recent *in silico* analysis predicts that the drug Withaferin-A would bind to an inhibitory region within the E2-E1 heterodimer complex, potentially deactivating the heterodimer and blocking viral entry. This study aims to investigate whether administering Withaferin-A can inhibit viral entry in dermal fibroblasts. Here, we propose an *in vitro* study, whereby CHIKV (Ross strain, E1 :A226) infected primary human dermal fibroblasts will undergo either treatment with Withaferin-A or DMSO (Withaferin-A solvent). By tagging a mouse anti-Chikungunya E2 antibody, we would conduct a time course to examine the expression intensity of the E2-E1 protein complex using flow cytometry. We expect a lower expression of E2-E1 glycoproteins in treated fibroblast cells, indicating that Withaferin-A was able to disrupt viral entry of CHIKV virions. If successful, future studies can investigate whether Withaferin-A binds to the inhibitory region competitively or if it allosterically changes the conformation of the E2-E1 protein complex, providing insight towards future studies for a long-term CHIKV treatment.

Engineering novel antibody-drug conjugates for treatment of MDR SCLC

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Small-cell lung cancer (SCLC) is the most lethal form of lung cancer. The current front-line treatment for SCLC is chemotherapy, however, patients can develop multidrug resistance (MDR). Previous studies have shown that Yes-associated protein 1 (YAP1) is associated with MDR. Antibody-drug conjugates (ADC) are innovative therapeutics that combine the properties of monoclonal antibodies (MoAbs) with the potent activity of cytotoxic bioactive compounds. Here, we generate MoAbs specific to antigens expressed on SCLC cells and conjugate them with verteporfin, a cytotoxic drug that inhibits YAP1. Kruger et al. created MoAbs that do not cross-react with other undesired cancer cells and normal cells using differential immunization for antigen and antibody discovery technology. Following this approach, we will engineer the same MoAbs against SCLC-specific antigens. Next, verteporfin will be conjugated through the catalysis of a site-specific enzyme known as the tRNA/aminoacyl-tRNA synthetase which incorporates the unnatural amino acid *p*-acetylphenylalanine. This method of homogenous ADC production previously demonstrated excellent pharmacokinetics and potent *in vitro* cytotoxic activity against cancer cells. Patient-derived SCLC cell lines, along with healthy donor cells, will be cultured and equal volume of medium containing ADCs, free drugs (positive control), or PBS (control) will be added to initiate the anti-cancer toxicity study. After conducting a cell viability assay, we expect to see our engineered ADCs exhibit high anti-SCLC toxicity compared to the controls. We envision our new class of potent biopharmaceutical medicine will serve as a novel method of targeted therapy for patients experiencing MDR for SCLC.

Mechanism of the ITPKC gene SNP that elicits Kawasaki disease

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Kawasaki disease is an acute form of pediatric vasculitis. It is characterized by fever, bilateral nonexudative conjunctivitis, lip erythema, and oral mucosa. Kawasaki disease is associated with a genetic mutation, a functional single nucleotide polymorphism (SNP) in the inositol 1,4,5-trisphosphate 3-kinase C (ITPKC) gene which is coupled with a child's susceptibility to the disease. However, the genetic mechanism of the Kawasaki disease is poorly understood. The purpose of this study is to establish an understanding of the mechanism of the ITPKC gene SNP to aid in the development of a diagnostic method for Kawasaki disease in pediatric populations. To test the hypothesis, 100 children under the age of 5 will be recruited. Half of the sample will have Kawasaki disease while the other half will be unaffected by the disease and function as the control group. To investigate the differences in cell populations between Kawasaki and controls, peripheral blood mononuclear cells expressing ITPKC will be stained with fluorophore-conjugated antibodies and processed with flow cytometry. Lastly, ELISA will quantify the expression levels of the ITPKC enzyme. The flow cytometry results will reveal the morphology differences of cells containing this SNP and the differences in cell populations between Kawasaki and controls. Furthermore, the results from ELISA will reveal potential variations in levels of ITPKC enzyme expression of Kawasaki in comparison to controls. Understanding the mechanism of Kawasaki disease will aid in the development of improved diagnostic tools potentially leading to earlier diagnosis of the disease.

Development of live-attenuated virus vaccine through mutation of SARS-CoV-2 NsP1

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SARS-CoV-2 evades the immune system by inhibiting cellular translation using the protein NsP1. The C-terminus of NsP1 competitively binds the 40s ribosomal mRNA channel, whereas the N-terminus likely promotes translation of viral RNA. Past research suggests C-terminal mutation of NsP1 as a promising step in the development of a live-attenuated virus vaccine against SARS-CoV-2. However, mutations at both termini have yet to be tested. We propose mutations at the N- and C-termini of NsP1 will result in a live-attenuated virus vaccine that induces a stronger immune response in host cells than with only one terminal mutation. The mutant virus will be generated with deletion mutations in the N- and C-termini of isolated SARS-CoV-2 viral RNA. To study the effect of the viral mutations on host cell translation, K562 cells that constitutively express GFP as a reporter for translation will be exposed to the virus. To confirm lowered infectivity, K562 cells will be treated with different concentrations of mutant virus. This will be followed by conducting a viral challenge in healthy mice using the mutated virus. Antibody production against the virus in mice will be quantified using ELISA. We

expect mice infected with both mutated termini to survive and produce the highest levels of antibodies, while mice infected with only one mutated terminus survive but produce fewer antibodies. This study will provide more insight on using NsP1 as a target for the production of live-attenuated virus mutants as vaccines against SARS-CoV-2 infection.

Effects of procainamide on *CTLA-4* expression in drug-induced lupus

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Lupus erythematosus is an autoimmune disease affecting over 5 million individuals worldwide, characterized by the overactivation of B and T lymphocytes. Of these, 10% suffer from drug-induced lupus (DIL), caused by high doses of medications such as procainamide, with 20% of procainamide users developing DIL. CTLA-4 and CD28 receptors on T-cell surfaces bind to B7 proteins on antigen-presenting cells, halting and promoting T-cell activation, respectively. Studies indicate that certain lupus symptoms are linked to abnormal *CTLA-4* expression, causing T-cell overactivation, though the nature of the relationship between CTLA-4 and lupus is nonunanimous. Other studies show that procainamide alters DNA methylation, while altered *CTLA-4* methylation has been tied to autoimmune disorders. Thus, we hypothesize that procainamide alters *CTLA-4* expression, eliciting DIL symptoms. We propose an *in vivo* experiment involving mouse models. One mouse sample would undergo long-term procainamide treatment with the other acting as a control. Isolated T-cells from both samples, taken from the thymus, would then undergo immunohistochemical staining, first with a primary antibody that binds to CTLA-4, then with a corresponding fluorescent antibody. Additionally, transcriptome data from scRNA-seq would be obtained to examine the expression of *CTLA-4* and other potential genes of interest. Results are expected to indicate low counts of CTLA-4 on the surface of procainamide-treated T-cells. By analyzing CTLA-4 distribution in the cell, we can determine if procainamide interferes with *CTLA-4* expression. If successful, a potential novel treatment involves using CTLA-4/CD28-specific monoclonal antibodies to counterbalance alterations in these pathways and neutralize the autoimmune damage inflicted by procainamide.

Levels of alpha-synuclein specific T-cells in dementia with Lewy bodies

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Dementia with Lewy bodies (DLB) disease shares several common features with Parkinson's disease (PD), as both diseases are characterized by the loss of dopaminergic neurons and the aggregation of α-synuclein proteins. Previous research has investigated the role of T-cell responses in the pathophysiology of PD and, namely, α -synuclein-specific T cell reactivity. α -synuclein-specific T cells are a class of T cells, moderated by $CD4^+$ T cells, that can recognize epitopes unique to α -synuclein protein. In PD, levels of α -synuclein-specific T cell reactivity were elevated following diagnosis then decreased throughout the progression of the disease. We hypothesize a similar progression in DLB, where levels of α -synuclein-specific T cells, including the $CD4^+$ T cell, will be highest at the onset of the disease and, hence, may be used as a potential biomarker. To address this, we will be conducting a study using transgenic mice models of DLB at the early, late, and middle stages of the disease's progression. A cell sample will be taken from brain tissue and grown in tissue culture media. The samples will be analysed using flow cytometry and fluorochrome isothiocyanate to determine the composition of $CD4^+$ cells at each stage. We expect the amount of $CD4^+$ cells will reach its peak in the early stages and progressively decrease in the middle and late stages. These results may provide a therapeutic target for the development of preventative solutions for DLB patients.

Combatting X-linked severe combined immunodeficiency through CRISPR-Cas9 technology

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X-linked severe combined immunodeficiency (XSCID) is a disease caused by a mutation of the interleukin 2 receptor gamma (IL2RG) that results in an absence of T lymphocytes in the immune system. This allows for typically non serious illnesses, such as the common cold, to become life-threatening. IL2RG is a gene located on the X-chromosome that encodes the IL-2RG protein. This protein is responsible for transmitting signals to the cell's nucleus in order to regulate the growth of T lymphocytes. These lymphocytes are imperative to the function of the immune system. When a defect in this protein occurs,

no signals are produced or transmitted and the production of T lymphocytes is evidently eliminated. The cause of this defect has not yet been identified. While bone marrow transplants exist as treatments and potential cures, they are difficult to obtain and significantly invasive. However, the fast-paced environment of CRISPR-Cas9 technology offers great promise to the world of immunology. Recent studies have shown that the use of the CRISPR-Cas9 has been applied to cancer treatments to promote the growth of T lymphocytes. This technology has the potential to be instrumental in the growth of the same cells for XSCID. CRISPR-Cas9 would be engineered to specifically target the mutated IL2RG gene, remove it and replace it with a healthy genome sequence. This engineered CRISPR would be injected directly into the patient and would be tested for validity through use of a blood draw test for levels of T lymphocytes in the blood.

Reducing mutation rates in HIV-1 to improve CRISPR-Cas9 treatment

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Human immunodeficiency virus type 1 (HIV-1) rapidly destroys the CD4+ T-cells of the immune system while evading detection from antibodies and CD8+ cells, making their host vulnerable to opportunistic infections. Although treatments like CRISPR-Cas9 exist that target proviruses without off-target effects, due to the mutagenic nature of HIV-1, mutated forms can escape and prolong infection. The high mutation rate stems from the mechanics of replication using reverse transcriptase (RT). This enzyme synthesizes viral DNA from template RNA, but as it lacks 3’ to 5’ exonuclease activity, it is largely error-prone with a high risk of mutation. We hypothesized that the use of directed evolution (DE) to encourage the development of 3’ to 5’ exonuclease activity in RT of HIV-1 would decrease mutation rates during replication, reducing strain variability, and rendering CRISPR-Cas9 treatment more effective. Efficacy of proofreading RT by DE will be tested using human T-cells infected with HIV-1 in vitro. Using protein engineering with DE, the proofreading function will be selectively developed in the HIV-1 genome encoding RT, and verified by comparing sequenced samples of T-cell DNA throughout the stages of replication. Its effectiveness in treatment will be assessed using CRISPR-Cas9 to treat infected T-cells with and without proofreading function. With sanger sequencing, the proofreading sample is expected to demonstrate a reduced mutation rate in treated HIV-1 infected T-cells. Overall, introducing a reduced viral mutagenic state in the patient as a form of preliminary treatment would render CRISPR-Cas9, an already promising technique, more effective against HIV-1 infection.

COVID-19: Is calprotectin a target for immunomodulatory drug development?

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To date, over 260 million individuals have been infected with SARS-CoV-2. Calprotectin, a heterodimer of S100A8 and S100A9 calcium-binding proteins, is released by neutrophils for various immune processes. Namely, it exhibits microbicidal function through heavy-metal chelation and serves as a proinflammatory ligand for innate immune receptors. Recent studies have revealed that calprotectin levels track closely with increasing COVID-19 severity, suggesting that calprotectin may play a direct role in exacerbating disease. Furthermore, inhibition of calprotectin has led to improved clinical outcomes in other conditions, such as sepsis. Therefore, this study aims to investigate whether decreasing calprotectin levels improves clinical outcomes in BALB/c mice with COVID-19. We will compare a control group of BALB/c mice with *Mrp14* knockout BALB/c mice, which do not express calprotectin. After infecting both groups with mouse-adapted SARS-CoV-2, we will observe markers of clinical severity, such as mortality and symptom severity, measure pro-inflammatory cytokines, especially IL-1, TNF-α, and IL-6, using ELISA assays, and identify organ damage using histopathology. To gauge the clinical potential of calprotectin, we will repeat these tests in BALB/c mice infected with mouse-adapted SARS-CoV-2 and compare a control group with an experimental group treated with narciclasine, a calprotectin inhibitor shown to improve clinical outcome in sepsis. We expect our results to reveal better clinical outcomes, lower proinflammatory levels, and decreased organ damage in the knockout and narciclasine groups compared to controls, demonstrating decreased COVID-19 severity. These results would help develop calprotectin inhibitors as new therapeutics for COVID-19 and possibly other diseases.

Preventing the metastasis of colorectal cancer using adoptive Treg therapy

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Globally, colorectal cancer (CRC) is the second leading cause of cancer death**.** The uncontrollable host immune response associated with gastrointestinal autoimmune diseases like Crohn's disease can cause impaired regulatory T cell (Treg) and T helper 17 cell (T_H17) balance and increase the risk of CRC development. Our study aims to replace current expensive and invasive therapies like chemotherapy and radiation with an adoptive regulatory T cell therapy. By re-establishing the Treg/T_H17 homeostasis in the gut, our treatment would allow for the suppression of autoimmunity and prevent CRC metastasis. We will purify circulating Tregs that express the Foxp3 master regulator from CRC patients. The Foxp3+Tregs will be activated using cell culturing upon stimulation with antibody-coated CD3 and CD28 beads. Subsequently, Treg expansion will be done by stimulation with interleukin-2 (IL-2). Upon the delivery of the expanded Tregs into CRC patients, we expect Foxp3+Tregs to suppress autoimmunity. We will use flow-cytometry to quantify the expression levels of Treg markers and measure Treg count followed by enzyme-linked immunosorbent assay (ELISA) to quantify cytokine secretion from T_H17 cells in peripheral blood mononuclear cells. We expect an upregulation of Foxp3 in the expanded Tregs, an increased Treg count, and a downregulation in T_H17 cytokine (IL-17, IL-21, IL-22) secretion by T_H17 cells, which would indicate increased Treg suppressive capacity thus a successful therapy.This adoptive Treg therapy will serve as a novel targeted and non-invasive treatment strategy for early-stage CRC in patients with gastrointestinal autoimmune diseases.

CAR T-Cell therapy as a potential treatment in BIA-ALCL

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Breast implant associated anaplastic large cell lymphoma (BIA-ALCL) is a fatal disease that develops 7-10 years after breast implantations due to chronic inflammation caused by foreign implants. 700 cases have been confirmed worldwide and have resulted in 36 known deaths. Current treatment is limited to surgical removal of the metastasized cells and implants, which can be painful and life-threatening for patients. CAR T-cell therapy showed effectiveness treating Non-Hodgkin's Lymphoma, which is similar to BIA-ALCL. This study further investigates the viability of treating early-stage BIA-ALCL using CAR T-cell therapy that targets CD30+, a BIA-ALCL marker. Immunodeficient, SCID/NOD mice bearing induced BIA-ALCL are treated with CAR T-cells for 17 days, and the CD30+ level in their peripheral blood mononuclear cells is measured using ELISA and flow cytometry to compare the effectiveness of the engineered T-cells. Cancerous mice treated by CAR T-cells are expected to have lower CD30+ levels compared to the ones without treatments, which indicates that CAR T-cell therapy is effective in treating BIA-ALCL at early-stage. CAR T-cell therapy reduces the risks of patients developing BIA-ALCL post-implantation, thus, improves patient life quality. This study extends the application of CAR T-cell therapy in treating lymphoma induced by chronic inflammation. Furthermore, this research contributes to research in early cancer detection, immunotherapy treatment and plastic surgery. Future studies can be expanded upon through clinical trials to investigate the safety and effectiveness of CAR T-cell therapy on individuals with breast implantations.

Introduction of mutant APOBEC3G into CD4+ T-cells to resist HIV-1

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Human immunodeficiency virus 1 (HIV-1) is a lethal retrovirus with high genetic diversity and replication ability. HIV infects human CD4+ T-cells, where rapid decline in CD4+ T-cells count is an indicator of HIV-1 infection. APOBEC3G (A3G) is a human anti-viral enzyme that blocks early stages of HIV-1 replication by counteracting reverse transcriptase, an essential regulatory enzyme for HIV-1. Nevertheless, HIV-1 overcomes A3G anti-viral activity by employing Vif accessory protein to degrade A3G. Due to a Proline/Glutamine point mutation in residue 129 (P129D), A3G in gorillas, also known as A3G-P129D, was found to be resistant against diverse, including human, Vif variants, thereby shedding light on a potential A3G-mediated gene therapy for HIV-1. As such, we hypothesize that transforming human A3G to A3G-P129D will prevent Vif degradation, thus ultimately blocking HIV-1 replication in CD4+ T-cells. Using CRISPR-Cas9 tools, P129D mutation of A3G will be introduced into human embryonic hematopoietic stem cells. Engineered stem cells will be transplanted into immunodeficient, i.e. SCID, mice, which will later be infected with HIV-1. Following infection, monoclonal antibodies will be employed to tag CD4+ T-cells, and thereafter, flow cytometry will be applied to compare CD4+ T-cell count in mice that

express A3G-P129D versus control mice transplanted with unmodified human CD4+ T-cells. We expect a notably higher CD4+ T-cell count in mice carrying P129D mutation than in the control group, hence corresponding to efficient prohibition of HIV-1 replication. Overall, our novel gene therapy approach could open the door to a potential long-term treatment for HIV-1.

Injecting MenSCs to induce β-cell regeneration in type 1 diabetes

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Affecting over 300,000 Canadians, type 1 diabetes (T1D) is an autoimmune disorder in which insulin-producing pancreatic β-cells are attacked by cytotoxic T cells. The destruction of β-cells leads to low levels of insulin, resulting in higher blood glucose levels and low glucose mobilization to tissues. While no treatment is widely used to increase β-cell production for T1D, mesenchymal stem cells have shown promising results in inhibiting $CD4^+$ T cell proliferation and β-cell regeneration. However, collecting bone marrow MSC is an invasive process, limiting further advances. We propose to investigate this process using menstrual blood-derived stem cells (MenSC), a novel type of mesenchymal stem cell with a higher rate of proliferation. We will induce β-cell regeneration using MenSCs in vitro by increasing the expression of neurogenin-3, pdx1, nkx 6.1 and pax to activate differentiation, as well as increase TNFR2 expression to convert the T cells into $CD4^+FOXP3^+$ Tregs. These cells will then be transplanted into the pancreas to slow T1D progression. We hypothesize that a dual approach via regeneration and immunomodulation will be most effective and decrease the need to administer insulin in T1D. This approach will be studied using rodent β-cell cultures as a model for observing β-cell regeneration in vitro and using MenSC differentiation to grow $CD4^+CD25^+FOXP3^+$ Treg cell populations in vitro for transplantation into NOD (non-obese diabetic) mice. We expect increases in β-cell populations due to regeneration and immunomodulation of $CD4^+$ cells by Treg cells. If effective, it holds the potential to develop a long-term treatment for T1D.

Using HLA-DR to establish contributing factors of primary biliary cholangitis

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Primary biliary cholangitis (PBC) is an autoimmune disease, slowly progressive, cholestatic liver disease that predominantly occurs in women. A recent meta-analysis found that the human leukocyte antigen (HLA)-DR07 and -DR08 genes are risk factors for PBC, contrasting protective factors HLA-DR11 and -DR13. PBC targets cells that have an accumulation of the E2 component of the pyruvate dehydrogenase complex (PDC-E2)—an antigen that triggers T-cell response in the liver. PDC-E2 has a high binding affinity to -DR08, while -DR11 does not show a positive response to this antigen. Thus, we hypothesize that knocking out -DR08 will drastically decrease T-cell activation in causing PBC. To study our hypothesis we propose knocking out HLA-DR8 and -DR11 using CRISPR-Cas9 in a mice model. The two genes will be knocked out individually then compared to study the onset of PBC. Western blot will be performed to confirm the knockouts, ELIZA analysis will be conducted to study liver function, chemokines and cytokines response. We predict that knocking out -DR08 will allow us to determine -DR07 involvement in PBC and, similarly, if -DR13 will compensate for -DR11 depletion. Overall, we anticipate to see reduction in T-cell aggression and liver cells' survival of the inflammation impact. Future directions include further studying a potential blocker to tone out -DR08 exposure and minimize PDC-E2 interaction, as well as map out HLA-DR genes involvement to gain a novel understanding of PBC and its genetic factors, and thus how to decrease risk and screen in early life.

Efficacy of omalizumab-mepolizumab combination in type 2 allergic asthmatic mice

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Over 250 million people worldwide are affected by asthma, a chronic inflammatory airway disease that causes breathing difficulties. 50-70% of asthmatics have Type 2 (T2) asthma, driven by T2 inflammation involving T-helper (Th)2 cytokine-mediated eosinophil activation, immunoglobulin (Ig)E production, airway hyperresponsiveness and remodelling. Omalizumab, an IgE antibody, and mepolizumab, an interleukin (IL)-5 antibody that decreases eosinophils, are used to

manage T2 allergic asthma. While combining monoclonal antibodies targeting distinct pathways have demonstrated additive and synergistic benefits in many diseases, it is unknown whether omalizumab-mepolizumab combination will improve T2 asthma treatment outcomes. Our research will compare the efficacy of saline, omalizumab, mepolizumab, and omalizumab-mepolizumab combination in an established BALB/c mouse model of allergic asthma sensitized with ovalbumin (OVA). Treatments will be administered by subcutaneous injection one hour before OVA challenge, once-daily for one week. Following the final OVA challenge, we will assess total and differential leukocyte count in bronchoalveolar lavage fluid (BALF). ELISA will be used to quantify Th2 cytokines (IL-4, IL-5, IL-9, and IL-13) and IgE levels in BALF and serum[10]. Lung histological changes will be assessed using staining. Additionally, hyperresponsiveness will be measured using airway resistance and elastance. Omalizumab-mepolizumab combinatory therapy may target several pathways in T2 inflammation simultaneously, potentially leading to additive or synergistic effects. Thus, we expect the combination to be more effective in reducing inflammation and histopathological changes in asthma compared to either monotherapy. Our study may reveal benefits of omalizumab-mepolizumab treatment that could inform the development of new regimens for improved T2 allergic asthma management.

Vitamin-D receptor nanoparticle as a novel treatment for multiple sclerosis

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Multiple Sclerosis (MS) is a chronic disease that affects the central nervous system due to inflammation of the myelin sheath, but the cause of MS remains unclear. It has been suggested that Vitamin D (VitD), which supports anti-inflammatory mediators by increasing levels of T regulatory cells (Tregs), and Epstein-Barr virus (EBV) both play a role in the pathogenesis and progression of MS. VitD levels have been found to be inversely correlated with EBV load in MS patients. Specifically, Epstein-Barr Nuclear Antigen 2 (EBNA2) competes with VitD for Vitamin D Receptor (VDR) binding, therefore decreasing VitD-VDR binding and ultimately inhibiting VitD from influencing Tregs production. To combat this, we will create a VDR-like nanoparticle to be initially injected to cells in vitro that will decrease EBNA2-VDR binding, allowing for more VitD-VDR binding. This will lead to an anti-inflammatory response caused by an increase of Tregs. These nanoparticles will be created using recombinant technology to insert VDR's protein sequence into the genome of yeast *S. cerevisiae*, where the translated protein can be isolated and added to a serum. Fluorophore targeting $CD3^+CD4^+CD25^+FoxP3^+$ will also be added that specifically targets Tregs. Therefore, fluorescence detection in a flow cytometer following injection can mean an increase of Tregs, implying decreased EBNA2-VDR binding. These findings are expected to validate the theory that EBNA2-VDR binding leads to the progression of MS. After further study in vitro as well as in vivo, these findings could give rise to a potential treatment for MS.

Outer membrane vesicles as a novel *Salmonella* vaccine

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Invasive nontyphoidal *Salmonella* (iNTS), an underestimated disease endemic to sub-Saharan Africa, disproportionately affects populations with poor access to healthcare. Existing vaccines cannot address the culprit *Salmonella* serovars and produce poor immunogenicity in infants, the highest-risk population. Furthermore, current vaccine candidates require complex synthetic chemistry and remain expensive, time-consuming, and serovar-limited. Comparatively, outer membrane vesicles (OMVs), which are secreted by gram-negative bacteria, provide several advantages: simple synthesis, low cost, potential for multivalency, and significant immunogenicity against pathogenic bacteria. We propose using OMVs of non-pathogenic *Escherichia coli* as a vaccine delivery platform for *Salmonella* antigens. *E. coli* mutant strain CLM37 cannot express its own O-antigen, a polysaccharide unique to different bacterial strains. As demonstrated in related studies, CLM37 will be transformed with plasmids containing the gene for *Salmonella* O:4-antigen, which is present in multiple *Salmonella* serovars. We expect CLM37 to express O:4 on its surface and consequently on secreted OMVs, which will be collected via ultracentrifugation and used to induce host immune response. To evaluate immunogenicity, mice will be injected with O:4-expressing OMVs or control (empty) OMVs. Collected sera will be assayed via Western blot to quantify antibody response to O:4. Furthermore, vaccine efficacy against *Salmonella* infection will be evaluated via opsonophagocytic assay and murine vaccine challenge. Respectively, we expect higher *Salmonella* killing in leukocytes primed with vaccinated-mouse sera, and

lower *Salmonella* colonization in mice immunized with O:4-expressing OMVs. These results would further establish OMVs as a versatile, cost-effective delivery platform, paving a pathway for future vaccines.

Prévention du mélanome: Mesurer la réponse immunitaire au gp100

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Le mélanome, un type de cancer de la peau, est le cinquième cancer le plus fréquent aux États-Unis. Malgré le développement de diverses approches thérapeutiques pour le traiter, certains patients n'y répondent pas. Prévenir son apparition est donc primordial, mais aucun vaccin prophylactique n'existe pour ce cancer à l'heure actuelle. Activer le système immunitaire à l'aide d'un antigène surexprimé, soit un antigène exprimé par les MHC de classe I dans plus de 90% des tumeurs mélaniques, est une approche thérapeutique dont l'efficacité a été démontrée dans des essais cliniques. Notre stratégie consiste donc à administrer la protéine gp100, un antigène surexprimé, ou un placebo dans un modèle murin et mesurer par cytométrie en flux l'amplitude de la réponse immunitaire. Nous nous attendons à observer une grande concentration de cellules dendritiques présentant le gp100 ainsi qu'une hausse de la concentration de lymphocytes T avec les récepteurs CD4(+) et CD8(+) chez les souris traitées avec la protéine gp100. Ensuite, nous injecterons des cellules de mélanome murin B16 dans les souris prétraitées avec gp100 ou le placebo, et le développement de tumeur sera monitoré. Nous nous attendons à observer un retard ou absence de tumeur chez les souris traitées avec gp100. Finalement, comprendre l'efficacité de la réponse immunitaire stimulée par gp100 permettrait le développement d'un potentiel vaccin prophylactique contre le mélanome.

The early detection of HIV using a Nef direct ELISA

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Human immunodeficiency virus (HIV), affecting approximately 86,000 Canadians, causes acquired immunodeficiency syndrome (AIDS) characterized by flu-like symptoms and opportunistic infections. Men who have sex with men (MSM) represent a disproportionate 48% of new HIV diagnoses lending to the Canadian Blood Services' requirement for MSM to abstain for 3 months prior to blood donation. To decrease this abstinence period, early diagnosis technology using an HIV capsid protein, P24, is available. While P24 rapid tests can detect HIV just 14 days after exposure, they are inaccurate with multiple studies finding a sensitivity of 76.9%. However, an auxiliary protein associated with HIV infection, known as Nef (negative regulatory factor), may be a better target in determining HIV exposure. We expect Nef to be detectable even earlier than P24, and at higher sensitivity, due to early excretion, while inside exosomes, and extremely high translation rates. We propose the use of a direct ELISA using anti-Nef monoclonal antibodies to detect Nef concentrations in the plasma of MSM patients. We hypothesize that Nef ELISA will provide earlier, and more sensitive HIV diagnoses compared to P24 ELISAs. To address our hypothesis, we will inoculate humanized mice with HIV. Once a day for 21 days, we will take a blood sample, centrifuge for plasma isolation, and run two ELISAs: Nef and P24. We expect to reach detectable levels of Nef protein in less than 14 days (before p24) and have greater sensitivity. This will decrease the abstinence period for MSM thereby making blood donation more accessible.

Oral delivery of antisense oligonucleotide to combat IgE-mediated food allergies

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Food allergy affects roughly 5% of children and 3-4% of adults in western countries, with its clinical presentation ranging from mild to life-threatening reactions. Most food allergies are type I hypersensitivity reactions, characterized by the production and binding of IgE to effector cells. High IgE concentration has been documented in the gastrointestinal (GI) tract of food allergic individuals, suggesting that GI tissues are important reservoirs for allergen specific cells. Recent research combating the IgE response has utilized antisense oligonucleotides (ASOs) to target a polyadenylation signal (PAS) in the IgE pre-mRNA, thus redirecting IgE release from the secreted form to membrane form. Previous work investigating ASO implementation has encountered barriers during oral delivery. To address this, we propose developing a coated oral formulation promoting the efficient and selective release of ASOs into the IgE-rich upper intestinal tract. We hypothesize this goal can be achieved by combining, (1) sodium caprate coated ASOs and (2) a controlled release gastroretentive dosage form

(CR®-GRDF) capsule, to maximize absorption in duodenum mucosa and protect ASOs from stomach acidity, respectively. We suggest administering this novel formulation to transgenic mice expressing humanized IgE using the same ASO designed by Marchalot et al. Using an indirect ELISA, we expect to see reduced IgE concentrations following the administration of our formulation when compared to controls (sodium caprate-ASO, CR-GRDF-ASO). Should our results be as expected, this new formulation may offer an avenue for human clinical trials following safety investigation.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Authors' Contributions
DB: Co-President and founder of the IgNITE Medical Case Competition, assisted authors with their submission, drafted the conference abstract booklet, and gave final approval of the version to be published.
BB: Co-President and founder of the IgNITE Medical Case Competition, assisted authors with their submission, reviewed the abstract submissions and ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.
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