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Mission Statement

The Queen’s Science Undergraduate Research Journal is an annual, peer-reviewed publication that seeks to enrich the student experience through engagement in the scientific community. Founded in 2015, QSURJ is an open-access journal that allows students to contribute through the systematic review and dissemination of undergraduate research.
Letter from the Editor

Dear Reader,

Queen’s University has celebrated over 175 years of research, with students walking the grounds today that carry the potential to unlock tomorrow’s greatest discoveries. From public health policy, to particle astrophysics, to comprehensive programs for those with Schizophrenia, our community of eager researchers continue to ask, examine, and challenge questions that enlighten us of the world we live in. Central to the field of research is the dissemination of the results generated in our academic spaces, as it encourages intelligent discussion and the building of a supportive community. I have had the pleasure of overseeing not only the amazing work of those inside the labs and board rooms, but also of those who are committed to the accessibility of publishing platforms through peer editing, marketing, and sponsorship programs. QSURJ has been working over the past few years to integrate more diverse content into the journal, and this year I am pleased to say that we have been able to foster a wider breadth of academic research spanning from space technology to biomedical research. I would like to thank this year’s incredible executive team for all of their work in putting together this publication - it would not have been possible without them.

Thank you for taking the time to appreciate this year’s issue of the Queen’s Science Undergraduate Research Journal. It has been a pleasure to oversee this publication, and I cannot wait to see what QSURJ has in store in the future.

Sincerely,

Lexi Koster
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The Alzheimer’s disease signature molecular events may result from the failure of antiviral defense mechanisms in the brain.

A review of the recent findings suggesting viral etiology of Alzheimer’s disease.

Jamil Muradov

Abstract
The possibility of the viral etiology of Alzheimer’s disease has become a recurrent theme in the recent literature. The role of Aβ in inflammation modulation and anti-microbial defence has been recognized. The viral infections have been implicated in the neuronal changes, characteristic of Alzheimer’s disease, at the transcription regulation, transcription, translation, and proteome levels. Although the direct mechanism by which herpesviridae cause induction of Aβ and oligomerization of Aβ have not yet been shown, for the first time, the direct role of Aβ in viral particle clearance has been demonstrated. Aβ may play a physiological role in the brain, and Alzheimer’s disease might result from a failure of the antiviral brain defence systems. In this review, the relationship between Alzheimer’s disease signature molecular events and the viral infection is discussed. Re-evaluation of role of Aβ allows proposing novel directions in Alzheimer’s research and disease treatment methods.

Hypothesis Statement
It is thought that the neuroimmunological responses, characteristic of the early stages of Alzheimer’s disease (or pre-dementia stage), serve to clear the viral invasion. In the latter stages, if the defence mechanisms fail, viral infection might become persistent and result in an altered inflammatory response, pathological Aβ oligomer accumulation, Tau-protein buildup, and, hence, brain degeneration.

1. Introduction
1.1 Overview of Alzheimer’s disease
Alzheimer’s disease (AD) is a neurodegenerative disorder that is characterized by memory deterioration and gradual cognitive incapacitation. This condition was first described by Dr. Alois Alzheimer in 1906. He noticed the degeneration in brain tissue of a female patient who had suffered from a poorly-understood mental illness. AD is characterized by the loss of neurons in multiple parts of the brain, such as temporal and parietal lobes, frontal cortex, cingulate gyrus, amygdala, and other parts of the brain. Post-mortem analysis of the AD patients’ brains showed high consistency in hippocampal size reduction, which is related to the memory deterioration. Distinct features of a pathological brain histology include amyloid plaques (senile plaques) and neurofibrillary tangles; the presence of Lewy bodies is also not uncommon.

1.2 Clinical Epidemiology of Alzheimer’s disease
Alzheimer’s disease (AD) is the most common type of dementia (significant decline in cognitive, memory, and learning functions of the brain) and makes up 50% to 70% of all dementia cases. Early AD symptoms include the difficulty with remembering recent events. Other cognitive dysfunctions include impairment of learning, difficulty with understanding languages, agnosia, apraxia. Interestingly, the memories from the childhood (both episodic and semantic memory) and motor memory are affected to a lesser degree. The progression of the disease is often associated with physical, mental, and social disability. The patients tend to suffer from depression, sleep disorders, disorientation, speech disorders, oculomotor deficits. In late stages of the disease, they often become dependent on the care-giving family members. The care-giving relatives, especially spouses, may experience significant levels of chronic stress, problems with immunity, and may suffer from clinical depression. In 2006, 12 international experts were asked to perform a meta-analysis review of epidemiological data on AD. It was estimated that, in 2006, 24.3 million people suffered from Alzheimer’s dementia. It was also projected that 81.1 million individuals will be affected by 2040. Overall, AD constitutes a significant social and economic burden: only in Europe 55 billion € is spent yearly on the health care costs, not including indirect costs.
history of AD is obtained with age of onset noted for each affected individual\(^2\). Early onset AD diagnosis can be made in patients younger than 65. After 65 years, AD is classified as late onset \(^13\). To assess global cognitive function, Mini-Mental State Examination and clinical dementia ranking (sensitivity of 92% and specificity of up to 96%) are used clinically \(^12\). Memory assessments can be useful in distinguishing the patients with dementia and the patients with memory problems due to the age: Memory Impairment Scale test can be used as a short, quick first-line tool in evaluating AD (sensitivity of 60% and 96% specificity) \(^12\). Additional tests can be used to assess executive and instrumental functions \(^12\).

Neuroimaging is another major method of evaluating AD. The brain CT scan is amendable to exclude hematoa, hydrocephalus, and brain tumors \(^12\). MRI can be used for the same purpose but can also be used to assess the brain atrophy. It was shown that MRI can show the AD-related hippocampal atrophy with the accuracy from 67 to 100\% \(^14\) and general brain atrophy with sensitivity of 85% and specificity of 88\% \(^15\). Additionally, MRI allows to assess the vascular degeneration.

Molecular genetic testing can be useful for further evaluation of the patient’s condition. An association of APOE ε4 allele and AD has been well established: approximately 35-45\% of patients with familial AD have at least one copy of the allele \(^16\). No major biomarkers are currently available to routinely diagnose the disease at its early stages.

### 1.4 Alzheimer’s disease signature molecular events

Since 1906, the causes of AD and molecular mechanisms underlying AD have been debated. Even though it is agreed-upon that genetic, environmental, and microbial causes all play a role in the development of the disease, the most common hypotheses about its pathogenesis are cholinergic hypothesis, Aβ hypothesis, tau-hypothesis, and inflammatory hypothesis \(^17\).

Currently, a number of various interrelated molecular pathways are suggested in AD causation. Amyloid precursor protein (APP), an integral membrane protein found in the synapses, is a precursor of multiple AD culprit molecules. Although its exact function is not currently established, its localization suggests it takes part in the regulation of synaptogenesis and synaptic activity. In a recent study \(^18\), an updated molecular mechanism of AD has been suggested: it is thought that normal processing of APP involves its cleavage by α-secretase, while, in AD, secretases β and gamma are likely to cause a disruption in the maintenance of normal Aβ peptide levels \(^18,19\). As a result, Aβ peptides spontaneously aggregate into soluble oligomers that, in turn, form insoluble β-sheet senile plagues \(^18,20\). The cleavage of the APP into Aβ proceeds through C99 APP intermediate (a marker of the pathological condition) \(^18\). Aβ-peptide can result in Aβ 40 and Aβ 42 formation (the latter plays role in attracting microglia to the site of neuronal damage and release of II-1β, TNF-α, IFN-gamma, which, in turn, further results in an increase of Aβ 42 \(^21\). Aβ-oligomers play role in astrocyte-neuron complex integrity; they are responsible for the gradual degeneration of these complexes and vascular degeneration in AD brain \(^22\). Importantly, Aβ-oligomers hyperphosphorylate tau protein \(^23\). Tau proteins stabilize microtubules and, hence, play an important role in the neuronal structure maintenance. When hyperphosphorylated by Aβ-oligomers (due to phosphorylation of improper residues), Tau proteins cannot associate with microtubules and microtubules quickly disintegrate \(^21\). This leads to the instability of neuronal membrane and the spontaneous association of microtubule fragments into the neurofibrillary tangles. Importantly, the tangles and senile plagues not only result in disruption of normal neuronal homeostasis, but also decrease the acetylcholine levels in the synapses responsible for learning and memory through inhibition of choline acetyltransferase and acetylcholinesterase activity, and vesicular transport disruption \(^24,25\). Conversely, glutamate levels in such synapses pathologically increase. Low-level chronic efflux of Ca++ through glutamate-stimulated N-methyl-D-aspartate glutaminergic receptor (NMDAR), which is a Ca++ channel, compromise the Ca++ dependent responses within the cell \(^4\). Although these events (neurotransmitter level fluctuations) play an important role in disease progression, they are not erasable in the pathogenesis of the disease.

### 1.5 Current pharmacological treatment options only target the symptoms of Alzheimer’s disease

The only 5 families of drugs, approved by the US Food and Drug Administration (FDA), for AD treatment (2015), all target the components of the synaptic transmission system, but not the primary causative events in the progression of AD \(^18\). These drugs are rivastigmine (acetylcholinesterase inhibitors), galantamine (acetylcholine receptor potentiators), tacrine (acetylcholinesterase inhibitors), donepezil (acetylcholinesterase inhibitors), and memantine (NMDAR inhibitor) \(^18\). Current diagnostic and treatment methods of AD are fairly reliable. However, at the time of diagnosis, the brain damage cannot be reversed or prevented. Although, over the past century, the understanding of molecular pathologies in AD has expanded, the causative factors of AD are yet to be identified. This would allow to create novel treatment methods that do not only target the symptoms of the disease, but can effectively prevent AD progression.

### 1.6 The shift in paradigm of Alzheimer’s disease understanding

In the recent decade, the focus in AD pathogenesis research has gradually broadened. It has been suggested that the neurotransmitter level fluctuations, Aβ, and Tau-related aggregation are not the only key players in AD. The function of Aβ, a key landmark protein in AD, is still poorly understood, but its role in antimicrobial defence has been implicated \(^26\). A large body of literature has investigated the role of brain immune defence (glial and astrocyte responses) in the pathogenesis of AD. The viral origin of AD has been fervently debated. It is agreed-upon that an inflammation is a normal physiological response to either exogenous (pathogens, physical damage) or endogenous (cancer cells, autoimmune conditions) stress. It is also known that, in AD, over-exaggerated response of glial cells to formation of Aβ oligomers and neurofibrillar tangles further promotes tissue damage and contributed to the progression of the disease \(^27\). However, the fact that substantial accumulation of amyloid-β oligomer levels can be present in completely healthy, cognitively efficient, and AD-free individuals, alongside with the highly stable evolutionarily conservation of Aβ peptide, suggests that it could possibly play a physiological role \(^28\). Conversely, it has been previously thought that Aβ is an abberant peptide, only present in pathological states. A big number of works \(^18,26-31\) on this topic have shown that viral induction of pathological Abeta oligomerization in AD should not be overlooked. In fact, it is possible that the brain degeneration is a result of a latent viral infection. In the current work, the causative factors of molecular mechanisms underlying AD progression are reviewed. It is thought that the neuroimmunological responses, characteristic
for the early stages of AD (or pre-dementia stage), actually serve to clear the viral invasion. In the latter stages, if defence mechanisms fail, viral infection might become persistent and ultimately results in a persistent inflammatory response, pathological Aβ oligomer accumulation, Tau-protein buildup, and, hence, brain degeneration.

2. Main Body

2.1 Aβ may play a role in brain immune defence

A key role of the Aβ signaling in the initiation of glial anti-pathogen response has been established. In AD, soluble Aβ oligomers and even Aβ fibrils were found to bind to a multitude of microglial receptors: SCARA1, CD36, CD14, αβ1 integrin, CD47, and several toll-like receptors. The pro-inflammatory role of Aβ is supported by the observation of the increased cytokine release upon stimulation of above-mentioned receptors and lack of response in receptor knock-out models. It should be noted that the cytokine response, normally, may carry out a defensive function: IL-1 (glial cytokine) was found to facilitate neural plasticity through oligodendrocyte-neuron interactions, reduce hyperalgesia, and prevent excessive phosphorylation of NMDA receptors in peripheral nervous tissue (trigeminal nerve) of mice. Further, the mutual activation of glial cells by Aβ and Aβ synthesis, caused by pro-inflammatory cytokines, does not necessarily cause an irreversible “extinction vortex” (“chicken or the egg situation”), in which pro-inflammatory feedforward stimulation of glia leads to an uncontrolled build-up of Aβ. In fact, Aβ signaling induces the phagocytosis of the amyloid oligomers by the glia. Both soluble and insoluble amyloid aggregates are degraded via a lysosomal pathway, with insoluble fibrils being more resistant to the protease. The pathologies in this system, related to the inefficient regulation of Aβ utilization or glial deactivation (poorly understood mechanism), often result in the buildup of fibrils and neural degeneration. Such causes include, but are not limited to, CD36 deficiency (plays a role in post-infection deactivation of glia), deficiency of Aβ proteolytic enzymes or uptake proteins. These mutations, however, are relatively rare. More prevalent molecular determinant of AD (sporadic AD) is an apolipoprotein E4 variant (APOE e4), which causes impaired phagocytosis of Aβ by microglia and astrocytes, excessive secretion of cholesterol, disrupted overall lipid metabolism, and other pathologies. Additionally, the altered structure of mitochondrial membrane (due to disrupted lipid metabolism) may result in higher susceptibility to reactive oxygen species.

2.2 Previously established links between Alzheimer’s disease and the viral infections

It is clear that a complex interplay between nervous and immune cells within the brain could not have been evolutionarily preserved without a purpose. All the above mentioned mechanisms evidently participate in a normal brain defence and neuronal plasticity, regeneration, and nutrition. However, it cannot be denied that an exogenous impetus (environmental toxicity, brain injury, microbial infection) can result in an impaired immune response which causes AD. The individuals that present AD-correlated genotypes (APOE e4) are at particularly high risk. Still, APOE e4 is not a necessary determinant of the disease. In an early work by Itzhaki and colleagues, the strong association between Herpes Simplex Virus type 1 (HSV-1) infection and AD risk was established. In fact, since then, a number of studies have outlined the role of HSV-1, human herpesvirus types 6 and 7, and Epstein-Barr virus in AD. It is known that herpesviridae have a unique ability to “highjack” the neural transport systems: even after a minor zoster infection the latent virion particles can hide in the trigeminal ganglion. Further, those particles can use both retrograde and anterograde transport to reach the intracranial nervous system. This allows for viral transport into the brain tissue and delayed reactivation of the viral particles. The post-mortem analysis of viral DNA levels in APOE e4 positive and negative patients showed that APOE e4 variant might play a role in viral infection susceptibility, which would suggest viral origin of AD. Additionally, a longitudinal study, conducted in Taiwan, showed that the likelihood of developing AD, in patients with severe herpetic infection, was reduced by 90% using aggressive antiviral medication. Unfortunately, until recently, no studies were able to further supplement this information by showing the functional relationship between molecular events in early AD and herpetic infection. An important step to be done is to show how exactly herpesviridae cause neural inflammation.

2.3 Role of HSV-1 in brain inflammation

Designing a study that would allow to observe a sequence of events from invasion of a neuron by a virus to degeneration of the neuron, due to accumulation of amyloid/Tau fibrils, is not easy. This task becomes almost impossible to perform in humans due to the ethical concerns. In a recent study, the progression of the neuroinflammatory and neurodegenerative events, in response to an HSV-1 infection, has been studied in the murine encephalitis models. The authors aimed to show that the latent HSV-1 infection has the capacity to reactivate and cause asymptomatic inflammation in the brain. Three experimental groups, infected with HSV-1 (intranasal inoculation), were euthanized at 7, 15, or 60 days post-infection, and the nervous inflammation marker levels were compared to those of the control groups. 7, 15, and 60 days post-infection (dpi) results were also compared to elucidate the differences and similarities in biomarker levels during acute infection (7 days), productive infection period (15 days), and during the latent reactivation phase (60 days). The presence of HSV-1 infection in the brain and trigeminal ganglion tissue was confirmed using the anti-HSV-1 antibody anti-ICP4 (viral antigen). It was found that anti-ICP4 antibody intensively stained the cortex and afferent sensory fibers of the trigeminal nerve. This, again, supports the possibility of the viral axonal transport. Toll-like receptor, interferon α and β levels were used to evaluate the adaptive immune response. It was found that TLR levels increased substantially from 7 days post-infection to 15 dpi. Interestingly, the highest increase was observed for TLR 2 and 3: receptors known to be induced by amyloid β. Interferon levels were also increased over the given period. At 60 dpi, TLR levels were elevated. Further, the levels of early degenerative markers were measured during productive and latent infection stages. Improperly phosphorylated Tau-protein (p-tau) and improperly-cleaved Tau isoform (TauC3) levels were evaluated. The rise in the levels of these markers was highly significant 15 dpi. Even though, the levels of these markers were also higher at 60 dpi for infected animals than those of control animals, an elevation in neurodegeneration marker levels was also noticed in the control mice (natural aging).

The authors were able to show that, during productive neural HSV-1 infection, immune resources of the brain are mobilized:
TLR, interferon, and other pro-inflammatory compound levels rise. Interestingly, in the preceding acute phase, the levels of some of TLRs and interleukin-related factors are lower, which is explained by HSV-1 caused mRNA degradation. This mechanism is thought to be employed by HSV-1 to establish latency. High TLR-4 levels can be thought to play a role in latent infection. HSV-1 was shown to cause an increase in Tau-levels, which suggests its role in neurodegeneration.

Unfortunately, the authors were not able to show any effect of HSV-1 on amyloid β levels. Additionally, all the findings primarily are concerned with the TLR levels. Although, they are known to be important in the glial cell activation, through PAMPs/DAMPs ligand binding, they only indirectly suggest that the glial cells were in the neuroinflammatory defence mode. Glial cells possess a degree of phenotypical flexibility which allows them to turn on/off the sets of genes needed to modulate the phenotype. As such, AD progression is associated with the increase of TREM2, APP, BACE1, and CD36 expression in the glia. A study that could show the increase of TLR4 levels, as a result of the glial phenotype modulation, would support the latent asymptomatic inflammation theory strongly.

2.4 HSV-1 infection results in increased levels of AD-specific molecules

Another study shows distinct HSV-1-induced APP expression in rats. These findings complement the work conducted by Martin and colleagues in 2014. The investigators were able to use the monolayer neuronal preparations from the rat cortex. The tissue samples were fixated and either treated with HSV-1 (experimental) or with the mock inoculate (control). A multitude of the organelle preparation, immunohistochemistry methods, and enzymatic assays were used to study the localization and expression levels of the APP cleavage pathway. It was found that, in the virus-infected cells, CTF (C-terminal fragment of APP that can be cleaved further to yield Aβ) was increased in the nuclear compartment. The same was true for AICD peptide (APP intracellular domain, resulting from APP CFF cleavage). Interestingly, AICD directly upregulates expression of NEP protein: an enzyme which can degrade Aβ.

Evidently, HSV-1 infection results in the elevated levels of the molecules that are eminent in AD. The problem, however, with this model is that it only shows the effects of an acute HSV-1 infection. Additionally, the relationship between Aβ and HSV-1 (neither quantitatively, nor functionally) is not elucidated.

Overall, these findings show that neurons and glia are able to quickly respond to a viral infection by changing certain protein levels. It is not clear, however, why Aβ oligomer aggregation phenotype would be evolutionarily preserved if it lacked a particular useful function. Two recent breakthrough studies have increased our understanding of the causative role of viruses in AD and Aβ function.

2.5 AD-specific transcriptional and translational events may be directly caused by a herpetic infection

For the first time, the researchers linked the viral activity with the functional genomics of multiple brain regions in the brains of pre-clinical AD patients, post-mortem. All patients had died before they developed any cognitive symptoms. This allowed for an assumption that the study results would represent the early stages of AD. The investigators created a computation model-probabilistic causal network (PCN) that would identify significant changes in the neuronal gene expression data of individuals with pre-clinical AD (in comparison with healthy controls). For this step, the study was focused on the entorhinal cortex and hippocampus: the regions that are affected by the neuronal damage the most. They found that the promoters of genes, regulated by C2H2 transcription factor, and the number of G4 DNA motifs available for transcription initiation were higher in pre-clinical AD patients. G4 and C2H2 DNA regions were found to be within the sequences responsible in multiple viral contexts: binding Epstein-Barr virus Rta protein to promote the expression of antiviral genes, regulating anti-HIV receptor transcription, and even interacting with HSV-1. Another finding of the study was identification of miR-155 expression in pre-clinical AD: a molecule involved in multiple virus-host interactions (EBV, HHV-6A, other herpesviridae). As it can be seen, the genome, proteome, and transcriptome-wide computerized study revealed the anti-viral defence activity in the pre-clinical AD patients. The difference in the RNA-seq based abundance of the virus in pre-clinical AD and healthy subjects was noted. All data were retrieved from the sequencing database at Mount Sinai Hospital Brain Bank. Depending on the threshold of RNA-seq analysis, the investigators were able to identify from two main groups of rosovloviridae (genus of herpesviridae) to a great number of individual variants in anterior prefrontal cortex and superior temporal gyrus of the patients. These findings were further strengthened by the addition of data from the Religious Orders Study, Memory and Aging Project, and Mayo Temporal Cortex Database. The presence of HHV-6A and HHV7 was consistent. Additionally, PCN also identified the consistent presence of the latency-associated transcript (LAT) and HSV-1 in a large proportion of subjects. The next step of such a massive study was to explore the relationship between clinical symptoms of AD and HHV6 occurrence. Both HHV6 and 7 were associated with AD-specific traits. This was complemented by the fact that the AD-traits were also associated with the increased activity in viral quantitative trait loci (host DNA regions active during viral infection). The genes that are perturbed by herpesviridae included multiple genes important in APP processing. Also, the genes, implied in AD-risk, had higher profiles of expression in HV6 infected individuals. This, as a consequence, could result in higher neuronal death rates in HV6-rich regions. Indeed, the presence of HV6 within certain regions of the brain was highly correlated with low neuronal per total cell count fraction. The HHV6 infection was even associated with the anti-viral perturbation on a translational level. Additionally, HHV6 and HHV7 were not found to be ubiquitous in all types of neural degeneration and were presumably specific to AD. The study very strongly established a possible causative role of herpesviridae in AD. It employs the information from multiple large databases. The authors are also able to show that viruses affect normal neuronal homeostasis at all of DNA, transcriptome, tRNA, and proteome levels. This study has a huge significance not only for AD research, but for other fields as well. A similar approach could be used to elucidate the pivotal points in the molecular mechanism of other diseases, such as cancer and other type of neurodegeneration. Still, the authors acknowledge that even though PCN shows interactions within the molecular network, future studies are needed to elucidate the order of the above mentioned molecular events. Additionally, in the study, only pre-clinical AD patients were analyzed. It is not known
whether they would have consistently developed the later stage symptoms.

2.6 Molecular events in early AD may serve to defend the brain from the viral infections

Previously discussed studies show how viruses are able to alter the expression patterns in the infected cells. It is logically correct to state that initial changes in the cell phenotype should evolutionarily be directed at defending from the virus. One more recent study casts light on why Aβ expression, a distinct characteristic of AD, is needed to protect the brain. For the first time, a group of researchers at Harvard showed direct evidence of Aβ in anti-viral defence in mouse encephalitis models. Mice, transgenically modified to produce higher levels of Aβ and infected with HHV6, were found to have significantly higher longitudinal survival rates than normal infected mice. Because human neuronal HHV6 receptors are different from mice (murine homologs in mice and CD46/CD134 in humans), a human neuroglioma monolayer was also used. Further, it was shown that oligomeric concentrations of cell-derived Aβ supplement protect neuroglioma cells from HSV1-caused death. The study also showed how exactly Aβ interacts with the viral particles. The binding of Aβ to HSV-1 and HHV6 A and B was assayed using modified ELISA. The authors incubated Aβ with heat-mobilized virion particles and measured Aβ oligomerization using Aβ antibodies. It was found that cell-derived Aβ avidly binds viral surface glycoproteins; the binding signal is also detected when neuroglioma cells are incubated with the viruses. Conversely, no surface binding was observed when viruses were incubated with Aβ-deficient neuroglioma cells. Then, the authors established the functional importance of Aβ binding to viral surface. It was determined that fibril-covered viral particles aggregated in the extracellular space, but were not able to invade the host neuroglioma cells. Surprisingly, right after neuroglioma monolayer inoculation with HHV6, rapid oligomerization of Aβ allows to prevent infection, and the oligomers do not form any plaques or tangles; after 3 weeks of persistent infection, however, AD-specific Aβ plaques can be observed in the culture. These findings do not prove that AD has purely viral pathogenesis. However, the fact that Aβ oligomers lower the HHV6 shows its role in antiviral defence. Of course, it cannot be refuted that Aβ oligomerization in excess leads to irreversible brain damage, and that viruses have a probable role in the acceleration of the process. AD is likely a result of the failure of the brain tissue to clear the pathogen. Further investigation of why Aβ fibrillation becomes malignant may elucidate novel molecular targets for pharmacological treatment. Determination of the causative mechanisms in AD would allow us to enhance the limited repertoire of the therapeutics available to AD patients and prevent long term brain damage.

3. Discussion

Physiological interactions of Aβ oligomer with the immunological response-related receptors in the CNS indirectly suggested the role of Aβ in normal brain defence. It might even facilitate synaptic plasticity, hyperalgesia modulation, and other adaptive processes. Failure of these processes causes AD. Since the 1990s, the role of viral pathogens in Aβ-related neurodegeneration has been recognized. The patients, with the identified AD-related gene variants, are known to be at particularly high risk, and it is possible that their brain defence system is less resistant to viral infection. In a recent study, direct induction and persistence of the brain inflammation, in response to the HSV-1 infection, have been shown. Molecular events of the inflammatory model were comparable with the AD signature molecular events. HSV-1 was also found to induce Aβ, but the absence of the plausible mechanistic explanation of the induction does not allow to establish causal relationship between HSV-1 and Aβ. A recent massive computational analysis study of the genomics, transcription profiles, proteome, and signaling levels in the brains of the AD patients showed key genes and transcription factors that may be directly affected by the virus to initiate a brain defence response. Finally, a direct role of Aβ in viral clearance was shown. Still exact sequence of molecular events from the viral invasion of the nervous system to induction of Aβ, altered inflammatory response, and accumulation of plaques has yet to be shown. Elucidating this pathway in humans might require significant efforts of multiple study groups.

3.1 Controversies in the viral etiology of Alzheimer’s disease

It was estimated that 67% of the world population, younger than 50 y.o., had an active herpetic infection within their lifetime, at least once. A logical question that might arise, then, is why only a fraction of those individuals acquires a persistent viral infection and develops the neurodegeneration. At the same time, given AD is caused by a persistent chronic encephalitis-like condition, the reasons why viral infection causes acute infection in some individuals versus chronic infection in others is not clear. Here, it should be noted, that, if neurodegeneration is considered as a normal process of aging, a viral infection might be viewed as a precipitating factor that fast-forwards neuronal death, in the susceptible individuals. Just like in Parkinson’s disease, a combination of genetic, environmental, and pathogen factors, likely, predisposes to AD. Additionally, viral infection might simply be an opportunistic “passenger” in AD. The reasons for which, in some individuals, the normal brain defence might fail to clear out the infection, becomes persistent, or causes excessive damage are also not well-established. It is possible that APOE e4 variant is one of the factors resulting in a compromised post-infectious Aβ reuptake. Also, according to the above described findings, it is possible that Aβ oligomers can be characterized as playing either a defence or a senile, pathological function. It is not clear whether two types of oligomers have any biochemical differences. In the future, it would be beneficial to identify the distinct molecular features of each type of plaques to explain how they contribute to viral clearance and neurodegeneration, respectively. APOE-related glial reuptake might be less efficient for the senile Aβ oligomers. Additionally, an elegant experiment to show Aβ induction mechanism by viruses has yet to be conducted. It is also possible that AD can be caused by the microbial pathogens, other than herpesviridae. Bacteria, fungi, and even prions were implicated in AD. It is also unclear how pathogens are able to invade the brain, but stay silent for several decades, before neurodegeneration is noticed. The Aβ is starting to be recognized as an anti-microbial peptide by the science community, but it is not clear how it pathogenic to form senile plaques. So, three main directions, in which AD research should be carried out in the future, are determination of the causative mechanism by which viral infection results in Aβ expression,
determination of the events that result in the conversion of normal Aβ oligomerization into senile oligomerization (Tau phosphorylation abnormalities should also be studied), and the mechanisms by which viral infections remain latent, due to the failure of the brain defence.

3.2 Development of novel treatment methodologies

The above-discussed findings allow to develop and test multiple novel diagnostic and therapeutic methodologies. First of all, the acuity of the herpetic viral infection can be correlated with the likelihood of developing AD. The screening of the patients with the history of acute herpetic infection, especially encephalitis, for the presence of Aβ, APP, P-Tau, anti-viral cytokines, etc. in the CSF and blood (also suggests BBB damage) can become routine and can be combined with the genetic screening for APOE variants.

In the prodromal stage of AD, identification of the latent viral condition and detection of elevated AD signature molecules might be crucial to prevent the neuronal loss as early as possible. Computerization of the viral infection-specific transcriptome screening methods might be very efficient. Current AD treatment methods are only symptomatic and have limited efficacy. Antiviral and anti-inflammatory therapies can be used to better manage AD. Antiviral drugs were previously shown to decrease pathological Aβ oligomerization, P-Tau accumulation, and HSV-1 titer in the HSV-1 infected cell culture. Antiviral therapy reduced AD signature molecule levels in the murine model. In a recent study, the effects of antiviral therapy in patients with HSV-1 were analyzed. HSV-1 patients have threefold increase in the chances of developing AD, compared to a diverse control group. However, it was shown that the timely antiviral intervention reduced the chance of AD by approximately 90%.

Besides antiviral therapy, targeting brain inflammation and oxidative metabolism is promising. Recently, an indirect modulator of oxidative stress and inflammation in CNS, CAD-31, was shown to diminish the effects of Aβ plaque damage in murine AD model. Stem cell therapy methods have also been tested. Ischemia-tolerant human mesenchymal stem cells were shown to reduce brain inflammation and Aβ pathological oligomerization in mice. In the future, counteracting the “angry” microglial phenotype using monocyte precursor-derived reprogrammed immune cells could also be possible.

3.3 Conclusion

Aβ oligomerization pathway components have been considered as potential therapy targets in AD. However, this approach has not given significant results. The role of Aβ in antiviral defence has to stimulate the field of science to reconsider the possible treatment strategies. The future questions to be answered are how viral infection results in Aβ expression, why Aβ oligomerization becomes pathological, and how latent infection results in slow progression of the disease. Understanding how viral infection can modify the neuronal transcription profiles can be important in identifying the early biomarkers of AD. Pathological oligomerization and reuptake (especially in patients with APOE variation) of Aβ could be modulated using re-programmed stem cells and inflammatory modulators. Finally, temporally-targeted pulse antiviral therapy might prevent the conversion of an active infection into a latent.

Studies of AD pathogenesis should not be limited to herpesviridae. Viral, fungal, and bacterial causes of AD are in the process of re-evaluation by the scientific community. The emerging body of knowledge in how AD is triggered by a multitude of factors will allow preventing the growth of disease prevalence. Microbial causes of other degenerative diseases (Parkinson’s disease, Multiple Sclerosis, etc.) also have become a recurrent theme in literature, and future works in the field are essential to establish microbial factors in other types of neurodegeneration.

4. References

See Appendix A.
Functional and Ethical Considerations of Developing a Therapeutic Cure for HIV Using CRISPR

Nicholas Grubic, Joey Nadasdi, & Robbie Kloosterman

Introduction to CRISPR and Human Viral Treatment

The breakthrough of clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) as a gene editing tool has revolutionized genetic manipulation. Initially discovered as a component of the bacterial adaptive immune system used to neutralize foreign DNA\(^1\), CRISPR was subsequently re-engineered to target the genome of eukaryotes, including human cell lines\(^2\,\,\,3\). Recent developments are evolving the CRISPR/Cas9 system into an adaptable and precise tool for genetic engineering. It uses a guide RNA (gRNA), complementary to a target sequence, that leads the associated Cas9 nuclease to the gene of interest and induces DNA cleavage. In addition to the gRNA, a protospacer adjacent motif (PAM) nucleotide sequence is required to immediately follow the target sequence. This sequence is typically the trinucleotide NGG, which is required for Cas9 to initiate a double strand break in the DNA, which can be repaired by either non-homologous end joining (NHEJ) or homologous recombination (HR) (Figure 1). These cuts and subsequent repairs allow for the knockout of specific genes, or the insertion of foreign sequences. More recently, CRISPR has been used to target a variety of human viruses\(^4\), including Epstein-Barr virus\(^5\), Zika virus\(^6\), and most notably, Human Immunodeficiency Virus (HIV)\(^7\,\,\,8\). Although the CRISPR/Cas9 gene editing system has demonstrated the potential to provide a cure for HIV, several limitations have sparked controversy in regard to its therapeutic value. The inconsistent fidelity and potential deleterious effects of CRISPR, as well as unanswered ethical concerns, call for further research in this field. In this article, we highlight recent advancements of the CRISPR system that may be applicable to HIV treatment, and ethical considerations that must be discussed in order to implement these techniques (Figure 2).

Current Applications of CRISPR/Cas9 in HIV Treatment

Despite the millions of HIV-infected individuals receiving antiretroviral therapy (ART) or highly active antiretroviral therapy (HAART), these therapies only suppress HIV replication, halting the progression of the disease and impeding acquired immunodeficiency syndrome (AIDS). Due to the viral latency of HIV, the effects of antiretroviral therapy, as well as the immune system’s natural response, can be avoided by the virus by remaining latent in a small number of immune cells. As a result, both a low-level viral genome expression and replication in tissues, as well as a reservoir of latently infected cells that serve as a reservoir for HIV-1 (known as the HIV reservoir) is evident. The goal of a therapeutic cure for HIV-1-positive patients would latent HIV reservoir as a result of this functional cure.

Over the past decade, the possibility of using the CRISPR/Cas9 system as a consistently successful intracellular defense mechanism against HIV-1 infection in human cells has seen great potential. One application of CRISPR/Cas9 in HIV treatment that has been investigated extensively involves disrupting the HIV co-receptors CCR5 and CXCR4, effectively restricting viral infection and making the HIV/co-receptor interaction a major developmental target for novel antiviral strategies aimed at treating/preventing HIV infection. CCR5 and CXCR4 are structurally related chemokine receptors of the seven-transmembrane G-protein coupled receptor family. The importance of these chemokine receptors for HIV entry has been known for quite some time. Over two decades ago, CXCR4 was first shown to mediate entry of T cell line-tropic HIV-1 strains\(^9\), whereas CCR5 was first shown to mediate entry of macrophage-tropic strains of HIV-1\(^10\). In their function as HIV co-receptors, CCR5 and CXCR4 physically associate with a specific component of the envelope glycoprotein of the virus, the CD4-activated gp120 subunit, which is responsible for binding to specific target cell receptors. Ultimately, HIV-1 entry is mediated through its surface envelope glycoprotein, gp120, by sequential binding to CD4 and then to a specific chemokine receptor, either CCR5 or CXCR4\(^11\). Among past studies, targeted disruption of CCR5 and CXCR4 in human cell lines, either individually\(^12\) or simultaneously\(^13\) have shown promise in potentially providing an effective and safe strategy towards a functional cure for HIV-1 infection.

Alternative to disrupting cell surface receptors, direct targeting of the HIV-1 proviral genome from infected cells, preventing viral replication and viral rebound after treatment discontinuation, is also an avenue that has been extensively experimented and analyzed for its feasibility in developing a therapeutic cure. Although various genome editing techniques that use engineered nucleases to introduce double-strand breaks at a targeted locus with subsequent repeat, such as zinc finger nucleases (ZFNs)\(^14\) and transcription activator-like effector nucleases (TALENs)\(^15\), have been harnessed in HIV treatment, the CRISPR/Cas9 system has shown the most promise in targeting different segments and conserved regions of the HIV proviral genome\(^16\,\,\,17\).

Strategies to Improve the Specificity and Application of the CRISPR System to HIV

The highly mutable and genetically diverse characteristics of the integrated HIV virus remain a major obstacle of utilizing CRISPR to eradicate the HIV reservoir. Although highly conserved regions within the HIV genome have been identified
as promising targets for gene editing\textsuperscript{21-23}, classical CRISPR-mediated approaches have variable specificity, which may induce off-target mutations\textsuperscript{24-26}. Also, due to the strict PAM sequence requirement of the popular Streptococcus pyogenes Cas9 (SpCas9) nuclease\textsuperscript{27}, there are limited regions available within the HIV genome to target with CRISPR\textsuperscript{28,29}. Despite the substantial potential of this technique, these drawbacks are important caveats of CRISPR that must be addressed in order to develop accurate and safe therapies for HIV infection.

One plausible approach to circumvent these limitations is to alter the specificity of the Cas9 nuclease. Through genetic modification, SpCas9 variants possessing the ability to recognize altered PAM sequences have been engineered\textsuperscript{31}. These alternative Cas9 nucleases, specific for novel PAM sequences, have been shown to effectively double the targeting potential of the CRISPR/Cas9 system within the human genome\textsuperscript{31} (Figure 3). Furthermore, modification of the CRISPR gRNA via sequence truncation has also provided advancements to this system. Truncated gRNAs (tru-gRNAs), which are shorter in length than their 20 nucleotide gRNA counterparts, have been shown to direct nuclease cleavage at the intended site with high efficiency. As well, they reduce mutagenic effects at closely matched off-target sites without decreasing the targeting range\textsuperscript{32,33}. Although contradictory effects of tru-gRNAs have been observed\textsuperscript{34}, this approach may circumvent the major concern of off-target effects due to CRISPR misbinding. This is especially important when considering the therapeutic/clinical benefits of treating HIV-infected individuals with CRISPR. Shortcomings of the current HIV research lack answers to questions regarding whether gRNAs of shorter or longer complementarity length possess strong site-specific activity, and if certain gRNA characteristics specific to the HIV genome, such as nucleotide content, may improve CRISPR specificity. Although further research is required, evidence suggests that improvement of the CRISPR target range and reduction in off-target effects is plausible. This advocates for a prospective sterilizing cure for HIV infected individuals using CRISPR/Cas9.

The introduction of new nucleases may also increase target range and efficacy of CRISPR-directed HIV treatment. Cpf1, a smaller and more simplistic nuclease, requires a T-rich PAM sequence to induce cleavage, contrary to the G-rich PAM sequence required by Cas9\textsuperscript{35}. Given the AT-rich proviral DNA of the HIV virus\textsuperscript{16}, this nuclease may be able to target novel regions of the HIV genome that have not yet been targeted by conventional CRISPR/Cas9 techniques, setting the stage for further research. Similar to recently implemented combinational gRNA CRISPR/Cas9 approaches that target viral genomes\textsuperscript{6,9}, the CRISPR/Cpf1 technique has also shown success in simultaneously targeting multiple sites within a genome\textsuperscript{37}. This approach may increase the likelihood of altering genes that are essential for HIV replication and infection, possibly providing a sterilizing cure.

A novel and alternative application of CRISPR involves sequence-specific gene silencing, known as CRISPR interference (CRISPRi). This technique is based off the standard CRISPR system but employs a Cas9 that lacks nuclease activity in order to target DNA without subsequent cleavage\textsuperscript{38} (Figure 4). The inactive Cas9 is co-expressed with a gRNA that is engineered to recognize a target sequence within a gene promoter or enhancer region. Various renditions of this system permit long-term gene silencing via post-translational modification of histones to produce tightly coiled heterochromatin, or short-term silencing by competing with transcriptional machinery for binding at the gene promoter\textsuperscript{39}. Even stronger suppression (up to 99% in eukaryotes) can be achieved by using several, non-overlapped gRNAs per gene\textsuperscript{40}.

CRISPRi has been used widely in bioengineering\textsuperscript{41}, stem cell studies\textsuperscript{42}, and viral disease research\textsuperscript{43,44}. However, of significant importance to the question at hand is the lack of research that focuses on investigating the feasibility of treating HIV with CRISPRi. This technique has the potential to circumvent current issues surrounding the treatment of HIV with CRISPR/Cas9, specifically viral escape. After the DNA is cleaved, non-homologous end joining (NHEJ) often re-ligates double stranded breaks. This is an error-prone process that can produce insertion or deletion mutations (indels) at the Cas9 cut site. Viral escape occurs when indels prevent further recognition of the target sequence by CRISPR but continue to permit viral gene expression. As CRISPRi does not involve DNA cleavage, NHEJ would not occur and indel mutations would not arise. Therefore, continuous recognition of the HIV target sequence by CRISPRi may be achieved, resulting in long term viral gene suppression. Recent studies focusing on the use of CRISPRi in the treatment of Epstein-Barr virus and Zika virus have reported great success\textsuperscript{7,43}. The favorable results of these studies should motivate researchers to investigate the feasibility and benefits of using CRISPRi in the treatment of HIV.

**Emerging Ethical Perspectives in the Treatment of HIV with CRISPR**

As is now evident, several functional limitations exist that prevent the treatment of HIV with CRISPR. However, given recent advancements in this field, CRISPR technology may offer a sterilizing cure in the future. Regardless of the functional viability of this treatment, the ethical limitations must still be considered.

The ethical, social and legal issues surrounding the use of CRISPR\textsuperscript{44} raises questions concerning the risk to benefit ratio of integrating this novel gene editing tool into HIV treatment. Heritable changes that may arise from germ-line mutations are of specific ethical concern\textsuperscript{45}. Should there be complications in the functional efficiency of this technology that cause alternations in germ cell DNA, the downstream complications in the offspring could be severe. Regardless of these concerns, there have been large advances in mouse trials, which have reached ethical-standards in germ-line gene editing\textsuperscript{46}. Germ-line modification via CRISPR could prove effective to prophylactically prevent HIV infection by altering CCR5, which serves as an HIV co-receptor, facilitating viral entry to immune cells. Again, the downstream effects of altering the human genome at the germinal level must be considered, as any off-target mutation would also be inherited by offspring. Before this technology can be implemented in the treatment of HIV, it must repeatedly and exclusively produce on-target mutations\textsuperscript{47}. Stakeholders, such as ethics boards must also be considered, and the large amount of these that must give approval before human clinical trials commence is nothing to scoff at\textsuperscript{48}. 
Perhaps one of the most recurring themes in this challenge is whether the potential risks, including brain complications\textsuperscript{44} and heritability of modifications\textsuperscript{45,49}, are surmountable and outweighed by the potential benefits. Further, environmental risks of ethical notability, including the potential to eradicate certain insect disease vectors with the same technology needed to treat HIV in humans, remain in question\textsuperscript{50}. This would entail eliminating one sex of the disease vector via CRISPR (e.g. female mosquitoes) in an effort to dually eradicate the entire species and the virus they carry. This ecosystem centered concern serves as a reminder that the range of stakeholders affected by treating HIV with CRISPR span further than governments and individuals. Moving forward, careful consideration that the majority of those suffering with HIV are in poverty-stricken regions is also of the utmost importance\textsuperscript{51,52}. According to the WHO, over 60\% of the world’s HIV cases are in sub-Saharan Africa, where over half of the population lives with less than $1 per day\textsuperscript{53}. Despite CRISPR’s relatively low cost, ethical concerns will arise if the technology is successful in treating HIV but is out of financial reach for those most in need.

The main ethical themes surrounding gene editing in HIV treatment, the roadblocks are not unsurmountable. This is due to the obvious drive for innovation, as demonstrated by the high number of research trials currently running, and the increased willingness to address the ethical concerns. However, with stakeholders such as governments, individuals and the environment potentially at risk, there is still no concrete answer as to whether the CRISPR system is ethically viable or not.

**Conclusion**

Overall, CRISPR/Cas9 is a relatively new gene editing technology that may possess the potential to cure HIV in the near future. However, several functional and ethical limitations are currently preventing CRISPR from achieving this goal. The highly mutable characteristics of the HIV virus leading to difficult CRISPR targeting, high occurrence of viral escape, and abundance of ethical concerns, call for advancements in this field. In this article, we considered how CRISPR is currently being used in alternative contexts and the feasibility and benefit of translating these approaches to HIV treatment. Furthermore, we assessed the ethical issues that must be addressed before this technology can be used in humans.

**References**

See Appendix B

Katelyn Gray, Bernice Ho, & Olivia Villeneuve

Abstract
Injury to the spinal cord initiates a complex molecular and cellular pathological process that results in devastating physical, social, and financial consequences. The pathogenesis of spinal cord injury (SCI) is characterized by an initial primary phase that is followed by a secondary phase in which the initial damage to neural tissue is propagated. Thus, secondary injury is responsible for the worsening of neurological deficits observed in the period following the injury. In order to improve clinical outcomes of spinal cord injury patients, there is a need for a highly effective neuroprotective therapeutic option that prevents the progression of secondary injury. Optimization of this process can be achieved by fostering a concentrated research effort that focuses on mechanisms of secondary SCI that exhibit the most potential as therapeutic targets. Acrolein, a toxic molecule that arises in cellular conditions of oxidative stress, provides a unique intersection between important mechanisms of secondary SCI. While current research in this field is taking promising steps towards the development of a neuroprotective agent, the ideal strategy is finding an inclusive approach to target multiple secondary SCI mechanisms.

Introduction
Spinal cord injury (SCI) is an insult or damage to the spinal cord resulting in temporary or permanent neurological deficits. There are two stages of SCI that have been defined for clinical and academic purposes. Primary injury is the result of initial damage to neural tissue that leads to localized cell death, while secondary injury is the consequence of a hostile injury environment and leads to further destruction of neural tissue surrounding the necrotic core (Oyinbo, 2011). Currently, there is a large body of scientific research devoted to elucidating mechanisms of secondary injury. The ultimate objective of this research is to gain the knowledge needed to develop therapeutics that block the progression of neurodegeneration and diminish SCI-associated morbidities. To optimize these efforts, it is important to determine which mechanisms of secondary SCI demonstrate the greatest potential as therapeutic targets. Ideally, a concentrated research effort will lead to a highly effective neuroprotective therapeutic option in a short time frame. In addition to presenting the rationale behind selecting an optimal therapeutic target, this report will provide a glimpse into current research and outline the gaps that exist within the literature regarding this topic.

Understanding the Ideal SCI Phase and Associated Mechanisms to Target
There are three phases of SCI; acute, intermediate and chronic (Fig. 1) (Neirinckx et al., 2014). The acute phase is synonymous to primary injury and occurs immediately, involves necrosis of the damaged neurons, mass release of excitatory neurotransmitters and disruption of ion balance (Neirinckx et al., 2014). This phase of injury is characterized by immediate structural and biochemical changes that are clinically inaccessible for intervention, making it an unfit target for neuroprotective therapeutics (Guly et al., 2007). The chronic and final phase of secondary SCI is characterized by demyelination, cystic cavity formation and glial scarification occurring months after the injury.

Figure 1. The Phases of SCI. A visual schematic of associated events during the acute, intermediate, and chronic phases of SCI. Specifically, inflammation, oxidative stress, and apoptosis are part of the intermediate phase. (Adapted from Neirinckx et al., 2014).

As mentioned above, secondary SCI is characterized as the further progressive destruction of tissue expanding axial and radial to the initial injury site (Oyinbo, 2011). This degeneration
contributes to SCI-associated morbidities and negative patient outcomes (Hausmann, 2003). However, using a contusion rat model with various degrees of SCI, Basso et al (1996) noted that tissue sparing at the lesion center was highly correlated to locomotor functional outcome. The preservation of as little as 5-10% of the nerve fibers is enough to allow for recovery of basic locomotion (Basso et al., 1996). Therefore, targeting the origin of continued pathogenesis by restricting mechanisms of secondary SCI may promote preservation of the healthy tissue in patients. Thus, it is imperative to determine the optimal therapeutic target within secondary injury as, intuitively, the inhibition of further neurodegeneration leads to improved may improve patient outcomes for humans.

Now that it is established that targeting secondary damage is beneficial to promote clinical outcomes, it is important to determine which secondary mechanisms are the most sensible to target therapeutically. Currently, there are 25 well-supported mechanisms of secondary SCI (Ramer et al., 2005). It would be time-consuming and ineffective for researchers to focus on each of these mechanisms. This issue is addressed by the identification of principal mechanisms that show most promise as therapeutic targets. This not only saves time and resources that would have been devoted to studying all 25 mechanisms but promotes comparison of key mechanisms, which may provide insight into which interventions are suitable for a clinical setting and are most likely to be efficacious when administered within a reasonable range of time following the incidence of injury. Most importantly, if a connection is found between processes of secondary injury, it could be the ideal target to attenuate several secondary mechanisms. The literature supports oxidative stress, inflammasomes-mediated inflammation, and apoptosis as key mechanisms of intermediate phase secondary injury (Fleming et al., 2006).

### Oxidative stress

An increase in the mitochondrial production of reactive oxygen species (mROS) occurs post-SCI (Azbill et al., 1997). This creates a condition of oxidative stress in which the levels of mROS cannot be neutralized sufficiently by the antioxidant defense system of the cell. Subsequently, lipid peroxidation, nucleic acid oxidation and DNA fragmentation in neurons proximal to the injury site ensue (Xu et al., 2005). Lipid peroxidation gives rise to a toxic byproduct, acrolein, which has a multitude of negative effects on the cell (Jia et al., 2012). Additionally, it has been demonstrated that acrolein stimulates production of ROS, thus amplifying the condition of oxidative stress within the cell (Luo and Shi, 2005). Acrolein has also been shown to mediate the release of pro-inflammatory cytokines from macrophages, which may contribute to the hyper-inflammatory state of the injury environment (Facchinetti et al., 2007; Chen et al., 2008). Hence, oxidative stress has a significant, multi-factorial role in continued SCI progression.

### Inflammation

Similarly, another key mechanism of secondary SCI is inflammation. Current research supports NLRP3 inflammasomes, a multi-protein complex, as a key mediator of post-injury neuroinflammation, which can be triggered by mROS production (Yaron et al., 2015). Upon NLRP3 inflammasomes formation, it acts as a molecular platform for caspase-1 activation, and subsequent processing of IL-1β, a pro-inflammatory cytokine (Agnostini et al., 2004). NLRP3 inflammasomes contribute to the progression of secondary SCI as it causes further neuroinflammation, mitochondrial dysfunction and initiation of apoptosis (Wu et al., 2017, Sagulenko et al., 2013). In addition, increased levels of IL-1β contribute to lesion expansion, increased proliferation of astrocytes, and reduced plasticity of the central nervous system (Boato et al., 2013). Thus, inflammation is an important mechanism of secondary SCI as it can consequently lead to massive cell death and prevention of functional recovery.

### Apoptosis

The many mechanisms of secondary injury lead to the initiation of apoptotic cascades resulting in neuronal and glial cell death (Nesic et al., 2001; Xu et al., 2005). An abundance of research involving human spinal cords support the key role of apoptosis in the progression of secondary SCI (Emery et al., 1998; Banik et al., 1997). In a study conducted by Emery et al., it was found that apoptosis was predominant in 14 out of the 15 spinal cords examined (Emery et al., 1998). While apoptosis can occur at any time during the intermediate phase, its greatest impact is that it facilitates the transition to the chronic, permanent injury manifestations of demyelination, cystic cavity formation and glial scarification (Neirinckx et al., 2014). Once this permanent damage has occurred, therapeutic interventions that attempt to preserve normal tissue are futile. Therefore, apoptosis is an important secondary SCI mechanism because it leads to these permanent events, in addition to the motor and sensory deficits seen in SCI-patients due to neuronal death. Preventing apoptosis in cells could potentially aid in stopping demyelination and glial scarification, thereby halting the progression to the secondary SCI.

### An Intersection of Important Secondary SCI Mechanisms

Acrolein, the previously mentioned byproduct of lipid peroxidation, provides a unique intersection between these important mechanisms of secondary SCI (Fig. 2). Acrolein exacerbates oxidative stress by amplifying mROS production (Luo and Shi, 2005). As mROS triggers NLRP3 inflammasomes formation, acrolein indirectly stimulates immune-mediated mechanisms (Yaron et al., 2015). This has been further demonstrated by evidence that the removal of acrolein from the injury environment diminishes the acrolein-mediated stimulation of the immune system (Chen et al., 2008). Furthermore, acrolein can initiate apoptotic cascades through two pathways. Firstly, acrolein can increase the release of inflammatory cytokines from macrophages (Facchinetti et al., 2007). These molecules are capable of activating caspases, which are proteases involved in DNA fragmentation and cell death (Nesic et al., 2001). Secondly, neuronal death is also achieved through mediating the translocation of BCL-2 associated death promoter (BAD) to the mitochondrial membrane (Tanel and Averill-Bates, 2007). This leads to the eventual activation of caspases by cytochrome c through a series of protein interactions (Fig. 2) (Tanel and Averill- Bates, 2007). Due to these connections between the key mechanisms of secondary injury, it is possible that by targeting oxidative stress, other mechanisms that contribute significantly to secondary injury will be attenuated.
Pre-clinical Advancements in Neuroprotective Therapeutics

It is important to examine how the mechanistic information presented in the literature translates to clinical applications. As discussed, oxidative stress, inflammation, and apoptosis are interconnected through the production and effects of acrolein. There are drugs in pre-clinical stages of research, such as N-acetylcysteine (NAC), methylene blue and minocycline, that target molecules within this pathway. These drugs are proven to be clinically safe as they currently treat other conditions, such as pulmonary diseases and infections (Dodd et al., 2008; Garrido-Mesa and Gálvez, 2013). Each drug has also shown promise of improving SCI clinical outcomes through the attenuation of secondary injury mechanisms either in vitro or with the use of animal models (Teng et al., 2004; Tanel and Averill-Bates, 2007; Lin et al., 2017).

NAC is a precursor to the antioxidant glutathione, a compound under investigation as a neuroprotective agent following injury to the nervous system (Chen et al., 2008). By increasing levels of glutathione, NAC diminishes the levels of mROS and promotes the detoxification of acrolein (Chen et al., 2008). Similarly, as a therapeutic, methylene blue targets the inflammatory response of SCI through reducing the formation of ROS (Figure 4) (Lin et al., 2017). Reducing the levels of ROS prevents both acrolein and NLRP3 inflammasomes formation, which inhibits the production and release of mature IL-1 and IL-8 spinal cord microglia (Lin et al., 2017; Facchinetti et al., 2007). Consequently, continued spinal cord damage from inflammation is prevented (Lin et al., 2017). On the other hand, minocycline, targets the apoptosis pathway. Minocycline prevents the release of cytochrome c from the mitochondria (Teng et al., 2004), which normally leads to apoptosis through the downstream activation of the caspase-9 cascade (Teng et al., 2004). By inhibiting the release of cytochrome c, caspase-9 will not be activated and this apoptotic cascade will not proceed.

From these three proposed treatments, methylene blue shows the most promise. Methylene blue acts at an early mechanistic step to decrease oxidative stress, resulting in reduction of immune-mediated events and prevention of apoptosis (Lin et al., 2017). While this treatment has significant potential, barriers to its success should be noted. Preventing ROS production is challenging as there is a very narrow therapeutic window. Studies have shown that impaired mitochondrial function and increases in ROS levels are observed post-SCI at the 1- and 4-hour time points, respectively (Azbill et al., 1997). Since these events occur very quickly following the initial impact on the spinal cord, prevention is difficult. Further research is necessary to determine if success of early research will translate to clinical efficacy.

Gaps in the Literature

As discussed, there is logical justification and scientific evidence that supports the importance of determining which mechanisms of secondary SCI are most suitable for clinical intervention. However, it is important to note that there are many gaps in the literature that provide a barrier to the development of an effective neuroprotective treatment option, besides the need to overcome a short therapeutic window. For instance, many studies often use contusion/ transaction models to study SCI (Hausmann, 2003), which discounts infection-originating SCI. Further studies should investigate if infectious SCI follows the same pathogenesis in terms of secondary injury mechanisms. Additionally, none of the aforementioned investigative compounds have advanced to preclinical trials, so it is uncertain if the promising results seen in the animal models and early research will translate to success in human trials. A final concern is that there are many secondary injury events that contribute to the progression of SCI. It is possible that targeting a few select mechanisms may not be sufficient for prevention of secondary injury. Future research should focus on an inclusive approach to treatments of SCI that is able to target multiple mechanisms of injury. While these issues are important to note, the research on targets to prevent secondary SCI should not be overlooked. Proper knowledge translation of the current advancements seen in animal models to future preclinical trials is necessary for the development of improved treatment options for SCI patients. Thus, inclusive approaches to neuroprotection that target multiple mechanisms, such as the actions of methylene blue, should be investigated further. This would optimally prevent the permanent secondary damage that often leads to greater burdens on patients.

Conclusion

An estimated 85 thousand people live with SCI in Canada, with new-injury rate of 50 per million people annually (Noonan et al., 2012). The astronomical prevalence and incidence has pushed scientific efforts to support the physical health of patients with SCI, in hopes to reduce the associated social, emotional, and financial burdens. For instance, treatment expenses, lifetime medical costs and lost earnings due to SCI can range upward to 10 million US dollars (Fehlings and Nguyen, 2010). Since primary injury often occurs unexpectedly, apart from achieving stabilization, the window for definitive specialized care of the primary injury is inaccessible. Therefore, treatment strategies are focused on combating the cascade of mechanisms contributing to secondary SCI, which causes the most pain and further neural degeneration. Of the 25 well-established mechanisms leading to secondary injury, oxidative stress, inflammation, and apoptosis are the three that demonstrate as the most promise targets for therapeutics (Oyinbo, 2011). As discussed, these are linked through the production of acrolein. Within this pathway, the clinically safe drug, methylene blue, has been shown to improve motor function by inhibiting all three mechanisms in rat models (Lin et al., 2017). Targeting these pathways will restrict further damage from occurring, resulting in improved patient outcomes. While current research in this field is taking promising steps towards the development of a neuroprotective agent, the optimal strategy is finding an inclusive approach to target multiple secondary SCI mechanisms.

References
See Appendix C
Sudden Cardiac Death: Genetic Markers & Risk Stratification

Matthew Preteroti

Year in, year out, there holds true one disease pathology underlying the majority of natural deaths worldwide: sudden cardiac death (SCD). SCD comprises close to 4,000,000 deaths worldwide every year and over the past quarter century this number has risen in a near inconceivable manner [2]. Cases of otherwise healthy individuals dying suddenly due to an underlying genetic predisposition to a pathology in which there a few symptoms other than death. But what if there was a method to risk-stratify patients through multichannel ECG-recordings? Samol et al. report in their study that the occurrence of a clinical event in patients who suffer from Long QT Syndrome (LQTS), a pathological cardiac predisposition to SCD, is associated with a prolonged, low-amplitude T-wave [8].

SCD manifests following sudden cardiac arrest, in which electrical instability of the heart leads to an arrhythmia or an irregular heartbeat, which is incapable of meeting the metabolic demands of the body [11]. SCD has many underlying etiologies however, there is one class which spans a gross majority: channelopathies. Channelopathies are a class of diseases characterized by dysfunction of ion channel subunits which regulate cardiac conduction. LQTS is the primary disease pathology of this class. Recent advances in this field have identified both risk stratification techniques and genetic markers that can be used in conjunction to allow identification of individuals prone to SCD as a result of inherited channelopathies. From this, many guideline-endorsed recommendations for primary and secondary prevention of SCD may be implemented at the level of both clinical practice and population health to reduce the prevalence and incidence of SCD [5].

During an investigation of whether multichannel ECG-recordings are indeed useful in risk-stratifying LQTS patients, Samol et al. uncovered that the occurrence of a clinical event, such as syncope, torsade de points (TdD), sudden cardiac arrest (SCA), or sudden cardiac death (SCD), was associated with a longer QTc-interval. The QTc-interval represents the time for both ventricular depolarization and repolarization to occur during cardiac conduction. The authors made use of 34 non-acquired LQTS-patients who were subjected to 12-channel ECG, treadmill test, and transthoracic echocardiography. Additionally, genetic analyses were performed in 29 patients. They observed that while undergoing the treadmill test that 12 patients underwent a cardiac event (CE) and the remaining 22 did not. From ECG recordings, they denoted that patients who underwent a CE had a significantly greater QTc-interval (519±43 ms) when compared to those that did not undergo a CE (458±42 ms) [8]. This makes sense as this is one of the most important risk factors for patients with LQTS. Concordant studies have established 500 ms as the threshold for risk of a cardiac event, in that QTc prolongation greater than 500 ms poses a serious risk in comparison to durations less than 500 ms [6].

Within the work of Samol and colleagues they identify that of the twelve patients that underwent a cardiac event, nine were female and three were male. This is interesting as it is well established that sex plays a key role in channelopathies, in that males typically have worse prognoses and are at greater risk of SCD when compared to females [4]. In an attempt to explain the variability within individuals of a particular channelopathy, one single genetic variant is most often insufficient, leading the consideration of both non-genetic and genetic modifiers to be accounted for. The most prominent non-genetic and genetic modifiers analyzed are demographic variables such as sex, age, or exogenous factors and coding or non-coding variants, respectively. Typically, early manifestations of symptoms may indicate an aggressive form of the disease, emphasizing the importance of hormonal changes such as testosterone however, there is evidence that sodium channels become leaky with age leading to a more serious phenotype [9]. Interestingly Samol et al. uncovered in their demographic data that on average subjects who underwent a cardiac event were five or more years younger than those who did not experience a cardiac event. Additionally, no cardiac events were recorded in any control group members who on average had an age of fifty-four years old. There are several exogenous factors that together with disease-causing variants can modulate the parameters of an ECG and thus alter genotype-phenotype correlation. For example, it has been shown that in Brugada Syndrome, a similar channelopathy, that a characteristic ECG pattern can be masked if the patient has a fever, excessive alcohol intake, or through eating large meals. Within LQTS it’s been shown that drugs or either electrolyte imbalance can prolong or unmask the QTc-interval [5]. Therefore, characteristic demographics are important factors that are becoming increasingly incorporated in risk stratification models of Long QT and associated channelopathies.

Four primary classes of channelopathies include Long QT Syndrome (LQTS), Brugada Syndrome (BS), Catecholaminergic polymorphic ventricular tachycardia (CPVT), and Short QT (SQT). In these genetic diseases, the prognosis is influenced by the type of causative mutation [1]. This is especially evident in LQTS where there is significant variability in patient outcome with regards to the three most common variants of the disease; LQT1, LQT2, and LQT3. LQT1 is caused by mutations in the KCNQ1 gene which encodes a potassium channel, LQT2 by mutations in the KCNH2 gene which encodes a separate potassium channel, and LQT3 by mutations in the SCN5A gene which encodes a cardiac sodium channel. Greater than 90% of SCD cases are due to mutation or polymorphism in one of these three genes [10]. Patients diagnosed with LQT1 have the most favorable outcome as they respond well to beta-blockers and do not require an Implantable Cardioverter Defibrillator (ICD). Whereas patients diagnosed with either LQT2 or LQT3 have more severe prognoses as they typically respond poorly to treatment with beta blockers and require an ICD to detect and
diminish an arrhythmia should one arise [6]. During the genotypic analysis of 29 patients Samol and colleagues were able to determine the underlying genetic mutation and specific protein responsible for the clinical presentation of Long QT. Of the 29, 15 had mutations within the KCNH2 gene, which manifests as LQT2, 9 had mutations in KCNQ1, which manifests as LQT1, and the remaining 5 had outlier mutations in less prevalent genes [8]. What is interesting is that prior studies have been able to determine triggers which pose a significant risk of causing a cardiac event and correlate them with known QT mutations. For instance, risk is significantly increased in LQT1 during physical exertion such as swimming, in LQT2 loud auditory triggers or postpartum periods, and in LQT3 during sleeping periods [9]. Interestingly during the treadmill test portion (physical exertion) of the study by Samol et al. the number of cardiac events triggered in patients was roughly greater amongst patients’ positive for LQT2 with those positive for LQT1. This in turn correlates highly with the increased QTc-interval as expressed by ECG analysis as a result of triggers that predispose to CE occurrence. Moreover, prior studies have been able to classify patients based on locus mutation position and found that patients with LQT1 locus mutations were at much lower risk (30%) of having a cardiac event prior to the age of forty when compared to patients with locus mutations at LQT2 (46%) and LQT3 (50%) [7]. Coinciding with the findings of Samol et al. that patients with LQT2 are subject to greater risk of cardiac event.

Within the genomic analysis of patients, Samol and colleagues went a step further and identified the specific nucleic acids and proteins underlying the clinical manifestation of Long QT. Of the 15 patients that were positive for LQT2 there were only recurrent mutations identified in 6 individuals meaning that the remaining 9 patients, whilst maintaining the clinical presentation of LQT2, had completely variable underlying mutations. For the 9 patients’ positive for LQT1, a more extreme trend was observed, in that there were no recurrent mutations identified and all mutated proteins were variable. This identification by Samol and colleagues raises a good point in that, when evaluating and classifying channelopathies such as LQTS it is important to bear in mind the concept of locus heterogeneity, meaning that not everyone with the symptoms of LQTS have a mutation in the same gene. Following the linkage analysis that discovered the first LQTS gene several other loci have been identified. Currently there are a total of thirteen known loci with variable effects on specific ion channels however, all are inherited in an autosomal dominant fashion and present as LQTS [10]. The difficulty in clinical classification arises as a result of the mutation spectrum in which one clinical assay is insufficient to give an overall picture of the genes in question. Mutations may be short-nucleotide variants, insertion/deletions, large rearrangements, or may even span the entire gene.

As LQTS is inherited in an autosomal dominant fashion that lends the possibility of multiple pathogenic variants being identified within a patient. In a recent review, it was discovered that roughly 8% of individuals carry either variants in greater than one LQTS gene or greater than one variant within a LQTS gene [1]. This in turn makes assessing pathogenicity or classifying via risk stratification for any one variant or the sum of variants increasingly difficult. There was even one case report of an individual carrying three LQTS variants [1]. This is again highlighted by Samol and colleagues whereby they identify one subject who was positive for both LQT1 and LQT2 as a result of two protein variants. This patient did additionally undergo a cardiac event.

The identification of pathogenic variants and triggers that can predispose a person to SCD are not the only issues in the future of risk stratification of Long QT patients. Currently there is very little research into the penetrance, expressivity, and phenotypic overlap associated with Long QT. Penetrance can be defined as the proportion of people who carry a certain genotype which expresses a characteristic phenotype or clinical manifestation whereas variable expressivity denotes that if you carry the genotype it may not be expressed in the same manner as others with the same genotype. Pleiotropy, or phenotypic overlap occurs in some cases when mutations in a single gene can possess variable effects resulting in different heritable cardiac channelopathies within the same multigenerational pedigree [3]. The accepted degree of penetrance and expressivity amongst channelopathies is highly variable primarily as a result of the number of cases analyzed among publications. It is thus important to assess the effects of variable penetrance and expressivity on the risk-stratification of Long QT patients.

This article discusses some of the current and relevant topics as they pertain to genetic markers and risk stratification of patients predisposed to SCD due to an inherited channelopathy called Long QT. Much potential for the identification and classification of specific pathogenic variants still exists as SCD remains a major cause of death, especially in young populations. The primary challenges ahead lie with identification of at-risk individuals as well as clinical measures in asymptomatic individuals that carry a mutation, as in many cases the first symptom is death. Additionally, the development of an index that can categorize genetic variants on a spectrum from benign to pathogenic to aid in the implementation of potential prophylactic screening or diagnostic measures. In an attempt to clarify the underpinnings of SCD-related diseases, future studies will require comprehensive genotype-phenotype correlations involving large cohorts of families. They should additionally be focused towards the adoption of personalized therapies in order to prevent SCD. The development of such therapies will require extensive interaction between families and specialists such as geneticists and cardiologists.

References
See Appendix D
The Public Health Implications of Administrative Policy Responses to E-Cigarette Use: A Content Analysis of Ontario’s University Campus Policies

Dylan Irvine & Aaron Bailey

Abstract

Purpose: To investigate Ontario university's institutional policy responses to the influx in e-cigarette use among adolescents and their associated regulation within campuses.

Methods: A review of relevant policy documents among Ontario’s 22 public universities was conducted. A manual search yielded relevant documents which were subjected to individual analysis by the authors. Interpretation and thematic grouping of their respective policies on e-cigarette use was conducted.

Results: 17 of 22 universities (n=17) have published policy documents relating to e-cigarette use on campus. The remaining 5 universities did not have a publicly available e-cigarette policy or do not mention e-cigarettes within their policy on smoking.

Discussion: 5 major thematic groups were identified, falling along a spectrum from blanket bans of e-cigarettes to the failure to reference e-cigarettes in relevant policy.

Conclusion: Administrative policies regarding e-cigarette use among Ontario universities are heterogenous and vary dramatically in scope. Future research in the arena of e-cigarette regulation and harm prevention would be beneficial to health promotion on university campuses in Ontario.

Introduction

The recent rise in e-cigarette use across North America has transformed a previously obscure area of tobacco control policy into a controversial debate within the public health community (Jenssen & Walley, 2019). Advocates for the use of e-cigarettes argue their importance as a harm reduction method and while skeptics voice concerns over tobacco industry involvement and the unknown long term health effects of their use (Miller, 2014; Jenssen & Walley, 2019; Hsieh, 2016; Green, Bayer & Fairchild 2016). While significant increases in observed and reported use among young adults have been observed in the United States (U.S) and Canada, post-secondary institutions have been slow to respond to this trend (Glasser, Abudayyeh, Cantrell & Niaura, 2018). This review will examine the institutional policy responses of Ontario’s 22 public research universities in order to perform a comparative analysis of their public health implications. This work intends to inform future policy discussions as post-secondary administrators look to modernize their tobacco control policy agenda’s, promote the interests of public health and protect students, staff and faculty from undue burden associated with e-cigarette use.

Background

Drastic increases in youth e-cigarette use that have occurred in recent years have captured the attention of public health authorities, medical practitioners, regulators and concerned parents in the U.S and Canada. According to the most recent data from the Food and Drug Administration (FDA), e-cigarette use in the U.S has increased by 78% over the last year, from 11.7% of high school students in 2017 to 20.8% in 2018 (FDA, 2018). Prior to this, a spike in e-cigarette use among high school students had increased drastically between 2011, with 1.5% of students to 13.4% in 2014 (Barrington-Trimis et al., 2016). Use among this demographic decreased between 2014 and 2016, although increases have been observed following the introduction of new e-cigarette products such as JUUL (Juul Labs) and Vype (Imperial Tobacco). As of February, 2018, JUUL controlled an estimated 49.6% dollar share of the U.S e-cigarette market (Willett et al., 2019). A prospective cohort study carried out by the Children’s Health Study examining data on e-cigarette use among 11th and 12th grade students demonstrated that over 40% of e-cigarette users reported cigarette smoking initiation after a 16 month follow up (Barrington-Trimis et al., 2016). This was in comparison to non-e-cigarette users, of which only 10.5% demonstrated cigarette initiation after 16 month follow up (Barrington-Trimis et al., 2016). These figures suggest that e-cigarette users were 6.16 times more likely to initiate cigarette smoking compared to non-e-cigarette users (Barrington et al., 2016). While these products show promise as harm reduction tools for those attempting smoking cessation, concerns surrounding adolescents who would otherwise be unlikely to use nicotine-delivering products are valid (Barrington-Trimis, 2016).

Current data suggests that the short or long-term use of e-cigarette products increases the likelihood of adolescents transitioning to cigarettes (Barrington-Trimis, 2016). This resembles the popular “gateway hypothesis” describing the transition from legal substances, such as alcohol, to illicit drug use (Kandel & Kandel, 2015). While this rise in e-cigarette use is too recent to provide insight into the long term health concerns surrounding their use, health effects of cigarette smoke are well documented. For those individuals who voluntarily initiate nicotine exposure through e-cigarette use and eventually transition to cigarettes, marked increases in cancer of the lungs, mouth and throat are observed (Hackshaw, Law, & Wald, 1997). A 24% increase in lung cancer is witnessed among smokers in comparison to non-smokers (Hackshaw,
Adolescent e-cigarette users are on average at about two times greater risk of transitioning to cigarette use (Barrington-Trimiš, 2016). Sex differences were also observed. Males were about 40% more likely to indicate susceptibility to cigarette use than females (Barrington-Trimiš, 2016). Rates of e-cigarette use manifesting into cases of cigarette use were also higher among those with reduced family income (Barrington-Trimiš, 2016).

Social environments, such as in that of a university campus, have also been associated with greater likelihood of cigarette use, physical dependence and addiction regardless of the individuals past e-cigarette use (Barrington-Trimiš, 2016). Further, the odds of indicating susceptibility to cigarette use for adolescents with friends using e-cigarettes was 2.45 times the odds for those with no friends using e-cigarettes after adjustment for personal e-cigarette use. A longitudinal cohort study carried out across 89 high schools across Ontario and Alberta, Canada, investigated the likelihood of never-smoking youth initiating cigarette smoking over a 2-year period (Aleyan, Cole, Qian, & Leatherdale, 2018). It was found that among the baseline sample of non-susceptible never-smokers, 45.2% of current e-cigarette users reported trying a cigarette after 2 years compared with 13.5% of non-e-cigarette users (Aleyan et al., 2018). The “susceptibility” of respondents was measured through the use of 3 valid and reliable survey questions which measured intention to try a combustible cigarette in the next year (Aleyan et al., 2018). Further, among the baseline sample of susceptible never-smokers, 62.4% of current e-cigarette users reported trying a cigarette 2 years later compared with 36.1% of non-current e-cigarette users (Aleyan et al., 2018). These findings contribute to the notion that e-cigarettes are contributing to the development of a new population of cigarette smokers comprised largely of low-risk youth who would otherwise be unlikely to initiate smoking cigarettes. This has driven public health authorities and researchers worldwide to investigate appropriate policy responses to the e-cigarette phenomenon.

Pharmacology and Pharmacokinetics of E-Cigarette Products

Nicotine is the main alkaloid found in tobacco and is responsible for the addictive properties of cigarettes (Le Houezec, 2003). Nicotine has positive effects on mood and cognition, and instills strong positive reinforcement tendencies in users which further contribute to physical dependence and addiction (Le Houezec, 2003). The likelihood that a substance will be abused varies depending on whether an individual is a frequent smoker as well as due to other factors including genetics (Armitage et al., 1975). Approximately 80% of this nicotine is metabolized and broken down in the liver and the rest by the lungs (Benowitz & Jacob, 1985). Nicotine is then transformed into cotinine, which goes on to be excreted primarily in the urine although it is also found in the saliva and hair (Benowitz & Jacob, 1985).

Module e-cigarette devices were originally created as a harm reduction initiative to aid in smoking cessation. New variations of these devices function through the use of nicotine salts, which display very similar pharmacokinetic profiles to cigarettes in the body in terms of absorption, metabolism and excretion (O’Connell et al., 2019). E-cigarettes as a harm reduction tool aim to increase smoker’s satisfaction by improving blood nicotine delivery and other sensory properties while potentially decreasing the amount and frequency used until the behaviour change can be completed (O’Connell et al., 2019). To evaluate the pharmacokinetics of nicotine in e-cigarette products utilizing nicotine salts in comparison to traditional nicotine found in cigarettes, a randomized, open-label, cross-over clinical study was conducted in 15 adult smokers in the U.S. using five different e-cigarette products after baseline data for conventional cigarette use was collected (O’Connell et al., 2019). The rate of nicotine absorption into the bloodstream was comparable from all e-cigarettes tested and took effect just as quickly as that of regular cigarettes (O’Connell et al., 2019). The rise in nicotine blood levels caused by e-cigarettes was described as moderately satisfying at relieving the desire to smoke (O’Connell et al., 2019). The results demonstrated that while delivering less nicotine than a conventional cigarette, the use of nicotine salts in e-cigarettes enables cigarette-like pulmonary delivery of nicotine that aids in the reduction of the urge to smoke (O’Connell et al., 2019). However, these properties have attracted the attention of public health professionals who are concerned about youth initiation. As previously discussed, this endemic of readily accessible and cheap e-cigarette products is likely creating a new generation of previously low-risk youth smokers. The trend of e-cigarette use among this demographic has become somewhat of a pastime, cultural norm and hobby. These factors and public health concerns for adolescents has resulted in the need for Ontario Universities to adapt new administrative policies regarding the use of e-cigarettes on campus.

Relevant Policy

Before individual administrative policy responses to e-cigarette use on Ontario’s university campuses can be explored, it is necessary that we present the legal and regulatory context of their development. It can be assumed that all institutional policy outputs on the subject of e-cigarette use or the use of combustible tobacco products adhere to the regulations set forth by the provincial Smoke-Free Ontario Act (SFOA) and Ontario Human Rights Code, the Electronic Cigarettes Act, the federal Tobacco Act, the Non-smokers’ Health Act, and relevant bylaws that are unique to the respective municipalities of the universities that will be examined.

Methods

In order to assess the scope and diversity of the current responses to this public health issue undertaken by post-secondary institutions in Ontario, a scoping review of relevant policy documents was conducted. All institutions designated as public universities by the government of Ontario in 2019 were included in the review (n=22). University websites were consulted and internal search queries were used to access publicly available policy documents exclusively concerning e-
cigarettes when available (n=0), and on-campus smoking policy positions referencing e-cigarettes when they were unavailable (n=17). The selected documents were then subjected to individual analysis by the authors, who interpreted, thematically sorted and recorded the elements of their respective policies on e-cigarette use. Common elements of institutional policy that organically emerged throughout the investigation were the basis for these descriptive groupings.

**Results**

17 of 22 universities (n=17) have published policy documents relating to e-cigarette use on campus. The remaining 5 universities did not have a publicly available e-cigarette policy or do not mention e-cigarettes within their policy on smoking. Figure 1 illustrates the detailed results of this review and Figure 2 depicts the spectrum of thematic groupings that emerged from this analysis. The majority of Ontario universities included e-cigarettes and vaporizing equipment as a tobacco product within the operational definition of "smoking". Others explicitly define e-cigarette and vaporizing products as separate from combustible tobacco and place these products under the same restrictions as tobacco products. Most refer to e-cigarette use as a smoking behaviour and restrict it to Designated Smoking Areas (DSA). Other campus policies included e-cigarettes within their definition of "smoking", but did not establish DSA’s. Instead, e-cigarette use was either restricted by applicable municipal and provincial laws or by institutional regulations resembling and sometimes exceeding them. A minority of institutions (n=5) did not have publicly available policy positions related to e-cigarette use or smoking, or do not reference e-cigarettes within their respective smoking policies and specify limitations on their use. The following subsections provide summary descriptions of the publicly available policy positions on e-cigarette use for each of Ontario’s 22 public universities.

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<th>Blanket ban</th>
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<th>Regulation of e-cigarette use reflects local bylaws</th>
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Figure 1: Summary table of university policy responses to e-cigarette use on campus
Figure 2: Spectrum of university policy responses to e-cigarette use on campus

Algoma University
The regulation of e-cigarette use at Algoma University is governed by the Tobacco and Smoking Policy, approved by administration in 2015. E-cigarettes are included within the document’s definition of “smoking”. Their use is prohibited in outdoor areas that are not DSA’s, university vehicles, buildings workplaces and enclosed public spaces. The policy is also intended to reflect the city of Sault Ste. Marie’s Smoking in Public Places and City Buildings 2003-2007 and the SFOA. The former by-law prohibits smoking in public places throughout the city of Sault Ste Marie and does not include any reference to e-cigarettes or vaporizing devices.

Brock University
Brock University mentions e-cigarette and vaporizer products within their Smoking and Vaping Policy. E-cigarettes are included in the policy’s definition of “Smoking”, while “Vaping” is defined separately. The contents of the policy apply equally to both categories, including cannabis. The Smoking and Vaping Policy applies to the “smoking and vapor of tobacco, nicotine or related products at Brock University” and prohibits the use of said products on or within all university property with the exception of DSA’s. The University also reserves the right to create “smoke and vapour-free corridors” on campus.

Carleton University
The Tobacco Smoking and Cannabis Consumption on Campus document governs e-cigarette use at Carleton University. This document includes e-cigarettes and vaporizing products within it’s working definition of “smoking”. Carleton University appears to exercise the precautionary principle, claiming that “[u]ntil such time as scientific evidence proves otherwise, electronic cigarettes (e-cigarettes) will be included in the definition, and their use is not permitted.” E-cigarette use may only occur in DSA’s that satisfy the requirements of the SFOA.

Lakehead University
Smoking and e-cigarette use fall under the jurisdiction of the Smoking on Premises Policy at Lakehead University. This policy prohibits smoking in university owned or leased buildings and public outdoor space on university property with the exception of DSA’s located in university parking lots. The document states that e-cigarettes are to be included within scope of these restrictions and they are not defined as a combustible tobacco product.

Laurentian University
E-cigarette use is regulated by the Policy on Smoking at Laurentian University. In this document, e-cigarettes are included within the working definition of smoking. Smoking and the use of e-cigarettes is prohibited inside all enclosed university buildings and vehicles, all outdoor areas, within 30 feet of university buildings. E-cigarette use is permitted within DSA’s. This policy is also intended to align with provincial and municipal bylaws and does not apply to areas referenced by the Federated Universities on-campus smoking policies.

McMaster University
Under the Tobacco & Smoke Free University Policy, electronic smoking devices are explicitly defined and included under the institutions definition of “smoking”. McMaster University prohibits the use of tobacco and electronic smoking devices outdoors on university property, in university owned vehicles or vehicles on university property, and within any university owned building. McMaster University does not include DSA’s on university property within their policy and exempts nicotine replacement therapy products, which does not include e-cigarettes within the policy document in question, from the application of the smoke-free campus initiative.

Nipissing University
The Smoking Policy regulates smoking and e-cigarette use at Nipissing University. Nipissing University does not allow smoking or inhalation within and on all university buildings and property and limits smoking and inhalation to outdoor DSA’s. Nipissing University's policy does not define e-cigarettes as “smoking” but extends these restrictions to them. Specific DSA’s are defined in the policy document, and the availability of smoking cessation programs is mentioned. Adherence to the City of North Bay Smoking By-Law No. 2012-097 is mentioned.

OCAD University
The Smoke Free Policy document from OCAD University does not define e-cigarettes, mention their use, or include them under the working definition of “smoking”. Smoking itself is prohibited in enclosed university property and within 30 meters of all university entrances and exits. There is no mention of DSA’s.

Queen’s University
Policy relevant to E-cigarette use at Queen’s University is available through the Smoking on Campus webpage. E-cigarettes are defined as “smoking devices”, which cannot be used in university owned or leased buildings, university owned vehicles, within a
minimum of 9 feet from entrances and exits to buildings, on patios and within 20 meters of sports field. Smoking on the Queen's University campus is also regulated by the Smoking and Vaporizing bylaw, which prohibits smoking and vaporizing in enclosed workspaces and designated public spaces. DSA's are not defined under either policy.

Royal Military College of Canada (RMC)
No publicly available documents from the Royal Military College of Canada were found throughout the course of this review.

Ryerson University
A description of Ryerson University's position is available through the Community Regulations of the 2019-2019 Undergraduate Calendars. This page does not reference e-cigarettes or include them within a definition of smoking. Smoking is prohibited within all university buildings, patios, parking lots, as well as within 9 meters of building entrances. Compliance with The City of Toronto Municipal Code Chapter 709 - Smoking is also cited for compliance which includes the same stipulations. No DSA's are outlined within the document.

Trent University
E-cigarettes are defined in Trent University's Smoke-Free Policy. The use of e-cigarettes at Trent University's Peterborough campus is prohibited within enclosed workspaces and enclosed public spaces, in sporting areas, on patios, and within 9 meters from a building entrance or 20 meters from a sporting area or area where children are known to be present, like a playground. Nicotine replacement therapy is exempted from the restrictions of this policy and is not defined. DSA's are also outlined within the document.

University of Guelph
The University of Guelph will regulate e-cigarette use on campus under their Tobacco & Smoke-Free Policy as of the spring of 2019. This policy includes e-cigarettes, vape pens, e-cigars, e-pipes and e-hookah devices under its working definition of “smoking”. The university will expressly prohibit the use of any tobacco product on university property and no DSA's are outlined. Nicotine replacement therapy is exempted from the restrictions applied to other tobacco products and is not defined.

University of Hearst
E-cigarette use is not addressed within institutional policy by the University of Hearst. Policy related to smoking is found within the “Procédures et directives en matière de santé et sécurité dans le milieu de travail” document, and states that municipal by-laws related to smoking, as well as provincial law including the Workplace Smoking Limitation Act 1990 and the SFOA.

University of Ontario Institute of Technology (UOIT)
The Interim Smoke-Free Campus Policy currently governs e-cigarette use at Uoit, to be replaced by a permanent policy in January 2019. E-cigarettes and vaporizers are included under the definition of “smoking”, which is prohibited on all outdoor and indoor university property. DSA's are not outlined.

University of Ottawa
According to Policy #58: Tobacco Use at the University of Ottawa defines e-cigarettes as separate from “smoking”. They are not expressly included within the restrictions on smoking within the policy, which include enclosed outdoor and indoor areas, as well as within 9 meters of building entrances and within vehicles owned by the university. Smoking is permitted in areas not defined by the policy and those with clearly marked signs. Classification of this policy was difficult given its vagueness. However, the authors determined it to be written in the spirit of regulating e-cigarette use in a manner similar to local bylaws related to tobacco use.

University of Toronto
The Smoke-Free Policy at the University of Toronto includes e-cigarettes within its prohibition of smoking on or within any property owned or leased by the university. The document also grants each University of Toronto campus the authority to implement DSA's at their own discretion, so long that they are outdoors and created in accordance with relevant provincial and municipal law. The University of Toronto is in the process of transitioning to a smoke-free campus and blanket ban on tobacco and e-cigarette use.

University of Waterloo
Policy 58 - Smoking regulates e-cigarette use at the University of Waterloo. It provides a definition of “e-cigarettes” but does not expressly include them when listing where tobacco smoking is to be prohibited. Vaporizing is referenced in relation to cannabis use, which is prohibited on university property. Smoking itself is prohibited within 10 meters from any building, 20 meters from sports areas, 20 meters from playgrounds, on patios and within any enclosed area or vehicle.

University of Windsor
The Smoking and Tobacco Policy at the University of Windsor does not include a definition of e-cigarettes and does not expressly include them within its scope. Smoking itself is exclusively permitted in marked DSA's.

Western University
Western University's Policy L16 - Policy on Smoking restricts conventional smoking and e-cigarette use to DSA's. The application of this policy to e-cigarettes as separate from smoking is explicitly included. E-cigarettes may not be used elsewhere on campus in accordance with provincial and municipal law.

Wilfrid Laurier University
In accordance with Policy 7.8 - Smoking, e-cigarettes are defined separately from smoking and included within the documents definition of smoking behaviour. Smoking itself is prohibited within all university buildings, vehicles, sporting areas, and patios and is permitted 10 meters from any building or doorway on university property. DSA's are not defined.

York University
York University's Smoking Policy is clearly applied to e-cigarettes. York University specifies that institutional smoking and e-cigarette use restrictions are those included within the SFOA, The Cannabis Act, and Toronto Smoking Bylaw 709. DSA's are not created or referenced within the policy statement.

Discussion
The results of this content analysis of publicly available university policy documents related to smoking and e-cigarette use in Ontario indicate that 5 thematic groupings have emerged. At one
extreme, several universities in the province have campus policies that do not mention e-cigarette use within their institutional policy documents (Hearst, Ryerson, Windsor, OCAD). On the other hand, a minority of universities have completely banned e-cigarette use alongside tobacco on university property without the creation of DSA’s (McMaster, Guelph, UOIT). Several campus responses fall in between these two categories, including the inclusion of e-cigarette use as a smoking behaviour and delegating regulation of their use to applicable provincial and municipal law without the creation of DSA’s or permitting their use within DSA’s. There is also a single case of a publicly available smoking policy being unavailable (RMC). These results indicate that there is clearly heterogeneity within university’s institutional policy responses to e-cigarette use in Ontario, ranging from their exclusion from policy to complete bans to accommodation.

While producing tangible recommendations for public health policy reform on university campuses is neither within the scope of this paper or the expertise of its authors, the academic and grey literature examining this topic in Ontario is limited. A 2018 environmental scan conducted by Leave The Pack Behind surveyed the content on Ontario college and university tobacco policies, focusing largely on the presence or absence of pharmacotherapy for smoking cessation, adherence to SFOA Requirements and brief details of the policy requirements (Leave The Pack Behind, 2018). In the U.S, Bayly et al. (2018) have included e-cigarettes in their examination of tobacco-free policies at 605 post-secondary institutions. Our review and analysis of Ontario university policy documents was conducted in order to use similar methodology to specifically evaluate university policies related to e-cigarette use in Ontario, which has not been done previously. While the description of institutional policy provided by Leave The Pack Behind is brief, this document proved to be a useful comparative tool for validating our thematic grouping of institutional policies.

As was stated previously, a comparative analysis of the impacts of differing policy responses to e-cigarette use on university campuses is outside the scope of this paper. It is, however, possible to review the existing literature on campus tobacco policy and e-cigarette regulation in order to investigate the potential implications our findings. While the body of research on the subject of e-cigarettes is largely concerned with prevalence and incidence, Brown, Hennes & Olson (2016) found that when asked, 42.3% of students surveyed at North Dakota Colleges & Universities did not know if e-cigarette use was permitted on their campus while 25.9% were aware of their campuses policy and its content. Whether or not the respondent’s campus prohibited e-cigarettes was a significant predictor of their reported awareness of their institutions position (Brown et al., 2016). 52.6% of students on campuses with e-cigarette prohibitions and 45.6% of those on campuses without such prohibitions reported awareness of their institutions policy (Brown et al., 2016). Campus policies on e-cigarettes were also not predictive of past or current use of e-cigarette products, and observed instances of e-cigarette use were not significantly different across campuses with and without policies specific to e-cigarettes (Brown et al., 2016). This study appears to be the most direct and comprehensive evaluation of campus e-cigarette policy that is currently available. Elsewhere, Llanes, Cabrables, Hernandez and Cooper (2019) have found that self-reported 30-day past prevalence of e-cigarette use increased markedly from 4.4% to 26.6% before dropping to 17.7% after the implementation of a tobacco free campus policy at a small university in the southern United States. Clearly, the rise in the popularity of e-cigarette use in student populations is inextricably connected to tobacco control policy and public health. Further research in the area of program design and evaluation is required to determine the effectiveness of the various approaches related to e-cigarette use seen within this policy sphere.

Limitations
The limitations inherent to our analysis and methods should be acknowledged. Primarily, it should be noted that the thematic coding of publicly available university policy documents was conducted manually through physical review of the documents and without the use of qualitative data software. While the criteria through which the documents were classified are correct and discrete, further thematic similarities and differences are likely to have emerged should the analysis have been conducted using such tools.

Conclusion
In conclusion, 17 of 22 recognized universities in Ontario had identifiable policy documents related to e-cigarette use on campus. This review also found that university policies across Ontario are inconsistent in type, scope and application. When administrative policy responses were present, measures included complete prohibitions of e-cigarette use on campus, restriction of e-cigarette use to DSA’s, and the use of regulatory frameworks modelled after provincial and municipal law, most notably the SFOA and municipal bylaws related to tobacco use. The subsequent examination of relevant research found that evaluations of campus policies specific to e-cigarette use are uncommon, while limited conclusions can be drawn from the current research surrounding the regulation e-cigarettes on university campuses in a similar manner to tobacco. These findings indicate that there potential for coordination and knowledge exchange between public health practitioners and university administrators working to address youth e-cigarette use in Ontario.

References
See Appendix E
ABSTRACT
The purpose of this experiment is to determine a relationship between the flux of muons and the zenith angle at which the pads are oriented, where the zenith angle will be varied in the North/South plane. The original prediction is that the relationship between zenith angle and muon flux is \( \Phi = I_0 \cos^2(\phi) \), where \( \phi \) is zenith angle, \( I_0 \) is muon flux at 0°, and \( \Phi \) is muon flux. The data being collected will also show whether there is a relationship between time of day and muon flux. This experiment is significant as it investigates the properties of muons, which are extremely high energy particles, capable of penetrating even lead; this means that muons are a background for many experiments and having an accurate measurement of their flux contributes to the scientific community. The key findings of this experiment are that the flux of muons is \( 58 \pm 3 \text{ m}^{-2} \times \text{min}^{-1} \times \text{sr}^{-1} \) compared to the accepted value of \( 70 \pm 1 \text{ m}^{-2} \times \text{min}^{-1} \times \text{sr}^{-1} \) (note that \( s \) is seconds, and \( sr \) is steradians – a measurement of solid angle), and that there is no statistically significant relationship between time of day and muon flux. The group concluded that, since the data fits the model \( \Phi = I_0 \cos^2(b \phi) \) with a 99.3% R-squared value (where \( b \) is a scalar adjustment factor), that the original prediction is justified. Another phenomenon that was observed was a statistically significant dip in muon flux between 3 and 4 a.m. which merits further investigation but is out of the scope of this experiment.

INTRODUCTION
Muons are charged, high energy elementary particles that decay quickly. When cosmic rays interact with upper atmospheric particles, pions are created which decay to high-energy muons and are sent towards Earth at relativistic speeds, which lengthens their rate of decay in Earth’s reference frame. These particles reach sea level with an average energy of 4 GeV, making them incredibly penetrating [1]. Muons have also recently been used as a means of non-invasive imaging in both the Egyptian pyramids, where it found a new secret chamber inside, and the Fukushima nuclear reactor where it has been used to assess internal damage to the structure under the rubble in situations where it would be too radioactively dangerous for humans to go [2] [3].

This experiment is being performed because the muon is an elementary particle, meaning that it is a fundamental building block of the universe, a further understanding of it confirms the current scientific model of the universe. Additionally, muons are an omnipresent source of background for other physics experiments [5] [6] [7].

The zenith angle is the angle from the vertical of a vector. The flux of muons is proportional to \( \cos^2 \phi \) where \( \phi \) is the zenith angle [5] [6] [8]. This is due to the decay of muons in air, in that muons originating from higher \( \phi \) value locations must travel farther to reach a detector, so the overall flux after accounting for the stopping power of air drops as a square-cosine function. The decay of muons is an important constant to the standard model of particle physics, and its value is currently being verified by the Mu2e experiment at Fermilab [9].

2 APPARATUS & DESIGN
Our apparatus consists of two paddles, one of which has dimensions \( L \times T = (51.5 \pm 0.5 \text{ cm}) \times (22.5 \pm 0.5 \text{ cm}) \) while the other has dimensions \( L' \times T' = (56 \pm 0.5 \text{ cm}) \times (31 \pm 0.5 \text{ cm}) \). A constant distance \( d' = 60 \pm 0.5 \text{ cm} \) separates these paddles. Through the center of this apparatus runs an axle, about which the paddles can rotate. There are several pairs of screws at one end of the apparatus acting as locking locations corresponding to known zenith angles. There is a secure pole at this end which can be placed between screws to hold the apparatus at varying zenith angles. This setup is shown in Figure 2 and Figure 3. When muons pass through the scintillator paddles, some of them will release a pulse of light that can be observed and measured [1].
When these paddles, of lengths $L$ and $L'$ and widths $T$ and $T'$, are placed parallel to one-another with separation $d'$, a solid-angle steradian calculation can be completed that describes the area of the sky, the normal to which intersects both planes.

Using the calculations in Appendix A, the solid angle encapsulated by the apparatus is $1.33\pm0.02$ sr. This represents the ratio of surface area swept, in steradians (sr), inside the region covered by the paddles, for any arbitrary radius $r$. From these calculations, larger paddles, as well as a smaller separation, would yield more counts, but with less accuracy, as a greater region of the sky will be encapsulated.

Because the indicator of a muon coincidence is a flash of light, it was important for us to prevent ambient photons from acting as false positives. As such, both scintillator paddles were covered in black tape and plastic garbage bags, which reduced the number of photons that interacted with the paddles. Because muons move at a large fraction of the speed of light, signals that are not sent by both paddles simultaneously can be discounted, which reduces false photon counts, as shown in Figure 4.

To make this happen, each scintillator paddle is connected to a wave guide and a photomultiplier tube, which amplifies the muons' signals and directs them to an output. Outputs from different paddles enter separate amplifiers and timing Single Channel Analyzer (SCA) units, which shape the outputs into uniform shapes and filter out any noise.

These signals then enter a coincidence unit, which will only allow signals sent by both paddles within a certain modifiable timing window, which filters photon noise. The number of coincidences are then sent to a computer, which uses a LabView program to record timestamps and counts in minute-long bins [8]. A diagram showing this setup is shown in Figure 5.

3 PREDICTIONS

When applying the previous steradian calculation to our scintillator setup, a solid angle of $1.33\pm0.02$ sr was found. Using the measured muon flux of $70\text{m}^2\text{s}^{-1}\text{sr}^{-1}$ from previous experiments, and the percentage of muons that can be picked up in scintillators, a theoretical flux when the scintillator paddles are parallel to the sky can be found by [6]:

\[
\text{Flux} = 70\times 1.33 = 93.1 \text{m}^2\text{s}^{-1}\text{sr}^{-1}
\]
Figure 5: A block diagram describing the basic electronic layout of the experiment's data acquisition.

\[ I = 70 \times P_{\text{eff}} \times \zeta \times T \times L, \]  

where \( I \) represents the frequency of muons counts, \( P_{\text{eff}} \) represents the percentage of muons stopped by the scintillator, \( \zeta \) represents the solid angle of the apparatus, \( T \) represents the width of the paddle, and \( L \) represents the length of the paddle. This expression evaluates to:

\[ I = (10.8 \pm 0.2 \text{s}^{-1}) \times P_{\text{eff}} \]

Plastic scintillator paddles, such as the ones in use in this experiment, have about 18% efficiency when they are in perfect condition, meaning that the ideal expected frequency is 1.1 Hz [8]. Because the paddles in use are old and depreciated, this value is not expected to be observed. By taking data samples over times greater than 24 hours, the group also considered the possibility of determining the variance in muon flux based on the time of day. However, upon further consideration, the group decided that this would likely not be a factor, as the cosmic rays that create muons, as well as the muons themselves, are charged particles, and therefore will be carried around the globe by Earth’s magnetic field. Tests were done to determine this relation, although no positive results were expected.

When the zenith angle is non-zero, muons passing through the paddles must travel a larger distance to hit both paddles, and therefore have more time to decay, resulting in a smaller flux. This relationship is well documented, and follows the relationship [6]:

\[ \Phi \sim I_0 \cos^2(b\varphi), \]  

where \( \Phi \) is the intensity of the muons observed, \( \varphi \) is the angle from the vertical, \( I_0 \) is the intensity observed when \( \varphi = 0 \), and \( b \) is a scalar adjustment factor. This relationship is predicted to hold strong at angles close to 0°, but will be less effective closer to 90°, as \( \cos^2(b\varphi) \) will equal zero at \( \varphi = 90^\circ \), while the true plot will approach zero, but not reach it.

4 PROCEDURE

The following procedure was used when collecting data:

1. The time that the apparatus was to have been rotated was confirmed with other group members.
2. After having arrived in the morning, the appropriate text file was opened, and it was verified that data collection had begun more than 24 hours before. It was verified that the angle on the text file corresponded to the angle on the apparatus. If there was a discrepancy, it was noted in the lab book.
3. After verification, the ‘STOP’ button on the LabView was pressed. The data collection would continue for another 2 cycles (2 minutes), at which point the ‘On’ light on the Timer unit would turn off.
4. The apparatus was rotated to the next appropriate angle by one group member, independent of the other present member. The order of angles was: 00°N, 15°N, 30°N, 45°N, 60°N, 15°S, 30°S, 45°S. If there were to be enough time, background noise was to be measured.
5. The ‘⇒’ button was pressed to begin the program. The program prompted the user to input a file name for the data to be collected.

5 DATA

The data collected was saved in text files with tab delimited columns, containing the number of counts, the date, and the time at which the data was taken.

There cannot be uncertainties on individual counts. The uncertainty is determined by taking the square root of the number of counts when summing all the counts together, or is the standard deviation on all the counts when referring to the mean. Refer to the analysis section for further uncertainty analysis.

A summary table of the total number of counts collected, and over what time interval the data was collected, is shown in Table 1.

<table>
<thead>
<tr>
<th>Degrees (°)</th>
<th>Counts</th>
<th>Samples (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00 N</td>
<td>14141</td>
<td>1551</td>
</tr>
<tr>
<td>15 N</td>
<td>31844</td>
<td>3761</td>
</tr>
<tr>
<td>30 N</td>
<td>10548</td>
<td>1531</td>
</tr>
<tr>
<td>45 N</td>
<td>6191</td>
<td>1436</td>
</tr>
<tr>
<td>60 N</td>
<td>3768</td>
<td>1480</td>
</tr>
<tr>
<td>15 S</td>
<td>13178</td>
<td>1761</td>
</tr>
<tr>
<td>30 S</td>
<td>23906</td>
<td>3826</td>
</tr>
<tr>
<td>45 S</td>
<td>6069</td>
<td>1500</td>
</tr>
</tbody>
</table>

6 ANALYSIS

For each angle, counts were collected and the mean counts per minute calculated. The error on each point is the error on the mean. The error on the angles was determined from the apparatus. The screws used to control the rotation of the paddles did not hold the apparatus completely steady, and the corresponding error associated which each position was measured with multiple phone level apps. The result of the compiled data is shown in Figure 6.
Figure 6: The number of counts vs. the zenith angle. Negative angles denote that the top paddle is oriented towards the North, while positive angles denote when the top paddle is facing South. The counts are all normalized by taking the mean of counts throughout the day and dividing by the mean at 0°. The mean at 0° was 9.14±0.03. The total counts throughout the day at 0° is 13132. The total counts are the sum of all the data collected throughout the day at 0°.

The data points at 15±1° and 30±1° were suspect, as they did not fit the cosine squared relationship very well, while all other points did. An explanation for this error is that the set of screws used to fix the position of the apparatus have gaps between that can be confused for valid placements for the rotation of the apparatus. The location between the fixed angle positions may have been mistakenly used. These angles were changed to the calculated values for the in-between screw locations, which resulted in a better fit.

To check the validity of the angle shift to the two data points, several methods were tried. Refer to discussion for more details. The modification made from Figure 7 to Figure 8 is that the background was removed from the calculated means.

To determine whether-or-not the time of day influences the muon flux, data from all days and all angles was shifted such that they all started at 12:00 AM and ended at 11:59 PM. Data for all angles was summed into one large data set which was binned into one-hour increments. This is shown below in Figure 9.

Figure 7: The normalized number of counts vs. the angle. Negative angles denote that the top paddle is oriented towards the North, while positive angles denote when the top paddle is facing South. Additionally, the angles at 15° N and 30° N were changed to 20° N and 35° N respectively, as the data is suspect, and may be explained by the apparatus being positioned improperly during data collection.

Figure 8: The number of counts vs. the angle. Negative angles denote that the top paddle is oriented towards the North, while positive angles denote when the top paddle is facing South. The mean of the counts caused by background was subtracted from all the means collected at different angles. The counts are all normalized by taking the mean of counts throughout the day, and dividing by the mean of the counts at 0°. This data used the modified angles.

Figure 9: Histogram of all the counts collected for every angle measurement. The mean of the counts per hour was calculated to be 2870, with a standard deviation of 50.
From this it was found that the counts from 3:00-4:00 AM are 4.6 standard deviations away from the mean.

The means with a removed background and modified angles vs zenith angle were then fit to a cosine-squared relationship. The amplitude was 0.9801, and the angular frequency was 1.117. These values are to correct our data, to fit the cosine squared relationship. The R-square value was 0.9931. This indicates that the data reasonably fits the model. A visual representation of this fit is shown in Figure 10.

To check if there was a relationship between time of day and the muon flux a sinusoidal fit was applied to the daily data binned in 1-hour groups. The R square value was 0.3735, and thus we can discount the possibility that there is a change in muon flux daily. A visual demonstration of this is shown in Figure 10.

7 DISCUSSION

When looking at the initial results for normalized counts as a function of zenith angle shown in Figure 6, it was surprising to see that the number of counts when the paddles were facing South, was fewer than their Northern counterparts, and not following the expected trendline. This error was then hypothesised to be due to human error when aligning the paddles, by setting the location to be between two valid screw locations, rather than at the pre-determined angles. This was tested by purposely taking a measurement at the location of one of the “accidental” angles (20°S). There is a discrepancy between this new point and the equivalent potential “accidental” points, which can be attributed to the re-attaching of the paddles to the rotating apparatus. Between the original data set collection and the re-measurement, the paddles were removed from the apparatus to take a background data set, having the paddles lie separate in the same plane. When re-attaching the paddles, not enough care was taken to ensure that the set up was identical to the original, which resulted in an offset of the scintillator paddles.

To double check that the re-attachment issue was the cause of the unusual drop, data was taken again with the offset still in place for a 0° zenith angle. The ratio of this data set count to original 0° count gave a “slant factor” due to the apparatus difference. The re-measured point (20°S) could now be adjusted with this slant factor to determine what its value would be, had the data collection apparatus been reattached properly. After the adjustment, the results were inconclusive, so the original data point was taken to be 20°S. Using this knowledge, the group assumed that the previously 30°S point – which didn’t fit the data – was actually 35°S. This result is shown in Figure 7.

Looking at the adjusted data, it still does not completely agree within the error bars shown on the graph, with theory. This is because the error bars on the graph are showing the statistical error due to the nature of our experiment being a counting experiment. In addition to this error, the solid angle is large enough that cos^2φ of nearby φ will give counts which are not at the correct rate. This effect will “flatten” the total counts for each angle – decrease the large angle counts (since smaller angles’ rates will also be present) and increase the counts at small angles (since large angles’ rates will be present). Based on the curve fitting result, this could be the cause of the increase in b value (related to the angular frequency of the model). If the experiment had perfect results, the a and b values would be exactly 1, and for our data, a agrees since it is 0.98 ± 0.04 with 95% confidence, while b is 1.12 ± 0.05, which confirms the “flattening” hypothesis, as shown in Figure 10. From the calculated value of a, our flux is found to be 9.0±0.4 μ/min. This translates, when the area and solid angle of our set up are a flux of 58±3 min^-1sr^-1m^-2. Comparing this to the theoretical flux of 70±1 s^-1sr^-1m^-2 this experiment’s flux corresponds to an efficiency of our experimental setup relative to the expected flux of about 1.2%, as opposed to the expected 18% efficiency. This, however, is expected, as the paddles used are old and do not function effectively. As a result, the voltage threshold set to discriminate photon noise from muons was set higher than would have otherwise been necessary, and the paddles’ efficiency was hindered.

Over the course of the experiment, many weather patterns were observed, including rain, snow, and clear skies. No significant trend was observed linking weather and the flux of muons, however more observation should be taken to make conclusive findings.

There was a solar flare which occurred while our set up was running which occurred on November 29th. Looking at the preliminary figures, it is seen that the detector did not see any increase in flux beyond the normal noise during the active period.

Regarding the effect of muon flux as a function of day, Figure 16 shows that over the course of the day there is no significant trend, since the R^2 value was around 0.3. Had there been an effect due to the sun’s position, there would be an evident sinusoidal relationship peaking at noon, and being at its minimum around midnight, when the sun is farthest away. Even though there is no trend of this nature, there is an interesting point around 3:00-4:00am where there was consistently a drop in
the number of muons counted. This data set is 4.6σ away from the mean, which corresponds to a difference of 247 counts, which is not impossible, however it is unlikely to occur without cause. Potential reasons for this drop could include a reduction in the power output of the grid or in Stirling Hall during this time, which may have resulted in the High Voltage power supply delivering inconsistent voltages to the photomultiplier tubes. A method to confirm this theory in future experiments would be to consistently measure the High Voltage output throughout the day.

This experiment was significantly limited by the electronics set up efficiency and data collection time. The efficiency was much lower than expected, resulting in longer time required to achieve meaningful number of counts. Another limitation was the length of the experiment and its relation to total data collection period. Since a data set requires over a day to collect, and the apparatus could not be updated over the weekend, each angle could only be measured once. If the experiment was to be repeated, it should be conducted over a longer period so that multiple trials of each data point are collected. Another suggestion would be to have multiple scintillator coincidence units set up at different angles collecting data simultaneously so that special events, such as weather or solar flares, could definitively be seen across all units.

8 CONCLUSION

A cosine squared relationship between muon flux and zenith angle was found. This agrees with the predicted model of $\Phi = I_0 \cos^2(\phi)$, with the addendum that there is an adjusted frequency term of $1.12 \pm 0.04$. The total flux on the Earth’s surface was found to be $58 \pm 3 \text{ m}^{-2}\text{ minute}^{-1}\text{ sr}^{-1}$. This is $1.2\%$ of the expected flux of $70\pm1 \text{ m}^{-2}\text{ s}^{-1}\text{ sr}^{-1}$, which can be attributed to electronic deficiencies or paddle efficiency. As well, the threshold set to discriminate photons vs. muons may have been set to high due to the inefficiency of the paddles, thus eliminating actual counts.

APPENDIX A - Solid Angle Calculations

![Diagram](image)

Figure 11: The geometric explanation of the mathematics describing the shift in scintillator paddle size.

The system’s solid angle describes the fraction of the sky that is observed by this apparatus. The first step to performing this calculation is to normalize the system to mimic identically sized paddles. This can be done using the geometry in Figure 11. Using the geometry of similar triangles, the mathematics describing the calculation for $d$ is:

$$d = d' \frac{L}{L'}$$  \hspace{1cm} (3)

The same geometry can be applied to $T$ and $T'$. The angle of the sky covered by the length-dimension of the scintillator paddles, represented by $\theta$, can now be expressed by:

$$\theta = \pi - 2 \arctan \left( \frac{d}{L} \right)$$  \hspace{1cm} (4)

Accounting for both the paddles’ dimensions, this calculation was done twice, this time using the width in lieu of the length, and representing the angle by $\beta$, is:

$$\beta = \pi - 2 \arctan \left( \frac{d}{T} \right)$$  \hspace{1cm} (5)

To calculate $\zeta$, the solid angle of this setup, an integral must be done to determine the percentage of the sky that is encompassed by the paddles. To accomplish this, the following integral must be evaluated:

$$\zeta = \frac{1}{r^2} \int_{-r \sin \left( \frac{\pi}{2} \right)}^{r \sin \left( \frac{\pi}{2} \right)} \theta \sqrt{r^2 - x^2} \, dx$$  \hspace{1cm} (6)

REFERENCES

See Appendix F
Design of a Portable Soil Analysis Instrument for Remote Teleoperated Rover Platforms

Emily Archer, James Xie, Alexander White, Edric Leung, Meghann Grenier, Janis Cheng, Lily de Loe, Raymond Ye, Andrew Downie

1. Introduction
Martian astrobiology seeks to understand the history of the Martian environment which can be extended to terrestrial systems and used in designing crewed planetary missions. However, typical experiments on Earth often require dedicated laboratories, skilled scientists, and a wide array of instruments to perform. On space robotics missions, data must be autonomously collected at the point of contact and analyzed with limited resources and tight operating constraints. As such, entire laboratories must be condensed and automated to be sent on a space mission.

The Queen’s Space Engineering Team (QSET) is proposing a portable instrument to receive soil samples, analyse their composition and transmission data back to a ground station. This project is part of a larger environment characterisation module to be mounted on a Mars rover system designed for competition at the University Rover Challenge (URC) at the Mars Desert Research Station (MDRS) in Utah. As such, the system is constrained to be less than 5 kg, have a footprint less than 12” x 12” x 12”, consume the harsh Utah desert in under 20 mins.

The National Aeronautics and Space Association (NASA) has defined a ‘Ladder of Life’ hierarchy for classifying the strength of evidence for extant life [1]. From this, any astrobiology instrument must be sufficiently sensitive, contamination-free, and provide repeatable results to support any evidence. Spectroscopy, chromatography, and electrochemical probes are among the most common technologies used in analysis of organic samples and there has been active research to miniaturize bulky instruments [2]. Colorimetric methods were selected for their ease of use, low cost, and can be miniaturized to the millilitre scale. Traditionally to perform a colorimetric analysis of a soil sample, a soil sample is mixed with an indicator and allowed to react. The liquid phase is placed into a cuvette where the spectra is taken, then all materials are disposed to waste. All sample-contacting equipment must then be cleaned before another sample may be examined. These processes must all be contained into a single unit and furthermore, waste cannot be discarded outside of the system.

2. Process Design

2.1 Analytical Instruments
Each sample cell is a 5/16” ID x 1 3/4” cylindrical chamber constructed from printed white acrylic resin with clear acrylic windows mounted on each face. Spectra are collected with an Avantes CMOS AvaSpec Mini (50 nm slit, 2.35 nm spectral resolution) and PCB-mounted ThorLabs LEDSW50 white LEDs (440 – 700 nm). Spectra will be taken over a 25 ms integration time and 25 averages to reduce background noise. The complete optical setup is shown in Figure 1.

Figure 1: Optical circuit diagram for measuring the spectra of soil samples

Five indicators were selected for the proof-of-concept design, shown in

The absorption signal is expected to follow the Beer-Lambert law within the concentrations observed on collected soil samples at MDRS. Calibration curves will be compared against soil samples at MDRS to determine concentrations within the soil. Future design iterations will seek to optimize the sample cell dimensions
to maximize sensitivity while minimizing liquid volume. Transitioning to a multi-LED or tungsten-halogen lamp light source would increase the spectral range to 300 – 1100 nm with

Figure 2: Process instrumentation diagram of the microreactor system, showing fluid paths in black and signal lines in blue (hatch for optical, circle for electrical).
existing equipment, however the former requires more complex optical coupling while the latter is delicate.

2.3 Sample Handling

Liquid phases are transported within the system via Adafruit 3910 peristaltic pumps to avoid fluid contact and can self-prime between samples. Pump sizes were verified to achieve a minimum safety factor of 1.5 based on first-order system friction estimates via the Bernoulli equation using the Fanning friction factor ($f$) for major losses given a tube roughness of 0.01 mm and tabulated coefficients for minor losses based on tube CAD geometry and manufacturer data [10]. The pressure loss across the filter was estimated using the Kozeny-Carman equation for a $\frac{1}{2}$" deep x 1" diameter cake of charcoal [10] and manufacturer data on the resins.

$$\frac{\Delta P}{\rho g} + \frac{\Delta (v^2)}{2g} + \Delta h = \frac{fL}{Rg} v^2_{avg}$$  \hspace{1cm} (1)

Mixing between the soil, indicator, and water is performed passively in a shortened $\frac{1}{4}$" ID static mixer (McMaster-Carr 3067K14) before the sample is filtered into the sample cell. Between each soil sample and indicator, fluid contacting components are rinsed with water and 70% isopropyl alcohol to remove residue. The primary error during operation arises from entrained droplets within the system (especially within the mixeare which may dilute or contaminate the sample. Custom components and tube paths have been designed to control liquid entrainment by limiting horizontal sections and corners which may collect liquid.

In each sample cell, small particulates which were not removed by the 28 $\mu$m filter will remain in suspension and bias results through scattering effects. As such, a delay between sample injection and measurement was estimated using the Stoke’s Law for $\text{Re} \approx 0.1$ to allow particles to settle across the diameter of the sample cell.

$$v = \frac{2(\rho_p - \rho_f)g r^2}{9 \mu}$$  \hspace{1cm} (2)

Given an average density ($\rho_f$) and viscosity ($\mu$) of water to be 1000 kg/m$^3$ and 0.89 cP respectively [10] and an assumed average particulate density ($\rho_p$) of 2650 kg/m$^3$ (quartz), Error! Reference source not found. shows the settling time and expected soil distribution based on grain-size distributions of Utah desert soils [11]. From this, a 1 min. settling time is sufficient to clear 75% of suspended particulates. This is acceptable as particulates < 1 $\mu$m which may cause colloidal scattering effects are not expected and any remaining particulates resulting in reduced light intensity are expected to stay in suspension through the duration of the spectra measurement.

![Figure 3: Settling time estimated from Stokes Law and % soil settled based on Utah soil grain-size distributions [11] against soil particulate size.](image)

3. Mechanical Assembly

The reactor internals, shown in Error! Reference source not found., are mounted in an aluminum 6061 frame with removable plates which may be individually populated to allow ease of modification/replacement of internal components. Topological optimization of each shelf was used to minimize
weight while maintaining a sub-mm deflection under expected shock loads. To reduce the effects of in-plane lateral forces while driving, carbon fiber shear walls are mounted to each exterior face. Passive thermal control will be used to maintain the internal temperature by sinking heat into the main chassis. Exterior walls will be painted white to further reduce sunlight absorption.

Custom components within the assembly are printed with acrylic resin which is chemically inert, lightweight, inexpensive [12], and allows for compact designs which would otherwise be unmachinable. Soak tests of each printed piece show negligible liquid permeation into the layer structure under the expected duty cycle of the instrument.

4. Controls & Operations

The rover operates over an internal network of microprocessors using the Robot Operating System (ROS) to manage events and data collection. The reactor is controlled using an Arduino Mega and Raspberry Pi 3B+, shown in Figure 4, serially linked to the network switch to receive commands from the ground station. This master-slave setup simplifies troubleshooting during operation, however the lack of distributed control structures limits redundancies should one controller fail.

Figure 4 outlines the ROS event sequences from sample drop-off in the hopper to final cleaning for subsequent samples. Cameras mounted within the enclosure will allow operators to track the sample movement and troubleshoot performance through manual control of each function. System health sensors including thermistors on the spectrometer and an accelerometer/gyroscope to identify instrument orientation will provide live data to the driver and used to identify sources of error.

Figure 5: Automated unit operation event sequence for analyzing a single soil sample.

5. Conclusion & Future Work

QSET has completed its manufacturing phase to produce a proof-of-concept teleoperated soil analysis instrument to be mounted on its rover platform, shown in Figure 6. The rapid prototyping approach and compact integrated fluid handling components available using 3D printing has been crucial to miniaturizing the instrument while maintaining a total cost of under $1500 (excl. spectrometer). Preliminary testing has successfully bench-operated the instrument, showing capabilities to receive, handle, and measure soil samples, as well as recycle solvents to extend performance.

This project will be proceeding into its calibration and testing phase, including a field-test at the MDRS from May 30 – June 1, 2019. Planned testing will include pump and spectrometer calibration to characterise internal losses, performance on real soil samples, and limits of detection and quantification for each analyte. System-level testing will focus on integrating the control system with the rover and perform field operation while on-board.

In future iterations, additional internal flow sensing and control systems will be implemented to improve volume and mass measurements. In-line conductivity and pH probes may be used to live-monitor filter performance as well as support in-situ measurements if placed along the sample injection line. QSET is expecting the system to undergo continuous development and upgrades over the next few years as more manufacturing technologies become available.

Figure 6: Proof-of-concept soil analysis instrument mounted on the rover platform during a bench-test.
Figure 4: 3D CAD of the entire assembly with major components labelled. The sieve (not shown) will be mounted on the top face of the structure.
Appendices

Appendix A


Appendix B


Figure 1. The CRISPR/Cas9 gene editing process. The CRISPR/Cas9 gene editing tool provides sequence-specific genome targeting using a complementary gRNA and Cas9 endonuclease. The Cas9 nuclease introduces double-stranded breaks following a PAM sequence. The break is repaired by one of two mechanisms: (1) Non-homologous end joining (NHEJ) which creates random insertions or deletions (indels) at the targeted site; or (2) Homologous recombination (HR) which creates precise changes based on the template DNA. Image courtesy of Transomic Technologies.

Figure 2. Curing HIV with CRISPR/Cas9 gene editing. To circumvent the current functional limitations of treating HIV with CRISPR, current approaches must be improved (CRISPR cleavage of viral DNA) or novel techniques using CRISPR must be developed (CRISPRi). Following these advancements, ethical considerations still provide a barrier towards reaching a widely accessible cure for HIV.
Figure 3. Representation of the total number of target sites in the human genome while using alternative PAM sequences that direct cleavage of SpCas9-variant nuclease. The NGG PAM sequence (blue) represents the number of target sites available accessible by the CRISPR/Cas9 approach (48%). All other PAM sequences represent the number of target sites accessible by using SpCas9-variant nuclease (52%), effectively doubling the targeting potential within the human genome. Adapted from Kleinstiver et al. (2015)11.

Figure 4. Mechanism of gene repression by CRISPR interference (CRISPRi). CRISPRi employs a catalytically inactive Cas9 (dCas9) to block binding of transcriptional machinery. A gRNA directs the dCas9 to a target sequence, which is typically a gene promoter or enhancer region. Image courtesy of Qi et al. (2013)22.

Appendix C

References


**Appendix E**


Appendix D


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