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Multiple Sclerosis Associated Retrovirus as a Prognostic Biomarker for Prescribing Disease Modifying Therapies in Multiple Sclerosis

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BACKGROUND INFORMATION

Multiple sclerosis (MS) is one of the most common neurological disease in young adults [1]. There are approximately 100,000 Canadians living with MS [2]. Despite the prevalence of MS, it is challenging to diagnose, especially early on in disease [3]. This is primarily due to the overlapping symptoms and pathology seen in other diseases, such as Lyme disease, clinically isolated syndrome, human-lymphotropic virus 1 associated myelopathy, neuromyelitis optica, acute disseminated encephalomyelitis and more [3.4]. Diagnosing MS requires the exclusion of all other possible diseases, and the McDonald criteria, which is the observation of two temporally and spatially separated lesions in the CNS [3].

MS is a disease of the human central nervous system characterized by chronic inflammation and demyelination [1]. The etiology of MS is unknown [1]. 85% of individuals diagnosed with MS begin with relapse remitting MS (RRMS), which is characterized by focal lesions of inflammation, demyelination, oligodendrocyte death, axonal destruction and blood brain barrier (BBB) injury [1]. Due to the variable location of the lesions sites in the CNS, MS has variable clinical symptoms. Often, MS clinically presents with symptoms including weakness, fatigue, numbness, tingling, cognitive dysfunction, abnormal gait and incontinence [5]. In the majority of patients, RRMS progresses to secondary progressive MS (SPMS) within 8-20 years of diagnosis [1], however this can be highly variable depending on the individual case [6]. SPMS is characterized by little-to-no new lesion formation, brain atrophy and neurodegeneration [7].

Although it is challenging to diagnose MS early in disease, it is important, as the majority of available disease-modifying therapies (DMTs) have higher efficacy at the early stages of disease [8]. There are no current treatments for SPMS [9]. DMTs for RRMS fall under two broad categories: classical, first-line, non-aggressive treatments, and aggressive, monoclonal antibody- based treatments, which have a higher risk

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and a higher reward [8]. Within these non-aggressive treatments, the two most prevalently used are interferon beta and glatiramer acetate [8]. Both of these DMTs demonstrate approximately a 30% reduction in relapse rate [8]. Glatiramer acetate reduces gadolinium enhanced lesions by 35-45%, and interferon reduces gadolinium enhanced lesions by 50-80% [8]. Natalizumab and alemtuzumab are two aggressive monoclonal antibody treatments used to treat RRMS. Natalizumab treatment causes a 60% reduction in relapse rate, and a 92% reduction in gadolinium enhanced lesions [8]. Alemtuzumab reduces relapse rate 50% more than interferon beta [8]. Although these treatments have higher efficacy, they are associated with more severe side effects come to non-aggressive, first-line treatments [8]. The most common side effects associated with glatiramer acetate and interferon beta is injection site reactions, occurring in 75% and 80% of patients, respectively [8]. In addition, glatiramer acetate is associated with asymptomatic lymphadenopathy (30% of patients), and interferon beta causes flu-like symptoms (75% of patients) [8]. On the other hand, infusion reactions are common in both alemtuzumab and natalizumab treatments [8,10]. Besides relatively benign infusion reactions, natalizumab causes hepatotoxicity and in rare cases, progressive leukoencephalopathy, a fatal neurological disorder [8]. Alemtuzumab has been associated with skin and thyroid malignancies, autoimmune disease (50% of patients), infection (70%) of patients, and thrombocytopenia (1% of patients) [8]. Due to the variable disease course of MS, patients and physicians are not able to adequately assess the risk of using aggressive DMTs.

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Discovering the etiology of MS has been challenging. Several factors, including autoimmunity, viruses, vitamin D, mitochondrial dysfunction, co-infection, microbiota, and oxidative stress have been thought to be implicated in MS [7,11, 12]. There is a growing body of research on the role of human endogenous retroviruses (HERVs) in MS. One HERV in particular, multiple sclerosis-associated retrovirus (MSRV), has been thought to play a role in MS [13]. MSRV is a W family HERV, and like other retroviruses, has long terminal repeats (LTRs), *gag, pol* and *env* genes [14]. There are several copies of HERV-W/MSRV gene sequences throughout the genome (20-100) [14], however almost all of them code for defunct protein [15]. One HERV-W envelope element, syncytin-1, is a fully functional protein essential for trophoblast fusion during placental development [15,16]. Despite there being no other functional HERV-W genes, MSRV envelope protein (Env) has been detected in the blood, brain and cerebrospinal fluid (CSF) of individuals with MS [15]. HERV-W elements are retrotransposably active, and one hypothesis suggests that recombination of multiple HERV-W elements allows for the successful production of MSRV-Env in patients with MS, however this is still debated [15].

RESEARCH QUESTIONS

MS is a variable disease that has the potential to cause devastating outcomes. Current DMTs have greater efficacy early on in disease, and aggressive therapies have the potential for better disease outcomes, however these treatments carry the risk of severe side effects. It would be valuable to identify a prognostic biomarker to predict disease severity, so an appropriate treatment regimen can be prescribed. The etiology of MS is unknown, which limits our ability to design pharmaceuticals that are capable of combatting the disease directly. There is mounting evidence in the literature that suggests that MSRV may play a role in MS. With this rationale, I will discuss the three questions that further explore the possibility of using MSRV as a biomarker to guide DMT prescription in Canada.

1. Can MSRV be used as a biomarker to predict MS severity?
2. Is it feasible to screen for MSRV in individuals diagnosed with MS in Canada?
3. Does MSRV contribute to the pathology seen in MS?

PROJECT NARRATIVE

Can MSRV be used as a biomarker to predict MS severity?

There are numerous reports detailing the increased presence of MSRV in individuals with MS. For instance, MSRV-Env/HERV-W expression has been detected via immunohistochemistry in the brains of all individuals

with MS, and there is increased expression at lesion sites [17]. The expression of the MSRV-Env in the brain is not seen in healthy controls [17]. Studies have reported that there is a six-fold increase of MSRV-*env* DNA in individuals with MS [18]. In addition, MSRV particle concentration in the CSF increases as MS clinically progresses [19]. Taken together, these findings demonstrate a correlation between MS and MSRV expression.

There is also evidence that MSRV expression correlates with active disease, and that it can predict future disease outcomes. MSRV transcript levels in the blood and CSF are higher during the active phase of RRMS compared to the remitting phase [19]. Furthermore, individuals who have detectable concentrations of MSRV in their CSF at the time of diagnosis have greater disability at 3, 6 and 10 years and they develop SPMS faster compared to individuals who do not have detectable MSRV at diagnosis [15]. These results suggest that MSRV can serve as a biomarker to aid in MS prognosis. MSRV expression data is briefly summarized in table 1.

By having a reliable biomarker, physicians will be more-able to assess the severity of the disease so they can adequately weigh the relative risks of using aggressive DMTs. This knowledge will also help individuals with MS decide if they think the risks of DMTs are worth taking based on the predicted severity of the MS. Using MSRV as a biomarker can provide novel knowledge of MS progression to both patients and physicians, allowing them to develop a patient-specific treatment plan that minimizes risk and maximizes patient outcomes.

One of the major limitations of these studies is that they are all conducted on relatively small sample sizes and limited to primarily European cohorts [15]. It is not clear if this research will prove to be relevant for individuals in Canada with MS, however MSRV expression in MS patients across European samples is consistent [20]. Due to the compelling nature of the aforementioned evidence, it is reasonable to investigate these findings in larger, Canadian cohorts to better understand if MSRV can be used as a prognostic biomarker for MS in Canada. Based on the findings of increased MSRV expression in individuals with MS across Europe, and the ability of MSRV expression to predict disease severity, it is rational to believe that these findings may be similar in Canadian cohorts.

In order for this initiative to become a reality, it will be essential for MSRV expression data to be shared between research groups through the means of a centralized database. By accumulating all of the data in a centralized location, it will allow for a widespread, efficient means of investigating this research question. Because many of the tools for investigating MSRV expression have been developed, such as PCR primers to specifically identify MSRV genes [18], and an antibody that is specific for HERV-W Envelope proteins [21], the majority of the foundational research has already been conducted. The future in understanding MSRV expression in the context of MS will rely solely on sample collection and storage in a centralized database.

Is it feasible to screen for MSRV in individuals diagnosed with MS in Canada?

As the tools have already been established to investigate MSRV expression, the bottleneck in assessing the role of MSRV in MS will be the feasibility of screening and the efficient storage of data. Diagnosing MS requires a multitude of tests that includes collecting blood and CSF samples, and imaging the CNS with magnetic resonance imaging (MRI) [3,22]. Blood samples are taken to look for infection [22], and CSF samples are used to rule out infectious agents, and to find evidence of BBB injury and oligoclonal bands [3]. Because these samples are already collected, and there are established protocols to analyze MSRV expression with antibody and PCR-based methods [18,21], it is reasonable to conclude that screening for MSRV in individual Canadian patients is feasible. This conclusion is summarized in table 1.

For this data to be useful, it must be collected on a large scale, and it must be accessible between researchers. Large scale collection will be essential to determine the efficacy of MSRV as a biomarker across the Canadian population, and collaboration between research groups will distribute the workload. The natural mediator of this initiative is the MS Society of Canada, who allocated $4.5 million in operating grants to Canadian MS researchers in the 2016/2017 grant application cycle [23]. As the research division of the MS Society of Canada's goal is to "provide the greatest benefit to individuals who are deeply affected by MS" [24], this proposed research initiative to screen for MSRV presence in biological fluids of individuals with MS to predict disease outcomes and guide DMT prescription is highly relevant to their cause.

Based on previous findings, it will be important to have long term monitoring of patients, ideally around 15 years. This will allow for enough time to track to the progression of RRMS to SPMS in sampled patients. Samples should be taken at RRMS diagnosis, during active MS attacks, and once a year. This should provide

TABLE 1 A brief outline of the measurement tools, feasibility and expression patterns of MSRV in MS patients.

Sample	What has previously been observed in MS patients	How will this be measured in the future.	Feasibility
Blood	Increased MSRV-*env* transcripts and circulating MSRV virions [15].	ELISA using GNbAC1 antibody (protein), and RT-PCR using MSRV-specific primers.	Protocols for detecting MSRV transcripts and protein from blood are established [18,21]. Blood tests are required for MS diagnosis [22].
CSF	MSRV-*env* detection at disease diagnosis predicts disease severity [15]. MSRV particle concentration increases as disease progresses [19].	ELISA using GNbAC1 antibody (protein), and RT-PCR using MSRV-specific primers.	Protocols for detecting MSRV transcripts and protein from CSF are established [18, 21]. Lumbar puncture and CSF evaluation is necessary for MS diagnosis [3, 22].

enough sample data to confirm the external validity of the findings in the previous European studies. It is reasonable to believe that similar patterns will be seen in large, Canadian cohorts, however it is necessary for this ground-work to be completed to accurately assess the efficacy of MSRV as a biomarker for MS prognosis in Canada.

By collecting data on MSRV expression in the blood and CSF from a large cohort of Canadians with MS, a better understanding of the relevance of MSRV as a biomarker for prognosis can be evaluated. If successful, this will not only serve as a benefit for guiding DMT prescription, but it may also shed insight into the underlying mechanisms driving disease in MS. As MS still has an unknown etiology, this initiative may pave the way for future research evaluating the potential role of MSRV as a driving factor in the pathogenesis of MS.

Does MSRV contribute to the pathology seen in MS?

The association between MSRV and MS may go beyond correlation. There is accumulating evidence that MSRV can contribute to the pathology seen in MS. As stated previously, MS is a chronic inflammatory, demyelinating disease of the CNS, that is further characterized by BBB dysfunction, oligodendrocyte death, defects in remyelination, and the presence of activated microglia, T cells and B cells in the CNS [1,7,11]. *In vitro* and *in vivo,* evidence has suggested the MSRV can contribute to or cause these pathological features seen in MS [15].

The surface domain of MSRV-Env/HERV-W is a potent TLR-4 agonist that activates and induces the upregulation of the proinflammatory cytokine IL-1β in microglia, and IL-1β, IL-6 and TNF-α in monocytes [25]. MSRV-Env stimulation of TLR-4 also inhibits oligodendrocyte precursor cell (OPC) maturation, which prevents the formation of mature, myelin forming oligodendrocytes [26]. Finally, MSRV-Env acts on endothelial cells of the BBB, causing an upregulation of ICAM-1, and proinflammatory cytokines IL-6 and IL-8, which allows for the increased transmigration of activated leukocytes in the BBB [27]. A brief summary of the inflammatory effects mediated by MSRV-Env is depicted in Figure 1.

Another study demonstrates that syncytin-1 expression in astrocytes causes the release of cytotoxic mediators, which then act on oligodendrocytes and cause their death [28]. Unfortunately, this study reported that these effects were mediated by syncytin-1, although there were no PCR-based, or antibody-based techniques to distinguish MSRV-Env, HERV-W Env and syncytin- 1 at this time. This highlights a major flaw in the field of research investigating the effects and expression of MSRV; all of the research prior to 2009 was not able to molecularly distinguish the RNA or proteins of HERV-W envelopes from each other [15]. Since 2009, a group has developed PCR primers that can accurately distinguish MSRV-*env* from syncytin-1 transcripts [18]. Based on this observation, it will be essential to re-evaluate many of the correlative findings of MSRV transcript expression in individuals with MS using updated PCR protocols. A monoclonal antibody IgG4, GNbAC1, has been developed that is specific for MSRV-Env/HERV-W/syncytin-1, however it is not able to differentiate between these proteins [21]. Further research will need to work towards developing a specific antibody towards MSRV-Env and syncytin-1 to accurately characterize *in-vivo* expression patterns.

In order to indirectly test the pathogenic effects of MSRV-Env in MS, a research group has begun conducting a phase IIb clinical studies on patients with MS, using the monoclonal antibody, GNbAC1 [29]. This antibody has been shown to be safe in healthy controls during phase I clinical trials [21], and it is currently being tested for its efficacy in patients with MS [29]. The results of this study are projected to be released at the end of 2017 [29]. If MSRV-Env/HERV-W/syncytin-1 is responsible for mediating the pathology seen in MS, a neutralizing antibody towards these proteins should mitigate this effect and prove to be a novel DMT for the

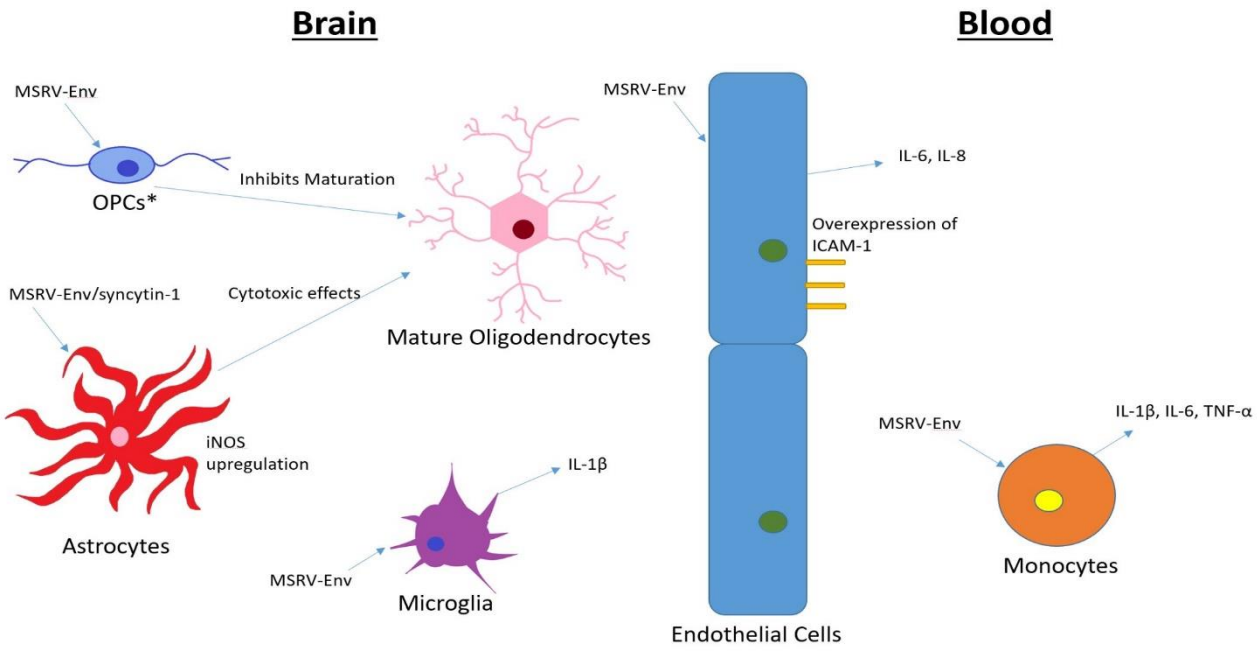

FIG. 1 A brief summary of the pathological and inflammatory effects of MSRV-Env. *OPCs are oligodendrocyte precursor cells.

treatment of MS in the future. Due to the large amount of evidence suggesting the MSRV can cause the pathology seen in MS, and the fact that MSRV is upregulated in individuals with MS, it is reasonable to expect that GNbAC1 will at least slow down disease progression in MS, if not stop progression completely.

Like the majority of research looking at MSRV, this clinical study is also being conducted in Europe. If the results of the phase IIb clinical study suggest that MS progression is limited through the use of this antibody, it will be essential to continue this trial in Canada. With approximately 100,000 individuals with MS in Canada and the potential for MS to cause severe, debilitating disease, the impact of this novel treatment could be immense. Not only could the results of this clinical trial provide a novel therapeutic to treat individuals with MS, it may elucidate the etiology of MS.

SUMMARY AND CONCLUSION

MS is a chronic inflammatory, demyelinating disease that has debilitating effects on a large number of Canadians [1,2,7,11]. First-line DMTs, such as glatiramer acetate and interferon beta are relatively safe, however they have marginal efficacy at slowing down disease progression [8]. More efficacious, aggressive, monoclonal antibody-based DMTs have been developed, however these therapies are associated with more severe, life-threatening side effects [8]. Due to the variable nature of disease progression in MS, it is challenging for physicians and patients to weigh the relative risk of using aggressive DMTs for treating MS. Thus, it would be valuable to discover a prognostic biomarker that can predict MS disease severity.

MSRV is a prime candidate to be experimentally investigated as a prognostic biomarker for prescribing DMTs in Canada. Evidence from European cohorts suggests that MSRV expression early in disease signifies more severe outcomes [15]. Clinical research surrounding MSRV expression should be expanded upon in Canadian cohorts to examine the external validity of these prior experiments to assess the potential role of MSRV as a biomarker in Canada. In order to accomplish this aim, the combined efforts of clinicians across Canada collecting samples and storing the data in a centralized database will increase the efficiency of data collection, while also providing a large number of samples from a large sample area. The Canadian MS Society is the natural mediator and financial backer of this initiative, as they are involved in funding MS research across Canada.

In addition to the potential of MSRV acting as a biomarker, there is research suggesting that MSRV is driving MS-related pathology. Currently, there is an ongoing investigation evaluating the use of GNbAC1, a

HERV-W Env-specific antibody, as a DMT for treating MS [29]. Like other research related to MSRV, this study is taking place in Europe. If the results of this study suggest that GNbAC1 is effective at mitigating disease progression, this will support the claims that MSRV is driving disease in MS, which will provide insight into the etiology of MS. This would be a ground-breaking study in the field of MS research, and it would allow for the further investigation of new therapies targeting MSRV in MS, while also opening up an avenue of research exploring why MSRV is expressed in individuals with MS.

There are many exciting opportunities surrounding MSRV research, however there are still significant challenges in the field. The literature surrounding MSRV/HERV-W/syncytin-1 is convoluted. Prior to 2009, there were no molecular methods to distinguish MSRV/HERV-W/syncytin-1 transcripts, and there are still no methods to distinguish the protein products [15]. As a result, the literature surrounding MSRV/HERV-W/syncytin-1 does not use consistent nomenclature, and many of the experiments reporting MSRV-env or syncytin-1 transcript expression prior to 2009 needs to be re-investigated using modern methods. Another avenue of future will work will be the location of a functional MSRV-*env* sequence within the genome of patients with MS. Although MSRV-Env protein products have been described, there is a lack of consensus as to how this protein is being produced [15].

With regards to using MSRV as a biomarker for prescribing DMTs, two major challenges are evident. Firstly, the efficacy of aggressive DMTs in patients with higher levels of MSRV expression needs to be investigated. Secondly, it will be important to consider the ethics of using MSRV as a biomarker. Aggressive DMTs carry the potential of severe side effects, which still need to be taken into account if they are prescribed on the basis of a high MSRV expression. In addition, in the early stages of researching MSRV as a prognostic biomarker, it will need to be determined if individual patients will receive the information regarding their relative expression level of MSRV in biological fluids. Nonetheless, these are challenges that can be faced once an effort has been made to explore the use of MSRV as a prognostic biomarker in Canada.

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