DEVELOPMENT AND COMMERCIALIZATION OF REMYELINATION THERAPEUTICS TO RESTORE NEURAL FUNCTION IN MULTIPLE SCLEROSIS

By

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Submitted in the partial fulfillment of the requirements for the degree of Master of Science

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Dedicated to all multiple sclerosis patients and their families...
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Multiple sclerosis (MS) affects around 400,000 people in the United States and above 2.5 million individuals throughout the world. There is currently no cure for MS, but the currently marketed anti-inflammatory drugs developed to combat MS are partially effective in delaying the disease. Although the exact cause of MS is not known, the onset is characterized by infiltration of immune cells and the destruction of oligodendrocytes and myelin leading to loss of neural function. Based on years of research in the laboratories of Drs. Trapp & Macklin, Renovo Neural’s approach is to use small molecules that can therapeutically stimulate oligodendrocyte differentiation eventually enhancing remyelination. The thesis will focus on Renovo Neural’s approach to develop compounds that stimulate remyelination, the company’s development plan and exit strategy.
1 Introduction

1.1 Internship at Renovo Neural Inc.

As part of my Master’s program requirement I joined Renovo Neural Inc. (RNI) as a business development intern in October 2010. The responsibilities that I primarily undertook during my internship period are briefed below:

- Assisted the company in putting together the strategic and development plans and milestones.
- Preparing initial marketing documents and presentations, quarterly newsletters, posters, etc.
- Assisted in conducting initial market research and due diligence on potential clients and competitors.
- Assisted in writing the commercialization and marketing sections of grants and also put together grants for submission.

1.2 Overview of Renovo Neural Inc.

RNI is a Cleveland Clinic spinoff that is at the forefront of the next frontier in drug development for Multiple Sclerosis (MS): Remyelination. RNI has been developing its remyelination assays since the company’s founding in mid—2008 and has offered them commercially since mid—2010. RNI’s assays are based on years of internationally credible research and development in the laboratory of Drs. Trapp & Macklin. The company was funded by a $ 3-million grant from the state of Ohio’s Third Frontier program. RNI is also evaluating and developing the compounds licensed from Cleveland Clinic, and screening additional small molecule libraries to generate pharmaceutical lead candidates for potential remyelination therapies.
1.3 Scope of Thesis

The scope of the thesis is to understand the basis of RNI’s hypothesis for the development of remyelination therapeutics. MS is one of the diseases for which the cause is not definitive and thus the therapeutic approaches are necessarily based on the best current knowledge of the disease and its causes. The thesis provides a clear scientific background for the development and commercialization plan of RNI’s remyelination therapeutic candidate for MS. The thesis also addresses the favorable market status where there is a present that currently is in need for a therapeutic that could restore neural functions and potentially cure MS. Finally it provides a recommendation on the exit strategy based on the market and company positioning.

The organization of the thesis is as follows:

1. A clear scientific background on the central nervous system, multiple sclerosis, its possible causes and available treatment options are described.

2. RNI’s technical approach along with background to support the hypothesis of development.

3. The commercialization plan with sections describing the development plan, market analysis and trends, market needs, RNI’s competitive advantage and value proposition, financial plan and finally the exit strategy.
2 Scientific Background

2.1 Overview of the Central Nervous System

The nervous system is the circuitry of the human body that essentially coordinates between every organ and tissue. The fundamental role of the nervous system is to control behavior. The nervous system of the body is divided into two broad components, the central nervous system (CNS) and the peripheral nervous system (PNS). (Iezzoni 2010)

The CNS consists of the brain and the spinal cord, which are continuous structures. The CNS functions as the command unit of the body. With such a critical role the CNS is protected by anatomic constructions that are bony structures, skull in the case of the soft gelatinous brain and vertebrae in the case of the spinal cord. The entire CNS is enveloped by the multi-membrane structure called meninges and is cushioned by the cerebrospinal fluid (CSF). The brain and spinal cord themselves are protected by a thin filmy layer called the pia matter that gives the shiny appearance when exposed, but is hardly visible to the human eye. (Iezzoni 2010) (Bordal 2010)

The primary roles of CNS are to integrate all the information it receives from inside and outside the body and signal the different part of the body to coordinate actions. The signals sent outwards are accountable to control body functions - both conscious (like muscle movements) and also autonomic functions (like heart functions). The various responses in the body are coordinated by the network that sends and receives signals from different part of the body to and from the CNS. The network is made up of specialized cells called neurons that conduct the signals or impulses from the brain to different parts of the body. (Iezzoni 2010).
2.1.1 Neurons

The brain is made up of nerve cells or neurons that are mostly similar to other cells present in the body. The human brain consists of billions of neurons that form a network through which information will be received or transmitted to different parts of the body by electrical signals. Other than the ability to transmit information, neurons differ from other cells by two important ways. Once neurons have differentiated they do not subsequently divide (it is a terminal differentiation) and if neurons are lost due to a trauma they are not replaced. (Dowling 2001) (Thompson 2000)

A typical neuron (Fig. 1) has a cell body that consists of the nucleus and the cytoplasm, the latter containing the Golgi apparatus, mitochondria and endoplasmic reticulum. A number of fibers with two types of extensions (dendrites and axons) branch out from the cell body of the neurons. Dendrites are responsible of receiving signals from other neurons and are short branching fibers extending out from the cell body. On the other

![Figure 1: A neuron & major parts](Thompson 2000)
a neuron will have only one axon branching out from the cell body that is responsible for carrying the responses back as signals to various parts of the body. (Iezzoni 2010) (Thompson 2000).

The single axon extending out from the cell body of a neuron then branches into number of smaller specialized terminals called synaptic terminals. The primary function of the axon is to conduct the information from the cell body of the neuron to the synaptic terminals as action potentials. The information passed through the axons terminates at the synapse, the place where the neuron transmits the information to the target neuron or cell. (Iezzoni 2010) (Thompson 2000)

2.1.2 Myelin

A protein sheath called myelin, a dielectric material surrounds the axons from the cell body to the point where the axon branches at the synapse. The myelin sheath acts as the insulating material that is responsible for the rapid and repetitive conduction of nerve impulses and also modulates the maturation and survival of the axons. The myelin sheath that surrounds the axons of the neurons in the CNS is produced by specialized glial cells called oligodendrocytes. (Iezzoni 2010) (Thompson 2000)

A single axon and its myelin sheath are not visible to the human eye, but the thousands of axons insulated with myelin sheaths appear whitish to the human eye. In mammals myelin accounts for 70% of the dry weight of the CNS. When viewed under a transmission electron microscope (TEM), the myelin appears to be a lamellar structure of dark and light lines that spiral around the axon, as shown in figure 2. Unlike most biological membranes, myelin has a high proportion of lipids (approximately 70%) with
most of the remainder (30%) being proteins. Immunocytochemistry studies have confirmed that CNS myelin in enriched with proteolipid protein (PLP) and Myelin basic Protein (MBP). (Sternberger, et al. 1978) (Siegel, Agranoff and Albers 1998)

Figure 2: Ultrastructure of myelinated axon in CNS (Tabira, et al. 1978)

Ax- axon, m – myelin sheath, * - Inner oligodendrocyte, arrow – outer oligodendrocyte

The electrical impulse is facilitated by creating an electrical gradient between the inside and outside of the axon’s membrane. Neuronal impulses conduct along the surface of the axon’s outer membrane, plasma membrane that has different protein molecules embedded within it. Some of these proteins create channels permitting inorganic chemical ions (sodium Na$^+$, potassium K$^+$ and Chloride Cl$^-$) to pass in and out of the cell. Na$^+$ and Cl$^-$ are the ions in the fluid outside the cell; their charges balance out to make the fluid neutral. K$^+$ is the common ion inside the cell and the organic anions of amino acid and proteins balance out positive charge of the potassium ion. The plasma membrane
mesh is very fine to allow the flow of relatively large anions to exit the cell and the protein channels inhibit the movement of the Na$^+$ into the cell. (Iezzoni 2010)

When the axon is at rest an electrical gradient is formed where the inside of the cell is slightly negatively charged compared to the fluid outside of the cell. When a nerve is stimulated, the gated ion channels in the resting neurons open allowing Na$^+$ to flow inside the cell and K$^+$ to flow outside the cell. The flow of inorganic ions across the membrane result in the reversal of the electrical gradient of the cell so that it is changed to be slightly positive inside the cell compared to outside. This reversal in the electrical gradient is termed as action potential and the impulses moving down the axon rapidly carrying the signal to its target. (Iezzoni 2010)

**Figure 3: Myelin wrapping around the axons** (Bloom, Beal and Kupfer 2007)

As can be seen in figure-3 above, the myelin forming cells form a series of discontinuous insulating sections of myelin along the length of the axon. The gaps between the myelin sheath produced by different oligodendrocytes are termed as nodes of Ranvier. In
myelinated axons the ion movements responsible for the conduction of impulses occur only in the nodes of Ranvier and they are the primary reason for the rapid signal transformation down the axon. The mammalian unmyelinated axons conduct at a velocity of 1m/sec whereas a myelinated axon of the same diameter conducts impulses 10 times faster. (Iezzoni 2010) (lazzarini 2004) (Thompson 2000)

2.1.3 Oligodendrocytes

Oligodendrocytes meaning “cells with few branches” are glial cells that are responsible for the synthesis and maintenance of CNS myelin. The myelination by the oligodendrocyte allows rapid propagation of signals across the axons. Individual oligodendrocytes can myelinate up to 60 different axons with (Figure-4) a wrapping of approximately 1μm. Myelination is a critical aspect for the normal functioning of an adult CNS as any damage to the myelin can lead to various functional discrepancies. (lazzarini 2004) (Armati and Mathey 2010)

![Figure 4: Oligodendrocyte wrapping axons (Scheinberg 1987)](image)
Oligodendrocyte Differentiation:

Oligodendrocytes are formed by a progenitor cell called oligodendrocyte progenitor cell (OPC). Developmentally oligodendrocytes are formed by the subventricular (SVZ) cells in the brain as they give rise to oligodendrocyte progenitor cells. The early OPCs express the platelet derived growth factor receptor α (PDGFαR) and proteoglycan, NG2. These early OPCs divide and migrate forming a network of stellate cells that cover most part of the CNS. Every OPC cell establishes an inhibition with the neighboring OPC that controls the distribution of these cells through the CNS. (lazzarini 2004)

The next step in the process of oligodendrocyte differentiation is the formation of late OPCs or oligodendroblasts from the early OPCs. The late OPCs then form the early oligodendrocyte or a premyelinating oligodendrocyte and then eventually differentiate into a late oligodendrocyte or a myelinating oligodendrocyte. The process of oligodendrocyte differentiation is termed as oligodendrogenesis and it is a temporal and spatial sequence that precedes myelination. The stages of differentiation of oligodendrocytes are shown in Figure 5 (lazzarini 2004).

![Figure 5: Stages of OPC development](courtesy: Renovo Neural Inc.)
The OPCs that differentiate into oligodendrocytes during normal conditions primarily myelinate the nearby axons. Once the process of differentiation is complete stellate NG2 and PDGFαR positive cells remain as a major component in the CNS providing a potential pool of progenitors that can used to treat demyelinated axons. (lazzarini 2004)

2.2 Multiple Sclerosis

Multiple Sclerosis (MS), a name that derives from “multiple scars”, is a neurodegenerative disease of the CNS with currently no cure. It is a chronic inflammatory disease of the CNS in which the fatty myelin sheaths (Figure-6) around the axons are damaged leading to demyelination and other symptoms. The damage to the myelin sheaths affects the ability of the nerve cells to conduct electrical impulses down their axons affecting the communication between the cells. There is no clear explanation on the cause of MS, but it is believed that MS is an autoimmune disease. MS is the most common neurological disease in young adults and is more common in females than males. (Lazzarini 2004) (Iezzoni 2010)

Figure 6: Normal myelinated axon vs. demyelinated axon (Iezzoni 2010)
MS has been considered a clinical entity since the late 19th century after a Parisian neurologist Jean-Martin Charcot gave a comprehensive clinical description. There were several pathologic descriptions that preceded Charcot’s clinical description. Charcot showed evidence of the damage to the CNS identifying MS as a disease. In 1868 Charcot provided the basic description of the disease through its description and by identifying features that differentiated MS from other conditions. These descriptions laid the foundation for the diagnosis of patients with MS and starting in the 1870s, MS was increasingly diagnosed across the world. Charcot and his colleague Valpian described the pathology of the condition as the destruction of myelin and described the disease progression that is currently recognized as clinical MS patterns. Since its first description as a clinical disease condition, MS has grown to be the most common demyelinating diseases causing neurological disability in young adults. (Lazzarini 2004) (Iezzoni 2010)

2.2.1 Clinical Subtypes of MS

In 1996 The National Multiple Sclerosis Society standardized MS into four subtypes based on the patterns of progression of the disease condition.

1. Relapsing-Remitting MS (RRMS)

2. Primary-Progressive MS (PPMS)

3. Secondary-Progressive MS (SPMS)

4. Progressive-Relapsing MS (PRMS)

Relapsing-Remitting MS:

RRMS is the most common form of the disease which affects 85% people with MS. In this form the patients have flare-ups or acute attacks with either full or partial recovery
(Fig – 7). The relapses are unpredictable and no disease progression is seen between the attacks. (National MS Society n.d.)

**Figure 7: Relapsing-remitting MS (a. full, b. partial recovery)** (National MS Society n.d.)

**Primary-Progressive MS:**

PPMS is characterized by a continual progression of disability from the onset of the disease condition, either with no remission or with minimal improvements. Around 10% of the people with MS are diagnosed with PPMS. In PPMS the patient does not suffer acute attacks and the diagnosis for PPMS is difficult compared to RRMS leading to delayed diagnosis of the disease condition often when patients are living with significant disabilities. (National MS Society n.d.)

**Figure 8: Primary-progressive MS** (National MS Society n.d.)
Secondary-Progressive MS:

Patients with RRMS eventually develop SPMS where the course begins with a typical RRMS disease course followed by progression of disability that may include occasional attacks and minor remissions. The characteristics of SPMS are low recovery following attacks, continuous worsening of neurological functions, etc. Based on natural history studies, 50% patients starting with RRMS develop SPMS over a period of 10 years and 90% over a period of 25 years.

![Graph showing increasing disability over time](image)

Figure 9: Secondary-progressive MS (National MS Society n.d.)

PRMS is the least common disease course where the progression of disability is observed from the onset of the disease course with or without recovery. Around 5% of the MS patients are diagnosed with PRMS and approximately 1% people diagnosed with PPMS covert to PRMS per year. (National MS Society n.d.) (Lazzarini 2004)
2.2.2 Causes of MS

Since the late nineteenth century different possible causes of MS have been postulated, but the exact cause of MS is still unknown. The current theory is that MS is caused by a combination of genetic, environmental, infectious, etc. factors. None of the various theories and explanations on the causes have yet proved to be definitive. The exact cause of MS is still the subject of ongoing investigations in immunology, epidemiology and genetics. (National MS Society n.d.) (Compston and Coles 2008)

The Immune system and MS

Autoimmune diseases are when the body’s own immune system identifies itself wrongly and attacks its tissues treating them as nonself. Currently the popular theory accepted commonly is that MS is an autoimmune disease where the body attacks or destroys the myelin sheaths surrounding the axons. However, it still remains unclear on the how much of the damage is caused by auto-immune attack and there are skeptics who do not accept that MS is an autoimmune disease. (Iezzoni 2010)

Research has shown that in MS two different factors that lead to an inflammatory immune response resulting the destruction of myelin sheaths. First is the involvement of
the killer T cells that are responsible for attacking diseased or damaged cells by binding with them and destroying them by the secretion of cytokines. In the case of MS the killer T cells identify the myelin sheaths and oligodendrocytes as nonself and damage them by binding and releasing lethal lymphokines. Helper T cells mediate the destruction by activating inflammatory cells that destroys foreign invaders but they are not active on all the myelin sheaths under destruction. In the studies performed myelin basic protein from myelin were injected into laboratory animals that precipitated EAE (experimental allergic encephalomyelitis) a chronic disease resembling MS. These studies showed that myelin probably stimulates the activation of myelin killer T cells that damages the animal’s own myelin. (NINDS 2009) (Iezzoni 2010)

The second aspect of the immune system is the malfunction of the blood brain barrier. Recent study has shown that when there is abnormal relaxation of the blood brain barrier, the defenses allows activated T cells, other inflammatory cells and immune system substances to enter the CNS that should in normal conditions be outside the CNS. The migrated T cells join with the B lymphocytes residing within the CNS and other inflammatory cells to attack and destroy the myelin sheaths and associated oligodendrocytes. (Bar-Or 2006) (Iezzoni 2010)

Genetics and MS

Although MS is not a hereditary disease in a strict sense, having a first degree of relative increases the risk of MS of an individual. In addition increasing scientific evidence suggests that genetics plays an important role in the susceptibility to MS. According to National Institute of Neurological Disorders and Stroke (NINDS), the risk of developing
MS in the US population is one tenth of one percent (0.1%), but increases to 1-3% for people with first degree relative (parent, sibling or child) with MS. Based on these studies it is also seen that if one of the identical twin has MS, the other twin has 30% chances of being affected with MS, while fraternal twins only have a 4% chance of both getting MS. (NINDS 2009)

**Table 1: Recurrence risk of MS in families** (Compston and Coles 2008)

<table>
<thead>
<tr>
<th>Genetic sharing</th>
<th>Relationship</th>
<th>Lifetime risk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>Monozygotic twin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibling with two affected parents</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibling with one affected parent</td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>Dizygotic twin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Child</td>
<td></td>
</tr>
<tr>
<td>25%</td>
<td>Half sibling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aunt/uncle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nephew/niece</td>
<td></td>
</tr>
<tr>
<td>12.5%</td>
<td>Cousin</td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>Adoptee</td>
<td></td>
</tr>
<tr>
<td></td>
<td>General population</td>
<td></td>
</tr>
</tbody>
</table>

Further studies on families with more than one MS patient showed that more than one gene is involved in MS susceptibility. Several research teams found that people with MS inherit certain regions of individual genes more frequently than other individuals. Human Leukocyte Protein (HLA), the gene (locus) for which is on chromosome 6 has specially drawn attention as HLA patterns of people with MS differ from normal healthy individuals. HLA plays an important role in influencing the immune system as they contain large number of genes related to the immune system. Research on patients in northern Europe and America have shown three different patterns of HLA that are more frequent in MS patients than other others. Recent studies have shown that different
combinations of HLAs correspond with the severity and progression, but this is still not useful as a diagnostic tool as not all MS patients have different predictable HLA patterns. (NINDS 2009)

Environmental Factors and MS:
The distribution pattern of MS throughout the world shows that the disease is more frequent in people in the colder climates than the people living in the tropical conditions. Migration studies throughout the world have a common conclusion, stating that if people under the age of 15 move from a high-MS region to a low MS-region the chances of acquiring the disease becomes similar to the low MS-region. Also people moving from high to low MS regions after the age of 15 have shown a similar level of risk to acquire the disease as in the high risk areas. This clearly shows that some exposure to an environmental factor that takes place before puberty plays the role in the acquisition of MS.

Other Factors and MS:
Additional considerations as potential causes for MS include the sunlight exposure and vitamin D that may play a role in the potential to developing MS. There are also epidemiological studies that have shown that MS occurs at a higher rate in individuals that smoke. Another major area of study in determining the cause of MS is the effect of exposure to various viruses, bacteria and microbes during childhood. But, as yet, there is no final conclusion on the relative importance of any of the factors that may have an effect on the likelihood of MS. The causes of MS are considered to be esoteric but it is important to understand the exact cause of MS to aid in the development of new treatments. (National MS Society n.d.)
2.2.3 Symptoms of MS

In MS patients, the damage caused to the myelin sheaths in the CNS and in some cases nerve fibers themselves, causes interference between the nerve impulses between the CNS and other parts of the body leading to the primary symptoms of the disease. Over the course of the disease, some of these symptoms are observed in intervals and a few are observed continuously. The symptoms in patients vary to be mild or severe, long or short depending on the area of the CNS affected. The most common initial symptom of MS is vision disturbances including blurred or double vision, blindness in one eye, color distortion, etc. Fifty five percent of MS patients have inflammatory problems of the optic nerve leading to an attack of the optic neuritis and is identified to be the first symptom in 15% of MS patients. The other common symptoms found in MS patients are muscle weakness, spasticity, partial or complete paralysis, fatigue, transitory abnormal sensory feelings like numbness, prickling, etc. (NINDS 2009) (National MS Society n.d.)

Approximately 50% of MS patients suffer from cognitive impairments such as difficulties in concentration, memory, judgment, etc. These symptoms are usually mild and mostly unnoticed by the patients and are brought to the attention by family and friends and detected through comprehensive testing. The cause of cognitive impairments is due to the lesions in the areas if the brain responsible for information processing and these impairments become more apparent when the information to be processed is complex. The disease course is said to have a correlation with the type of memory problems in individual patients but do not show any relation with the severity of the dysfunction or duration of illness. (NINDS 2009)
Depression is one of the common symptoms of MS patients, and about 10% patients suffer from severe maniac-depression. Around 5% people with MS suffer from weeping/laughing syndrome, episodes of inappropriate euphoria or unrelated emotional state. This is believed to be caused due to a demyelination in the brainstem, area that controls facial expression and emotions and is observed in severe cases. As progression of the disease course a few other symptoms observed in MS patients are sexual dysfunctions, loss of bowel and bladder control, etc. An unpredictable symptom is the effect of a patient with MS on the immediate family due to mental drain resulting from various emotional and in few cases financial burdens. (NINDS 2009)

2.2.4 Diagnosis of MS

Currently there are no physical signs, symptoms or laboratory tests that can be used to determine if a person has MS. In 2001, the International Panel of Diagnosis of MS updated the criteria and guidelines for the use of magnetic resonance imaging (MRI), visual evoked potential (VEP) cerebrospinal fluid analysis to speed the diagnostic process. These tests could be used to find a second lesion in the CNS for a person who has experienced an attack or relapse. (National MS Society n.d.)

Medical Exam and Neurologic Exam:

The physician takes a careful exam to identify past or present symptoms, gathers information on family history, and also performs various tests that evaluates mental, emotional & language functions, movement & coordination, etc. In many cases the medical history and neurologic exams gives information that meets the diagnostic criteria and other tools are used to confirm the diagnosis or provide additional information. (National MS Society n.d.)
**Magnetic Resonance Imaging (MRI):**

MRI is the best technology that is used to determine MS plaques or scarring at different parts of the CNS. MRI is also used to differentiate old lesions from the new or active. But MRI cannot solely be used as a diagnostic for MS as other disease condition have lesion in the CNS and also a few people, importantly older people, can have spots in their brain as seen in MS.

**Visual Evoked Potential (VEP):**

Evoked potential (EP) are the recordings of the nervous system’s electrical response to stimulus to specific sensory pathways. Damage to myelin will result in slow response; EPs can provide evidence of scarring of myelin along the nerve pathway. VEP is considered as most useful in diagnosing the disease.

**Cerebrospinal Fluid Analysis:**

Analysis of cerebrospinal fluids detects the levels of immune system proteins and the presence of oligoclonal bands that indicate the presence of immunoglobulins in CSF. These are found in about 90-95% of MS patients, but still CSF fluid analysis based on oligoclonal bands cannot be used as a reliable diagnostic as other disease condition also contain the oligoclonal bands.

**2.2.5 Current Treatments for MS**

MS does not have a cure. The better understanding of the disease pathology and the role of the immune system has led to various therapeutic advancements in the last 20 years. Patients with MS receive different disease treatment based on the different disease conditions.
Treating MS flares to restore baseline functioning in patients

Disease-modifying treatments to slow the progression of the disease

Treatment of symptoms of MS like optic neuritis, fatigue, pain, etc.

**Treating MS Flares:**

Flares or relapses are treated only when they are clinically evident of the disease activity such as loss of vision, muscle weakness, etc. The treatment aims to shorten the flare and restore normal functioning of the patient. Corticosteroids are the primary treatment of MS flares, as corticosteroids play an essential role in the metabolism of carbohydrates, proteins and fats and also other important biological functions such as regulation of inflammation. Studies have shown that corticosteroids shorten the length of MS flares with their anti-inflammatory and immunosuppressive effects. But there is no data suggesting these compounds reduce long-term disability or alter the course of the disease. (Iezzoni 2010)

Corticosteroids do not resolve acute flares in about 10% cases, where an intensive treatment called plasma exchange or plasmapheresis is recommended by physicians. Plasma exchange or plasmapheresis is the process of removing antibodies circulating in the blood with the assumption that these play a role in triggering the flare. There is contradictory evidence on whether plasma exchange can improve long term functioning in patients, but they might shorten acute flares. Treatment of MS flares has significant side effects and the potential side effects of plasma exchange are blood clotting problems and infections. (NINDS 2009) (Iezzoni 2010)

**Disease-modifying Treatments:**
In 1993 FDA approved the first anti-inflammatory medication Betaseron® that might slow the course of MS. The disease modifying medications are primarily used for treating relapsing-remitting sub-type, except for Betaseron® and Novantrone® that can be used for secondary progressive MS. Though there are currently seven marketed therapies although none of these work towards curing the disease. The currently marketed treatments aim to:

- Reduce relapse rates
- Prevent fixed disability directly associated to relapse
- Provide symptomatic management of fixed neurological deficits
- Prevent disability arising from disease progression
- Improve patient’s quality of life.

Below are the currently market therapies:

Table 2: Currently marketed MS therapies (National MS Society n.d.)

<table>
<thead>
<tr>
<th>Generic Name/Brand Name</th>
<th>Approval Year/ Mode of Administration</th>
<th>Mode of Action &amp; effectiveness</th>
<th>Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon beta-1b/ Betaseron®</td>
<td>1993/ subcutaneous injection alternative days.</td>
<td>Fewer flares, longer time between flares, no further increase in lesion area.</td>
<td>Flu-like symptoms, injection site reactions, other common symptoms.</td>
</tr>
<tr>
<td>Interferon beta-1a / Avonex®</td>
<td>1996/ Intramuscular Injection once a week</td>
<td>Reduced risk of disability progression, fewer flares, reduction in number of active lesions.</td>
<td>Flu-like symptoms, depression, poor concentration and other common symptoms, etc.</td>
</tr>
<tr>
<td>Glatiramer acetate / Copaxone®</td>
<td>1996 / subcutaneous injection everyday</td>
<td>Blocks myelin damaging T-cells / Reduction in annual relapses and lesions.</td>
<td>Injection site reactions, post-injection reaction, etc.</td>
</tr>
<tr>
<td>Drug</td>
<td>Year</td>
<td>Formulation</td>
<td>Mechanism of Action</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------</td>
<td>------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Mitoxantrone / Novantrone®</td>
<td>2000</td>
<td>Intravenous infusion 4 times a year; lifetime limit 8-12 doses</td>
<td>Inhibit the activity of T cells, B cells &amp; macrophages / slows MS disability, used as back-up for patients not responding to other disease modifying treatments</td>
</tr>
<tr>
<td>Interferon beta-1a/ Rebif®</td>
<td>2002</td>
<td>Subcutaneous injection 3 times a week</td>
<td>Lower relapse rate, higher proportion of relapse free patients, lower number of active lesions, delay in progression of diseases</td>
</tr>
<tr>
<td>Natalizumab/ Tysabri®</td>
<td>2006</td>
<td>Intravenous infusion every 4 weeks.</td>
<td>Monoclonal antibody to hamper immune damaging cells from blood stream/ reduced risk of disability progression, etc.</td>
</tr>
<tr>
<td>Fingolimod/ Gilenya®</td>
<td>2010</td>
<td>Oral capsule form</td>
<td>Prevents lymphocytes from entering the CNS/ reduced relapses, reduced risk of disability progression, reduced brain lesion.</td>
</tr>
</tbody>
</table>
3 Technology Platform

3.1 Technology Background

Demyelination is a pathological process in which the myelin sheaths around the axons are lost. In most cases of CNS demyelination the target is oligodendrocyte, and this type of demyelination is referred to as primary demyelination. Secondary demyelination is referred to when the myelin degenerates due to axonal loss. MS is due to primary demyelination by inflammatory damage to myelin and oligodendrocytes. There is no current knowledge on what exactly triggers demyelination in MS, thus there are two components to the treatment based on the current knowledge of the disease condition. The first is to prevent the damage from occurring and the second component is to repair the residual damage. Over the years various anti-inflammatory and immunomodulatory therapies have made progress towards accomplishing the former, there are no current therapies that work to repair the damage caused. (Zhao, et al. 2005) (Franklin and Ffrench-Constant 2008)

3.1.1 Remyelination

Remyelination is the process of regeneration of myelin sheaths around the demyelinated axons of the CNS (Fig – 11). Remyelination can restore conduction of nerve impulses that will eventually restore neural function and is increasingly believed to employ neuroprotective role to axons that will be used to protect neurons from degeneration as a result of a brain injury or as a result of a neurodegenerative disease. . Remyelination is a spontaneous regenerative process in the CNS that is a default response to demyelination. The reconstruction of the tissue by remyelination is complete except for one caveat in that the myelin sheath thickness and length developed by remyelination is less than the
original produced during developmental myelination. Remyelination forms a thinner and shorter myelin sheath than expected for a given diameter of axon (Fig -11). (Charlotte, Franklin and Zhao 2009) (Franklin and Ffrench-Constant 2008)

**Figure 11: Remyelination of axons** (Franklin and Ffrench-Constant 2008)

**a.** Following demyelination in the CNS, demyelinated axons has two possible fates, generation of thinner and smaller myelin sheaths through remyelination or degeneration of the axons leading to progressive decline. **b.** Transverse sections from adult rat cerebellar white matter under a light microscope showing myelinated axons of various
diameters in the left panel, demyelinated axons in the center panel and remyelinated axons by thin myelin sheaths four weeks after induced demyelination on the right panel.

3.1.2 Importance of Remyelination

Chronic demyelination predisposes axons to degeneration which is the major cause of progressive functional decline in MS patients. Axonal degeneration is an irreversible process, and genetic studies have demonstrated that myelinating cells play a role in axon survival. The requirement of myelin for axonal survival comes from observations of genetic mouse models where mice lacking the oligodendrocyte genes cnp, that encodes phosphodiesterase or Plp, that encodes a integral myelin protein show long term axonal degeneration in the presence of myelin sheath with no or subtle defects. The above observations were further supported by studies in humans with mutations in the Plp gene. Experimental studies have shown axons are protected from demyelination-mediated degeneration by remyelination and the beneficial properties of myelin could be trophic, protective, organizational or a combination of all. (Lappe-Siefke, et al. 2003) (Trapp and Nave 2008) (Charlotte, Franklin and Zhao 2009)

3.1.3 Remyelination Process

The remyelination process is mediated by the generation of additional mature oligodendrocytes. This is evident by the following observations (i) a greater number of oligodendrocytes were found in the area of remyelination than in an equivalent area that is not undergoing remyelination and (ii) remyelination is seen to be occurring in areas that have been experimentally depleted of oligodendrocytes. There has been a long debate on whether oligodendrocytes contribute to remyelination. In a lesion, death of oligodendrocytes is followed by repopulation of oligodendrocytes lineage cells, it is clear
that some other cell mediates it as oligodendrocyte neither can divide nor migrate. (Prayoonwiwat and Rodriguez 1993) (Zhao, et al. 2005) (Charlotte, Franklin and Zhao 2009)

The current consensus is that remyelination is mediated by the new oligodendrocytes derived from a population of precursor cells referred to as oligodendrocyte precursor/progenitor cells (OPCs). These cells are identified using markers of which NG2 and PDGFRα are the most frequently used. Other markers like O4, oligo 1 and oligo 2 are also used, but they require later-expressed markers to differentiate different stages as they expressed throughout the oligodendrocyte lineage. Studies performed showed that when oligodendrocytes are transplanted into experimental models of demyelination in comparison to transplantation of OPCs do not myelinate. The other supporting study performed showed that when demyelination was induced by galactocerebroside antibody where many oligodendrocytes cells survived and OPCs were removed from the white matter by x-irradiation, there was no remyelination seen within the lesion. (Keirstead and Blakemore 1997) (Crang, Gilson and Blakemore 1998) (Franklin and Ffrench-Constant 2008) (Charlotte, Franklin and Zhao 2009)

The overall process of remyelination is characterized by three different steps, namely activation, recruitment and differentiation (Fig -12). In response to demyelination, local OPCs undergo a switch from quiescent to an active state and proliferate and migrate to rapidly fill in the demyelinated area of the CNS. The activation phase not only involves change in morphology but also up regulates several genes that may be associated with the generation of oligodendrocytes during development- for example that encode transcription factors OLIG2, SOX2, Nkx2.2 and Myt1. OPCs are activated by injury-
induced changes in the microglia and not necessarily due to primary demyelination. (Franklin and Ffrench-Constant 2008) (Charlotte, Franklin and Zhao 2009)

Figure 12: The phases of remyelination (Franklin and Ffrench-Constant 2008)

The activation phase is followed by the recruitment phase where the activated OPCs proliferate rapidly and migrate at the site of demyelination. Though the phenomenon is known for years there is no clear idea on what environmental mitogens are associated with demyelination that promote the recruitment phase. Based on studies it is hypothesized that growth factors PDGF and FGF-2 promote the recruitment phase. PDGF is a neonatal OPC mitogen and survival factor responsible for regulating the number of OPCs in developing white matter and has also seen to have increased level of expression following demyelination. FGF-2 is also developmental OPC mitogen with increased
expression following demyelination. (Zhao, et al. 2005) (Charlotte, Franklin and Zhao 2009)

After the OPCs have migrated to the site of demyelination they must differentiate into remyelinating oligodendrocytes. The process of differentiation also includes three distinct steps: establish contact with the axon to be remyelinated, generate the myelin sheath and wrapping the compact myelin around the axon. Though being the basic capability of oligodendrocytes it is still not clear on how the axo-glial contact is established, how the interaction regulates in individual cell process, morphological changes that constitute the myelin. With the above said, growth factors were among the first to be studied and have shown to contribute to the regulation of differentiation. FGP for example plays a key role in inhibiting differentiation, retaining the recruitment stage until the conditions are amenable to differentiation, thus regulating the transition from the recruitment phase to differentiation phase. In order to identify various regenerative factors involved with remyelination, various studies were performed that provided evidence for a role of inflammatory response to demyelination. Though the relation between inflammation and regeneration is recognized in many other tissues, it is obscure with myelin regeneration. (Franklin and Ffrench-Constant 2008) (Charlotte, Franklin and Zhao 2009)

Macrophages have shown to play an important role in the removal of myelin debris that has been generated during demyelination. The removal of myelin debris is critical as a study showed that myelin contains proteins that inhibitory to remyelination as it prevents OPCs from differentiating into myelinating oligodendrocytes. Observations showed that activation of macrophage enhances myelination, as yet undefined regenerative factors by macrophages. (Kotter, et al. 2006) (Setzu, et al. 2006)
3.1.4 Causes of Remyelination Failure

Studies have shown that spontaneous remyelination eventually fails leaving the axons and in cases the entire neuron vulnerable to degeneration. There is no clear data that suggests the reason for remyelination failure. Certain observations showing the presence of quiescent OPCs in chronic MS lesions suggest that the failure of differentiation of OPCs into myelinating oligodendrocyte leads to failure of remyelination. (Franklin and Ffrench-Constant 2008) (Albert, et al. 2007)

Non-disease related factors:

There are various studies that have shown non-disease related factors like age, sex and genetic background to have an effect on the efficiency of remyelination. Remyelination efficiency like all other regenerative processes decreases with age. Studies have shown that age associated effects of remyelination is due to decrease in efficiency of both recruitment and differentiation phase of oligodendrocyte. Within aged adults females have shown to have better remyelination efficiency than compared to males, but not the same with young adults. Another consensus based on a study is the impaired response of macrophage with aging leading to poor clearance of debris; therefore inhibiting remyelination due to the presence of differentiation-inhibitory myelin proteins. (Franklin and Ffrench-Constant 2008)

Disease related Factors:

Adding to the non-disease related factors there could be some disease-specific reason for the failure of remyelination. If understood theoretically the reason for remyelination failure could be due to deficiency of OPCs, failure of the recruitment or failure of differentiation of OPCs. Experimental studies have shown that OPCs are efficient to
repopulate the areas that have been depleted, and even in cases where the same areas was
demyelinated repeatedly, it was never depleted of OPCs nor did prevent subsequent
remyelination. But in cases of sustained demyelination to a tissue it is seen that part of
the impairment of remyelination is due to the OPC deficiency. The second mechanism for
remyelination failure is due to OPC recruitment involving proliferation, migration and
repopulating the areas of remyelination. Studies show that only a proportion of lesions
account for failure of recruitment phase with studies showing the reason or the same
being disturbance of OPC migration. Other study suggest that in cases OPCs have to be
recruited from surrounding intact tissue and larger lesions require greater OPC
recruitment and with aging older OPCs are less responsive to recruitment signals.
(Franklin and Ffrench-Constant 2008)

Currently the best evidence supporting the failure of remyelination is the failure of
differentiation and maturation of OPCs. The presence of OPCs that fail to differentiate in
MS lesions was shown initially by oligodendrocyte-lineage marker O4 and then shown
by OPC marker NG2 and also PLP that shows premyelinating oligodendrocytes. There
are a few explanations on the failure of differentiation in the demyelinated lesions like the
presence of inhibitory factors of precursor differentiation like Notch and accumulation of
glycosaminoglycan hyaluronan. (Franklin and Ffrench-Constant 2008)

The other hypothesis is the possibility of either presence of negative factors or absence of
positive factors based on the studies showing both environmental factors and intrinsic
factors guide the stages of remyelination. Efficient remyelination depends on the timing
of action based on the presence and absence of the negative or positive factors
respectively. The above was articulated as the dysregulation hypothesis where the failure
of remyelination due to inappropriate sequence of events (Fig – 13). Though the failure of remyelination for a varied disease like MS is most likely to be multiple factors this hypothesis is useful for the understanding remyelination failure in most cases. (R. J. Franklin 2002) (Franklin and Ffrench-Constant 2008)

![Figure 13: Schematic representation of dysregulation hypothesis of remyelination failure](image)

**Figure 13: Schematic representation of dysregulation hypothesis of remyelination failure** (Franklin and Ffrench-Constant 2008)

### 3.2 RNI’s Approach

MS is an inflammatory disease of the CNS where after 5-15 years majority of the patients enter into a progressive phase where continuous and irreversible neurological decline is observed. The current anti-inflammatory or immunomodulatory therapies aim to reduce the white matter lesions and delay the relapse of MS delaying only the progression of the disease. The most well documented neuroprotective phenomenon in the MS brain is
remyelination as myelin restores conduction of nerve impulses and provides trophic support for axon survival. RNI’s approach is to develop a complementary treatment that will combine anti-inflammatory therapy and remyelination therapy to reduce neurological decline and restore neural function and potentially cure MS. The challenge that was seen was to bridge the gap between regenerative biology of oligodendrocytes to regenerative medicine. (Trapp, Nishiyama, et al. 1997) (Trapp and Nave 2008)

Oligodendrocytes are responsible for remyelinating axons post damage in the CNS. Studies have suggested that spontaneous remyelination eventually fails in chronic MS lesions. Studies identified NG2-positive stellate-shaped elongated OPCs and newly differentiated oligodendrocytes in both adult human brain and MS lesions. The above study provides two significant aspects, first evidence that OPCs are present in many chronic MS lesions and remyelination is not limited due to the absence of OPCs. Second it suggests that remyelination is often limited at the stage of OPC differentiation and thus molecules regulating OPC differentiation and remyelination represent therapeutic targets to promote remyelination. (Chang, et al. 2002) (Albert, et al. 2007)

OPCs differentiate in a sequential manner from the regions of the SVZ, and then migrate from the SVZ as bipolar/multipolar cells and are highly mitotic. They colonize the CNS and form a network of stellate-shaped cells and differentiate into premyelinating oligodendrocyte. After the completion of myelination there is a sizable population of undifferentiated OPCs that remain in the adult CNS. It is estimated that OPCs make 2-9% of the CNS cell population and recent fate-mapping studies have shown that adult OPCs are capable of remyelinating throughout their life. Mammalian CNS has limited capacity to regenerate post injury and partly due to inhibitory signals. Successful remyelination
not only requires generation of oligodendrocytes from OPCs but also requires overcoming the inhibitory signals of remyelination. (Dawson, et al. 2003) (Dimou, et al. 2008)

RNI’s Hypothesis:
RNI’s approach is to use small compounds to therapeutically stimulate OPC differentiation eventually enhancing remyelination to restore neural function in MS patients. Two technological advances lead to the identification and evaluation of such molecules; (i) an optimized method that can produce highly pure population of OPCs from mouse that will be used as the starting material for the screening, (ii) a high-content cell based screening system that can score differentiated oligodendrocytes in a relatively high-throughput manner. With the above technological advances a compound library of 14,000 compounds was screened to find potential hits that can stimulate oligodendrocyte differentiation and promote remyelination. The chart below depicts the process for the primary screening of the compounds leading to the initial hits.

![Figure 14: High content screening process](image-url)

(Courtesy – Renovo Neural Inc.)
Based on the primary screening of compounds RNI hypothesizes that compounds that promote oligodendrocyte differentiation in vitro will potentially promote remyelination in mammalian CNS. Data from Dr. Trapp’s lab showed that when these compounds were tested for oligodendrocyte differentiation they showed multi-fold increase in remyelination than the control. Below is the dose-response curve that was obtained by EC-50 studies conducted on one of RNI’s lead candidates. From the below curve it is evident that there is multi-fold increase in remyelination potential compared to control.

![Standard Curve](image)

**Figure 15: Dose-response curve of lead X** (Courtesy – Renovo neural Inc.)

Based on knowledge of previous studies and RNI’s primary screening, RNI hypothesis is to therapeutically stimulate OPC differentiation into myelinating oligodendrocytes that will remyelinated the demyelinated axons restoring neural function and potentially cure MS.
4 Commercialization Plan

4.1 Development Plan

RNI will develop remyelination therapeutics that will be a complementary treatment to the existing anti-inflammatory therapeutics. Below is the analytical framework for RNI’s development process.

![Analytical framework of development process](image)

**Figure 16: Analytical framework of development process**

4.1.1 Drug Discovery Phase

**Determine Target Disease & Develop Hypothesis for Treatment:**

RNI’s target disease is MS and based on studies it is known that remyelination fails due to failure in oligodendrocyte differentiation. The approach to be taken by RNI is to
therapeutically stimulate oligodendrocyte differentiation using small molecules that will eventually enhance remyelination in MS lesions.

**Screen Compound Libraries for Leads:**

The high throughput screening system was applied to compounds from small molecule libraries to obtain leads that show therapeutic potential to stimulate oligodendrocyte differentiation. RNI has a competitive edge as it already has an exclusive license to 13 compounds, which has generated a total of 31 leads including analogs and derivatives, obtained from the initial screening of 14,000 compounds. These lead compounds have proved to be potent during the primary *in vitro* screening in the laboratory of Drs. Trapp & Macklin at the Cleveland Clinic. Below is the list of the compounds identified in the patent application:

Primary compounds – 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 19, 13

Analogs of 7 – 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 7.10, 7.11, 7.12

Derivatives of 7 – CO1, CO2, CO3, CO4, CO5, CO6, CO7

Analogs of 19 – 19.2, 19.3

Analogs of 13 – 13.1, 13.2, 13.4, 13.5, 13.6

**Hit to Lead:**

RNI currently is currently positioned in the hit to lead phase of the development process. The hit to lead is a significant phase in the drug development process as the lead compound determines the success of the drug at the clinical stage. RNI has designed its studies to obtain the best lead from the list of licensed compounds that will be developed further. RNI will perform secondary screening for the above hits with improved and
efficient high throughput screening system that provides reliable and unequivocal evidence of remyelination potential. The secondary screening will help RNI to narrow the identified compounds down to the best 2-3 hits from the initial set of 31 hits. The next phase will be the EC-50 studies to be conducted on the three best hits from the primary screening and the resulting dose-response curve will further validate the potency of the hits. Based on the results of secondary screening RNI will obtain its best lead for further development and design lead optimization studies.

**Lead Optimization:**

Lead optimization is considered the decisive phase in drug discovery as the objective is to generate a preclinical drug candidate. In the early lead optimization stage, RNI will conduct the below studies to evaluate if lead candidate is druggable in its present form before designing and screening analogs and derivatives:

- In silico evaluation to predict tumorigenicity, mutagenicity & phototoxicity.
- Toxicokinetics & pharmacokinetic studies in mouse.
- Short toxicology studies in mice at different dose concentrations.

The results from the initial studies will determine whether these drugs can be developed further or derivatives and analogs should be designed to make it druggable. These studies will be completed within few weeks and RNI will design analogs and derivatives based on the results.

If required RNI will design analogs and derivatives to refine its lead compound to make it druggable. The designed analogs and derivatives will be screened as described earlier and the data obtained will be used to establish structure activity relationships (SAR). Based
on the results and SAR analysis the lead molecule will be refined to optimize its pharmacological properties and druglikeness. The lead candidate will be refined until a druggable candidate is generated that will be effective and safe when administered to humans. The lead optimization step will be the final stage of the drug discovery phase and the developed lead candidate will enter pre-clinical animal studies.

4.1.2 Preclinical Testing

RNI will conduct preclinical trials with the primary goal to evaluate safety and efficacy profile of the lead compound developed. During these studies the results will be analyzed to generate the best drug formulation, determine the route of delivery, dosage & duration of exposure, safety and efficacy of the drug. The pre-clinical study process for RNI’s remyelination drug will have five major steps before it can enter clinical trials:

- Efficacy Studies
- Pharmacology Studies
- Toxicology Studies
- Drug Formulation

Efficacy Studies:

In vivo studies will be designed in animal MS models to determine the efficacy of the drug candidate in detail. MS cuprizone mouse models will be used for studies and analysis to determine if the compound will have the desired therapeutic effect. The studies will also determine the threshold concentration and also concentration at which maximum therapeutic effect is seen.
Pharmacology Studies:

RNI will conduct pharmacology studies to understand the safety and efficacy of the lead candidate, where pharmacokinetic and pharmacodynamic studies will be performed and analyzed to assess the interactions between the animal models and the compound tested. Animal pharmacodynamic studies will be performed to understand the physiological and biochemical effects of the drug on the body. These studies will be performed to understand the interaction of RNI’s lead compound with proteins, cells, etc. Studies performed will evaluate the drug action that is expected to be stimulating the differentiation of OPCs. RNI also will evaluate if the compounds have the potential to inhibit factors deterring differentiation. The results will analyze the peak concentration, plasma concentration for the desired level of response. These will determine the therapeutic window which is an estimate of the dosage that can effectively treat the disease.

Pharmacokinetic studies will be performed to assess the fate of the compound once administered into the body. ADME (Adsorption, distribution, metabolism and Excretion) studies will be conducted to understand the effect of the drug level and kinetics of the drug that influence the pharmacological activity of the drug. RNI will build specific ADME animal models for MS remyelination. RNI will determine that the test compound does not contain any impurities that will affect the ADME studies. Based on the in vitro dose-response studies, RNI will determine the best concentration of its lead and will be used to conduct the whole animal studies. The results of the study will determine the gastrointestinal absorption and the overall elimination of the compound. RNI will also conduct intravenous dosing to determine the amount of chemical absorbed as the material
excreted is composed of both the unabsorbed as well as the eliminated chemicals. The intravenous study will provide the rate of metabolism, elimination and also eliminate the variability associated with the oral absorption. The data obtained from the pharmacokinetic studies will be useful in designing the toxicity studies, setting the dose levels, and also determining the mechanisms of toxicity. RNI will conduct pharmacokinetic studies with both oral and intravenous dosage of compounds at three different concentrations. Each concentration and each mode of administration will be done in both male and female, five each.

**Toxicology Studies:**

Based on the pharmacokinetic studies data like concentration-time curve, steady state concentration of the substance in blood or plasma, distribution, etc RNI will design its toxicology studies. RNI will conduct both *in vivo* and *in vitro* toxicology studies. RNI carries the expertise and infrastructure to conduct the toxicology studies in house. RNI will conduct two levels of toxicology studies. The first will be acute toxicology studies, where the maximum tolerable dose (MTD) and no observable adverse effect level (TOEL) will be determined. These acute studies will be performed at three different concentrations based on the pharmacokinetic studies and also the short toxicology studies in both male and female mice.

The other set of toxicology studies that RNI will conduct are the sub-acute studies, to determine the toxicity after repeated dosage of the drug. The sub-acute studies will be conducted at different concentrations with 10 mice models of each male and female to understand the toxic effects of the compound with both. As MS occur in highly young adults, higher in female than males RNI plans to conduct fertility and developmental
studies in both male and female mice. RNI will also conduct carcinogenicity trials to study of there are any tumor developments in animal models. RNI will conduct further toxicology studies on particular tissues in the body based on the data obtained from the pharmacokinetic studies. If the pharmacokinetic studies show that the substance accumulates in the bone marrow, long term studies will be performed to test the effect of the compound on hematopoietic function. The toxicology studies will take 12-18 months to be completed and these results will initiate the IND process and designing of the clinical trials.

**Drug Formulation**

It is critical for RNI to scale-up and formulate its compounds into possibly an oral medicinal drug, as it an easier route of administration for patients. RNI at this point does not carry the expertise to scale-up its compound into a drug and thus will hire a CRO with the best expertise in the drug formulation process. The scale-up process will be conducted simultaneously with the other preclinical studies.

**Potential Pitfall:** The potential pitfall at this stage would be that the compound cannot be developed as an oral drug, but will be an injectable medication. Based on the MS therapeutic industry, it is evident that injectable medications have been accepted by patients and physicians.

**4.1.3 Investigational New Drug (IND)**

On completion of its pre-clinical trials RNI will apply for an IND to get approval from FDA to initiate phase I clinical trials. RNI will hire a consultant who will help put together the IND application according to the specific guidelines and requirements set by
the FDA. RNI will apply for a sponsor-initiated IND that will primarily contain the following:

- Animal pharmacology & toxicology data showing evidence for the product to be safe for initial testing in humans.
- Information on chemical composition, formulation, manufacturing methods & stability of the drug.
- Detailed protocol of the proposed clinical studies showing safety for subjects under trials, capability of conducting the trials and adhere to the IND regulations.

The development timeline for RNI up to IND will be typically shorter than what is seen in the industry. This is because RNI already has pre-evaluated hits and stands in the hit to lead stage. Over this RNI also has a developed infrastructure for pre-clinical development of remyelination therapeutics. Below is a gantt chart that will depict the timelines for each study in the preclinical phase:

![Figure 17: Gantt chart for development timelines](image-url)
4.1.4 Regulatory Compliance:

One of the critical aspects while conducting preclinical studies is to comply with the regulations set by FDA to obtain IND approval. RNI will regularly monitor its studies and determine that everything is done following the guidelines. These guidelines have been followed by RNI since its discovery phase and will be followed through the development process. There are two major systems set-up by the FDA to regulate clinical trials and drug development.

**Good Manufacturing Practices (GMP):**

GMP is the quality system that regulates the manufacturing and testing of the pharmaceutical compounds used in the drug developed. RNI will comply with all GMP guidelines and will work towards improving its efficiency and effectiveness with better manufacturing practices.

**Good Laboratory practices (GLP):**

GLP is a system to control the laboratories and research organizations in the preclinical to ensure reliable results. RNI will design its preclinical studies based on the guidelines made to plan, perform, observe, record and archive the data obtained for each experiment.

4.2 Industry, Market and Competition

4.2.1 Introduction of the industry: Therapeutics for MS

The MS therapeutic market is entering a new phase of growth with development and innovation of new therapeutic platforms. The existing beta-interferon based drugs along with copaxone and tysabri are the currently marketed therapies. The existing therapies
have created a patient base in the market for MS but the market has a high degree of unmet medical need. The high market potential and the unmet need have gained significant interest from big pharma/biotech entities to enter and expand their presence within the MS market place.

4.2.2 Industry Analysis:
Despite several marketed disease modifying drugs and major advances over the last 15 years MS still represents a medical challenge without a cure. The current treatments available slow the progression of the disease, manage symptoms and of best improve the patient’s quality of life. Beyond the loss of freedom, mobility, etc, MS has a major social and economic impact on the life of the patient. MS has been a major economic burden, with data from 15 countries showing that the average lifetime cost per MS patient is around $1.2 million. (Brandt 2010)

The factors driving further growth and development on the MS therapeutic industry are:

- A major unmet need for a definite cure.
- Introduction of more effective and less invasive therapies.
- The socio-economic factors coupled with MS.
- Biosimilars entering into the anti-inflammatory market.

4.2.3 Market Analysis:
In 2009, MS therapies generated revenues over $7 billion across the seven major markets around the globe. Over the years the MS market has been stable with consistent growth with limited competition with just four active participants and an unmet medical need. Currently, the MS therapeutic market is in the transition stage entering an exciting phase
that is likely to benefit the patients with easier mode of administration and standard of care. With the launch of Gilinea an oral therapy from Novartis and seven other drugs in the late stage of development, the market is expected to change significantly as these drugs address improved efficacy and customer compliance. Although there are several advances over the years, curing MS still remains a challenge to the medical community. (Huyng 2010)

The major players in the market currently are Avonex (Biogen Idec), Betaseron (Bayer Schering Pharma), Rebif (EMD Serono), Copaxone (Teva Pharmaceuticals), Tysabri (Biogen Idec) and Novantrone (EMD Serono). Currently the market is concentrated by Biogen, Teva and EMD Serono with approximately 90% of revenues of the market.

4.2.4 Market Growth:

The global market has been driven over the years by the performance of the four major drugs Avonex, Betaseron, copaxone and Rebif (ABCR). Over the few years due to limited competition there has been a steady increase in the cost of treatment. With the launch of new products with improved efficacy and customer compliance, the market trend is to change to a competitive environment for the MS therapeutic industry. With revenues crossing over $ 7 billion across the globe in 2010 and with the introduction of various new pipeline drugs and biosimilars the market is expected to grow and bring in
revenues over $9 billion by 2014 and looks steady until 2019. (Huyng 2010)

![Multiple sclerosis market composition](image)

**Figure 18: Multiple sclerosis market composition (Huyng 2010)**

The United States has been the world’s largest market for MS therapeutics with ABCR being the frontline treatment option through the years. The market growth in the US has been double fold due to the high unmet medical need. In 2006 US generated revenues of approximately $3,241.9 million and are expected to grow to $7,314.8 million by 2013 with a CAGR of 12.3%. The introduction of new oral therapies is expected to drive the market growth further. Within the US ABCR are expected to grow at 8.3% whereas new therapies are expected to grow at a CAGR of 66.8%. (U.S. Multiple Sclerosis Market 2007)

### 4.2.5 Customer Profiles:

MS affects more than 2.5 million people worldwide with around approximately 400,000 patients within the US. The distribution of MS has been very uneven, with the northern
part of the world i.e., North America, Russia, Europe etc having high prevalence rate than the southern parts like Africa and Asia.

The age group with the highest prevalence rate has been estimated to be between the age groups of 29 – 45 making it the most common neurological disability in young adults (MS international federation, 2010). The prevalence rate for women is higher than that of men with a ratio of 2:1 respectively (MS international federation, 2010).

The annual cost per patient is predicted to be growing with a CAGR of 5.9% since 2003 from $15,062 to $28,536 by 2013 (Frost & Sullivan, 2007).

Figure 19: Average annual cost per patient forecast (U.S. Multiple Sclerosis Market 2007)

4.3 RNI’s Market Position

4.3.1 Competitive Advantage:

The current MS therapeutics in the market has huge numbers in revenues though they do not play a considerable role towards curing the disease. All current therapies are focused
on delaying the disease course, but none work towards restoring neural function. Currently the money pumped into research is more focused on developing anti-inflammatory drugs with a better mode of administration than working towards finding a cure for the disease. Remyelination being the next frontier and RNI being among the first to develop remyelination therapies is the major competitive edge over the existing market players. Below are the competitive advantages over the existing and future market players:

- Proprietary pre-screened candidates for further development.
- Infrastructure for in-house pre-clinical studies.
- Highly trained research team in the field of remyelination.
- Working under the guidance of credible names in the field of neurosciences.

4.3.2 Intellectual Property

RNI has exclusive rights to compounds and methods promoting oligodendrocyte precursor cell differentiation covered by PCT application PCT/US09/63720.

4.3.3 Value Proposition:

RNI’s value proposition from a market stand point is the fact that no current marketed therapeutics work towards restoring neural function lost during MS. RNI is creating itself a blue ocean in the existing red ocean of anti-inflammatory therapies. From a technology stand point RNI is among the first movers in the development of remyelination therapeutics for MS. RNI already has a set of tested compounds and currently stands in the hit to lead phase of development saving years on timelines and millions on cost for drug discovery.
4.4 Financial Plan

4.4.1 Development Cost

Table 3: Development cost

<table>
<thead>
<tr>
<th>Development Step</th>
<th>Cost</th>
<th>Timeline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Discovery Phase</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hit to Lead</td>
<td>$500,000</td>
<td>3-6 months</td>
</tr>
<tr>
<td><strong>Lead Optimization</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In silico Evaluation</td>
<td>$50,000</td>
<td>1 month</td>
</tr>
<tr>
<td>Toxiokinetic &amp; pharmacokinetic studies</td>
<td>$100,000</td>
<td>2-4 Weeks</td>
</tr>
<tr>
<td>Short Toxicology Studies</td>
<td>$100,000</td>
<td>2-4 Weeks</td>
</tr>
<tr>
<td>Data Analysis</td>
<td>$75,000</td>
<td>1 month</td>
</tr>
<tr>
<td>Derivative &amp; Analog Designing</td>
<td>$400,000</td>
<td>8-12 months</td>
</tr>
<tr>
<td>Lead Optimization Consultant</td>
<td>$150,000</td>
<td></td>
</tr>
<tr>
<td><strong>Lead Optimization Phase Total</strong></td>
<td>$875,000.00</td>
<td>12-15 months</td>
</tr>
<tr>
<td><strong>Pre-clinical Testing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Efficacy Studies</td>
<td>$450,000</td>
<td>3-6 months</td>
</tr>
<tr>
<td>Pharmacology Studies</td>
<td>$450,000</td>
<td>3-6 months</td>
</tr>
<tr>
<td>Toxicology Studies</td>
<td>$750,000</td>
<td>12 months</td>
</tr>
<tr>
<td>Drug Formulation</td>
<td>$500,000</td>
<td>12-18 months</td>
</tr>
<tr>
<td>IND</td>
<td>$150,000</td>
<td>1-3 months</td>
</tr>
<tr>
<td><strong>Pre-clinical Testing Total</strong></td>
<td>$2,300,000</td>
<td>18 - 24 months</td>
</tr>
</tbody>
</table>

Total Cost out of Pocket: $3,675,000
In direct Costs (@ 40%): $1,470,000

Total Cost: $5,145,000

4.4.2 Sources of Funds

RNI will solicit grants and also seek angel investments to fuel its compound development and obtain IND approval.
Grants:
RNI will actively look into grant opportunities as they are a significant source of non-diluting funds for start-up companies. RNI will solicit NIH small business innovation research (SBIR) phase I grant to develop its best leads for MS through hit to lead stage of development. Post the phase I, RNI will solicit a SBIR phase II grant to develop its lead compound through the pre-clinical trials and obtain an IND. RNI will also actively look into grant opportunities from other sources like National MS Society, Ohio Third Frontier, etc.

Private Equity:
RNI will run its development through soliciting grants at various institutions, but will also look for private funding from angel investors. The primary reason to look for private funding would be to speed up the development process as grants can potentially delay the development process significantly.

Uses of Funds:
The grant funds that will be obtained will be used to develop the compounds that promote remyelination in MS patients. Being a drug development company RNI will always need cash for ready execution to pace the development. In the initial 3-4 years of development there would be continuous out-flow of cash for various studies and development of the compounds.
5 Exit Strategy

The recommended exit strategy for RNI’s remyelination MS therapeutic drug development project will be to exit post IND. RNI’s exit strategy post IND approval should be to license-out the developed lead compound to a giant pharma/biotech entity. RNI post IND exit recommendation are based of the following reasons (i) RNI is a start-up and does not carry the expertise and financial resources to conduct clinical trials (ii) RNI’s long term focus is to develop therapeutics for other neural diseases and will focus on utilizing its expertise in developing new therapeutics and, (iii) being an hypothesis there is no clear data that suggests the efficiency of remyelination of these compounds on adult MS lesions. Keeping in considerations the above factors RNI will plan to exit post IND and focus on its goal to develop therapeutics for other neural diseases.

Being one of the first movers towards developing remyelination therapeutics with a huge unmet need in the MS therapeutic industry, it will attract big pharma companies towards the project. RNI will look for strategic buyer’s that are existing players in the MS industry to acquire the developed compound with IND approval and move to clinical trials. The current market players in the MS industry have been keenly looking into developing remyelination therapeutics, and would certainly be interested in acquiring developed compounds to save years on research & development cost and timelines. Based on recent mergers & acquisitions in the healthcare industry and comparable RNI expects the developed compound for MS to be sold for 80-100 million dollars and additional milestone payments.
6 Bibliography


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