Canurta

ALS and Cannabis

Prepared By: Inayah Rehman, Healthcare Researcher
University of Toronto, HBSc.
Date: August 25, 2023

Intro

Many know Amyotrophic Lateral Sclerosis (ALS) by the viral "Ice Bucket Challenge" that took the world by storm in 2014, nearly a decade ago. Sports fans may recognize ALS as the condition Henry Louis "Lou" Gehrig experienced, causing the baseball legend to retire early; ALS can be transmitted through soil, dust, and dirt, leading to an increase in ALS presentation in outdoor athletes[1]. However, ALS is far more than a trend or a sports headline - it is a complex condition that afflicts 4-8 in 100,000 people worldwide with debilitating and life-threatening manifestations[2]. ALS research has increased steadily over the past two decades, averaging more than 6% annual growth in research publishings[3]. Despite this hopeful upswing, substantial breakthroughs have been rare and there remain many unanswered questions[4]. ALS continues to baffle the world with complex disease pathways and limited therapeutic options.

The neurodegenerative condition is classified by the degradation of upper and lower motor neurons of the central nervous system (CNS), primarily resulting in symptoms such as muscle shaking, cramping, and weakness, starting in the limbs and then affecting secondary muscles such as those of the digestive and respiratory systems[5]. Respiratory failure has been

cited as the most common cause of ALS-related death[5-6]. The rate of disease progression can differ greatly among patients; as such, survival rates vary from less than 5 years (for 80% of the population) to over 10 years (for up to 20% of the population)[5,7]. The condition also affects quality of life through symptoms not related to motility, such as cognitive defects and mood disorders including anxiety and depression in up to 50% of patients[8].

ALS is generally categorized into familial ALS, where multiple members of a family are affected, and sporadic ALS, which entails no known hereditary component and makes up over 90% of the population[9]. ALS is known to be highly heterogeneous in origin, a term that refers to the vast number of unique mutations that produce ALS symptoms. More than 20 genes have been recognized as possible genes of origin thus far, with the most common mutation amongst patients being in the 72nd open reading frame of chromosome 9 (C9orf72)[10]. This gene was concurrently found to be significantly implicated in the onset of frontotemporal dementia and various other neurodegenerative conditions[11]. Even then, this gene combined with the next four most common genetic variants [or neurofilaments] in ALS, only accounts for a portion of overall cases[5].

The population cohort most commonly afflicted with ALS is individuals aged 40-70, with a male to female ratio of 1.2:1[12]. Given Canada's ageing population, it can be extrapolated that the incidence rate of neurodegenerative conditions will increase. In fact, experts estimate that

global incidence rates will more than double over the next 17 years[13]. As such, it is more important than ever to explore potential therapies ahead of the surge of new patients in upcoming years. Currently, the most common legally approved medicine in Canada and the European Union for ALS is riluzole, which is an orally administered glutamate antagonist - this classification indicates that the drug inhibits glutamate receptors[14]. Glutamate is the main excitatory neurotransmitter in the central nervous system and is used in communication between neurons in the propagation of nerve impulses[15]. Though exact disease mechanisms are unknown, it has been found that glutamate levels are increased in ALS and that this may lead to excitotoxicity (high levels of calcium influx resulting in neuronal damage and death)[16]. Riluzole acts to inhibit the release of glutamic acid, the molecule that glutamate is derived from via cleavage of a hydrogen atom[17]. Despite the proven effects of this medication, Riluzole only exhibits a significant effect in the final stage of the condition and only extends survival by a matter of months[18]. Adverse side effects are also reported, including an abnormal increase in liver enzyme concentration, nausea, vomiting, weakness, and balance issues[14]. Aside from Riluzole, symptom management is the primary other medicated route, including options such as invasive ventilation for breathing difficulties, wheelchairs and physical therapy for mobility issues, and gastrostomies or nutritional supplements for digestive difficulties[4]. Many

of these options are invasive and do not conserve quality of life. As such, the market is in dire need of novel therapies.

As of mid 2023, Albrioza (sodium phenylbutyrate and ursodoxicoltaurine) was approved as a therapy by Health Canada due to the compound's reduction in protein misfolding, oxidative/endoplasmic reticulum stress, and inflammation among other functions, thereby reducing neuronal apoptosis[19]. Masitinib was also approved around this time as an add-on therapy to riluzole, targeting immune cells (macrophages, mast cells, and microglia) by inhibiting the activity of tyrosine kinase enzymes but resulting in adverse effects in up to 85% of patients[20]. Lastly, edavarone was approved in late 2022, reducing the effects of oxidative stress and slowing motor neuron degeneration but not reliably altering disease progression in the short-term and not significantly affecting patient outcomes in the long-term[21]. These therapies are newly approved and await more reliable evidence of significant progress, though they are available to all provinces[22].

Canurta's ALS Therapy

Canurta offers a unique approach to ALS treatment by pioneering a holistic approach in their product design. The proposed therapeutic is formulated with multiple compounds that each offer unique advantages both to symptom management as well as targeting specific disease mechanisms. The strongest advantage of this therapy is that it offers a

nonspecific approach where others have targeted individual mechanisms/pathways. It is possible that the lack of current effective treatment options is due to their specificity while the condition itself offers a high degree of heterogeneity[23]. As such, a multifactorial product would offer a strategic method of targeting a range of symptoms and differing disease progression. Additionally, unlike other emerging hemp-based pharmaceuticals such as those in the United Kingdom, of which 40% were found not to contain their advertised amount of cannabinoids and an additional 20% contained inactive compounds, Canurta's patented harvesting strategy ensures that molecular yield is as high and potent as possible[24-25]. Canurta prioritizes honesty and integrity in its pursuit of non-toxic and effective pharmaceuticals rooted in botanical compounds.

Canurta's product is a combination of cannabinoids, terpenes, and the novel flavonoid Cannflavin A. Benefits of each are listed in Table 1.0. Cannabis itself has also been proven to help alleviate the most common symptoms such as fatigue (experienced by 90% of patients), muscle spasms (84% of patients), cramping (74%), difficulty sleeping (60%) and pain (59%)[26]. Case studies of 6-month usage of cannabinoid-based medical products have resulted in a dramatic decrease in pain and inflammation, and improved quality of sleep, and comprehensive reviews conclude that cannabinoids alleviate muscle spasticity, nausea, vomiting, and chronic pain[27-28].

Table 1.0 Benefits of each compound in Canurta's ALS multifactorial product[29].

Compound Class	Benefits
Cannabinoids	Inhibits protein misfolding, improves muscle spasticity and spasms, alleviates pain, bronchodilation, improves sleep, decreases weakness. Anti-inflammatory properties, protects nerve cells from damage, reduces vomiting. Muscle relaxation, reduces anxiety, protects nerve cells from damage, improves weakness. Immunomodulation, gene transcription modulation. Decreases anxiety and nausea.
Terpenes	Anti-inflammatory, reduces pain, decreases glutamate induced excitotoxicity. Decreases damage from oxidative stress. Improves depression. Improves anxiety, decreases hyperactive glutamatergic signaling.
Cannflavin A	Anti-inflammatory, promotes longevity

Biomarkers

Due to its heterogeneity in a number of disease variables including age of onset, survival rates, and etiology, ALS has proven to be difficult to study[23]. In order to streamline study design, it is important to narrow down an

efficient method of tracking disease onset and progression. Though multiple different avenues have been explored thus far, some are better studied than others and each has a unique list of benefits and drawbacks.

Neurofilaments, which are structural proteins found primarily in the axonal region of neurons, are regularly released into cerebrospinal fluid (CSF) and the bloodstream following neuronal damage (specifically due to the membrane rupturing)[30]. Due to their association with cell degeneration, neurofilaments seem to serve as a reliable biomarker for detecting ALS. There are multiple subunits of neurofilaments: light (NfL) medium (NfM) and heavy (NfH) chains, α-internexin, and peripherin[31]. Through the implementation of electrochemiluminescence based assays, levels of NfL and phosphorylated NfH (pNfH) in the CSF and blood were found to be about 20 times higher in ALS patients than healthy controls, thus making this filament a strong variable for ALS detection[32-33]. Neurofilaments have been recognized as validated biomarkers for ALS in recent literature and may also serve as pharmacodynamic biomarkers for assessing treatment effect by tracking NfL levels over time and correlating these levels with treatment intake. NfL levels may even be able to predict the rate of disease progression, as they have demonstrated this capability in multiple sclerosis patients, for whom the highest serum NfL levels were strongly correlated with the fastest rate of disease progression[34]. An interesting recent development in the field is the Food and Drug Administration's (FDA's) approval of tofersen for ALS solely

due to the drug's proven availability to decrease NfL levels, despite its performance at the clinical trial level, thus representing the importance that NfL levels are granted in the field as well as their credibility in clinical trials[35].

Neurofilament levels can provide the benefit of early disease detection and thus prevention since they can be measured and interpreted months before symptoms manifest[36]. However, it must be acknowledged that elevated neurofilament levels are not exclusive to ALS and can occur in a range of other neurological conditions, which might make result interpretation complex. For example, elevated levels themselves without symptoms or familial history of ALS could signify multiple sclerosis, frontotemporal dementia, and CNS inflammatory diseases like neurosarcoidosis[37-38]. Elevated NfL is a common trait among all motor neuron diseases, of which ALS is the most common[39]. As such, it is important to take into consideration that, while NfL levels are strong indicators of ALS, they must be validated by ALS symptoms to ensure construct validity in experimental design.

Protein byproducts found in urine and blood also hold potential as biomarkers for ALS. These proteins can be released during phenomena such as muscle breakdown (elevated creatinine), inflammation (elevated acute phase proteins), and altered metabolism (elevated neurotrophin receptor p75 extracellular domain), all of which are associated with ALS[40-42]. The advantage of using urine and blood for biomarker discovery, as opposed to lumbar tap

for CSF, is their easy accessibility and the ability to collect samples repeatedly over time. These protein products are not specific to ALS either and are even more broad than neurofilament levels; protein levels in urine and blood can be influenced by factors like diet, hydration status, and kidney function[43].

The reasoning for not considering various elevated protein byproducts especially complicates the usage of inflammatory cells for the purpose of ALS progression tracking. Though molecules like interleukin-6 (IL-6) were found to be elevated in the blood and CSF of ALS patients and were demonstrated to accurately indicate disease progression in astrocytes for up to 12 months following diagnosis, their role is to modulate any immune reaction in innate immunity and thus should not be considered as a sole diagnostic tool for neurodegenerative conditions[44-45]. Similarly, regulatory T-cells (Tregs) in the immune system offer anti-inflammatory activity in innate immunity and were found to be downregulated in ALS patients, reflecting neuroinflammation in patients[46]. One study found that cannabinoids upregulate Tregs via the promotion of dendritic cells that synthesize Tregs[47]. As such, a direct correlation could be made between inflammatory cell levels and Canurta's treatment effect, but would be more helpful as a supplementary biomarker to limit the chance of other inflammatory causes explaining the results.

The mode of testing is an important factor to consider in study design, as it affects patient comfort. CSF is the source of a variety of protein biomarkers for ALS as it is within the CNS and directly reflects changes in the

system. Several proteins linked to ALS have been identified in CSF samples from patients, including NfL, TDP-43 and tau[48]. These proteins play roles in processes like oxidative stress management, RNA metabolism regulation, and cytoskeletal function, which are all known to be disrupted in ALS[30,49-50]. Using CSF as a means of discovering biomarkers offers the advantage of reflecting changes occurring within the brain and spinal cord. However, collecting CSF requires a puncture procedure (lumbar tap), which is more invasive compared to collecting urine or blood samples[51]. As such, the tradeoff between patient discomfort for multiple strong biomarkers must be given extensive consideration.

Saliva, a non-invasive biofluid, has garnered attention as a potential reservoir of biomarkers for ALS. Saliva contains proteins that can provide insights into physiological processes. In the context of ALS, specific salivary proteins like cystatin S and statherin have shown differentiation in concentration between healthy controls and ALS patients with over 80% accuracy[52]. However, they are less thoroughly researched than neurofilaments, and their role in relation to ALS is not yet understood. The rise of additional supportive research could make these proteins highly beneficial for clinical trial usage.

Novel research about extraction is also emerging; for example, membrane affinity-based techniques were recently implemented to isolate extracellular vesicles (ECVs/EVs) from saliva, and were found to possess

greater purity than those isolated from blood but no significant protein expression differences were noted between healthy controls and ALS patients[53]. Next-generation sequencing of these vesicles can also provide insight into ribonucleic acid (RNA) levels. Given the novelty and inconclusive nature of this research, it can be concluded that salivary proteins are not currently a reliable biomarker.

While these are more direct methods of extracting biomarkers, digital health products can offer a strong advantage to the field. For example, single-use at-home spirometers that relay data to a cellphone app have recently been explored to track ALS respiratory decline[54]. Given the gravity of respiratory decline in relation to ALS-related mortality, at-home monitoring offers a time-efficient, accurate, and favourable method of data collection[55]. Additionally, researchers have just created the first wearable cuff to monitor muscle atrophy via changes in muscle size and volume and await further testing to glean feasibility in patients with neurodegenerative conditions[56]. Lastly, cognitive function can be monitored using at-home wireless electroencephalography tools combined with interactive assessments to determine cognitive decline; such tools were found to be user-friendly and useful for gathering frequent data that accurately captures trends[57].

ALS and FTD

Frontotemporal dementia (FTD) is a form of dementia that specifically results in the degradation of the frontal and temporal lobes of the brain. As mentioned in the introduction, ALS and FTD are closely related. In fact, the conditions can co-develop; 15% of ALS patients develop FTD symptoms[58]. This fact is perhaps better understood in the context of their shared onset commonly due to mutations in the C9orf72 gene. Since a correlation has been established between the conditions and a shared developmental pathway has been identified, it can be predicted that Canurta's ALS therapy would likely have an effect on FTD that should be concurrently explored within ALS patients.

A major similarity between the two is their shared accumulation of a protein known as TAR DNA-binding protein 43 (TDP-43), a molecule that normally modulates RNA processing. Neurological conditions can cause misfolding of these proteins, resulting in abnormal aggregates - in fact, over 95% of ALS patients and majority of FTD patients exhibit these aggregates, which were found to propagate through disease progression[59]. The commonality in pathways and biomarkers highlights the difficulty in monitoring treatment effect, as the conditions are too similar to differentiate in early clinical trials. Currently, no reliable biomarker has been discovered that sets the two apart enough to compare treatment effect individually.

However, if upcoming literature is able to identify a distinguishing factor, it could be highly advantageous to test Canurta's product on FTD patients[60].

Suggested Protocol

A strong clinical trial design is a pivotal component of effective research. It is important to consider both therapeutic approval requirements and optimal methods of data retrieval to obtain desired information. Given the overview of major biomarkers and their retrieval methods and the nature of the condition, this paper suggests that a combination of neurofilament and inflammatory molecules in CSF and blood, digital at-home monitoring, and self-reported symptoms should be tracked in a clinical trial to monitor the effect of Canurta's ALS therapy. This clinical trial aims to evaluate the safety and efficacy of Canurta's cannabis-based therapeutic in treating ALS while utilizing digital health technologies to remotely monitor disease progression. The study seeks to address the urgent need for effective treatments for ALS patients, exploring the potential benefits of cannabinoids and leveraging digital tools for comprehensive disease monitoring.

This partially decentralized clinical trial would employ a multi prong approach, leveraging clinical and lab data as well as at-home user-friendly health tracking technologies to enhance patient accessibility and comfort while optimizing data collection efficiency. The study should be randomized and double-blind to ensure there is no bias affecting results, maintain

objectivity, and limit the placebo effect[61]. The trial should follow a stratified design, including ALS patients diagnosed based on standardized criteria and grouped by disease severity within specific parameters, rather than using time from diagnosis, thus providing a more accurate and generalizable overview of disease progression[62]. Patients can be selected through a multivariable prediction model (e.g. family history of ALS, aged 40-70, and male) to achieve accurate patient recruitment[63]. Subject groups should include individuals with ALS risk factors, individuals at early stages of disease (following diagnosis), and late-stage individuals to determine the effect throughout disease progression. Trial duration should meet a minimum requirement of one year for accurate safety data, as per the recommendations of the FDA[64]. Participants should be randomized in a 1:1 ratio to receive either the cannabinoid therapeutic or placebo.. The study should be longitudinal, as many ALS studies are, to provide long-term data regarding disease progression and treatment effects over time, but an endpoint should be carefully considered as survival as an endpoint is time-consuming and can be difficult to interpret - instead, more specific endpoints such as respiratory function decline, muscle symptoms, and/or cognitive function (among others) should be considered[65]. One primary reliable outcome measure is changes in the ALS Functional Rating Scale - Revised (ALSFRS-R), which is arguably the most reliable tool for monitoring disease progression via a 12-item questionnaire that allows for quantitative rankings

of various disease facets[66]. This outcome measure has been used in major trials such as the Phase III clinical trial testing the effect of edaravone[65]. Perhaps the most significant primary outcome measure is NfL levels in the blood and CSF, as these are the most reputable biomarkers and appear to be of high priority for the FDA[64]. Secondary outcome measures should include respiratory function (as measured by pulse oximeters and at-home spirometers), physical capability, as self-reported by patients via questionnaires, and, when further developed, muscle atrophy measures as deemed by wearable cuffs. Using this multifaceted approach, the accumulation of data reflecting both biological values and self-reported values ensures that confounding variables are limited and results best reflect ALS disease progression.

Participants in the treatment group should receive the investigational cannabinoid therapeutic, administered as an oral/topical treatment (depending on whether the product is an oral pill vs. oil). It is worth noting that the FDA outlines that drug administration can often be completed via intrathecal (spine) injections for early stage trials. Following the progress of the trial, treatment can be self-administered and subjects should follow a preset regimen for administration to ensure optimal and standardized timing between doses. Participants in the control group should receive a placebo, closely matching the cannabinoid therapeutic in appearance and administration method. Following treatment administration, subjects should

follow an optimized timeline for symptom tracking and reporting, including the at-home components of self-reporting symptoms and submitting data from devices for physiological and cognitive changes. Data collection should thus be frequent given this structure in combination with clinical visits during and following treatment to obtain plasma and CSF volumes for protein level detection. CSF should be withdrawn using a lumbar tap and blood samples via venipuncture. Simoa was found to be the most sensitive and accurate analysis tool to measure NfL levels[67].

Statistical analyses will be conducted to compare the treatment and control groups for primary and secondary endpoints. The trial will be conducted in compliance with relevant ethical guidelines, and informed consent will be obtained from all participants. Data privacy and confidentiality should be maintained throughout the study. It is recommended that data compilation be streamlined by integrating each component into a mobile application with the following features:

Activity Trackers: Wearable devices like fitness trackers that monitor movement, steps, and physical activity levels display data and create trend graphs. This data can help researchers assess changes in mobility and activity patterns.

Smartwatches: Smartwatches equipped with various sensors can provide continuous monitoring of heart rate, sleep patterns, and other physiological parameters.

Symptom Tracking Apps: A mobile app designed specifically for ALS patients would allow users to track and record their daily symptoms, pain levels, energy levels, and overall well-being. The ALSFRS-R can be integrated into this platform.

Medication Reminder Apps: These apps help patients manage their medication schedules and track adherence.

Motor Function Assessment Apps: These apps guide patients through standardized video assessments that measure motor function, strength, and coordination. The collected data can be shared with healthcare providers for evaluation.

Voice Diaries: Patients can record their voice periodically to document changes in speech patterns and vocal strength over time.

Text Diaries: Apps that allow patients to type and save notes about their symptoms, experiences, and daily challenges can provide valuable insights during medical consultations.

Environmental Control Systems:

Pulse Oximeters: These devices measure oxygen saturation levels and heart rate, which can be important indicators of respiratory function.

Respiratory Spirometers: Devices that track breathing patterns and respiratory rate can assist in monitoring respiratory health.

Conclusion

In sum, this clinical trial aims to evaluate the safety and efficacy of Canurta's cannabis-based therapeutic in treating ALS by monitoring NfL levels in the blood and CSF while utilizing digital health technologies to remotely monitor disease progression frequently. The study seeks to address the urgent need for effective treatments for ALS patients, exploring the potential benefits of cannabinoids and leveraging digital tools for comprehensive disease monitoring. Participants will utilize wearable devices and digital health platforms to remotely collect data, including vital signs, mobility metrics, and patient-reported outcomes. Regular virtual visits will be conducted to monitor treatment response, assess safety, and ensure protocol adherence. A mobile application will allow participants to record daily symptoms, medication adherence, and any adverse events. The app will also feature video-based assessments to track motor function.

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