

## RESULTS OF CANNABIS RESEARCH INTO AUTO-IMMUNE DISEASES (SPECIFICALLY MS) LEADS TO NEW BROADER IMMUNE THERAPY RESEARCH

- **Successful in vitro (using human cells in lab) and in vivo (in mice) trials to stop the progression of MS using a unique cannabis strain**
- **In some cases the damage was also significantly reversed**
- **The Technion has found a chemovar that can effectively kill CD4 T cells that have become destructive instead of productive, which causes the damage to the body by the immune system in MS**
- **The Technion is isolating and identifying the molecules within the strain that is responsible for the therapeutic effect, with the goal of creating a pharmaceutical product to safely treat MS without the detrimental side effects found in current MS treatments**
- **The Company is now engaging with the Technion to plan concurrent clinical trials to test the same chemovar on other damaging immune malfunctions (cytokine storm) that can be caused by destructive T cells in other diseases**
- **Clinical trials in Israel using whole Cannabis extracts are very different from traditional pharmaceutical clinical trials and do not require phase 1 of safety and toxicity**

On the 26th of February 2018, Cann Global Limited (ASX: "CGB" or "the Company")'s medical Cannabis research division, Medical Cannabis Research Group Pty Ltd (MCRG) entered into a research agreement with Professor David (Dedi) Meiri and his team at the Technion, Israel's renowned university, to research the possibility of the use of cannabis in the treatment of autoimmune disease, specifically multiple sclerosis (MS).

See the following link to the announcement released by the Company at that time:

<https://www.asx.com.au/asxpdf/20180226/pdf/43rxp6bh4v8lrk.pdf>

The Technion's lab is widely considered to be the leading cannabis research lab globally and has the unique ability to comprehensively profile cannabinoid composition for a variety of cannabis strains and to purify single Phytocannabinoids (molecules synthesised by the cannabis plant) and create suspect profiles for examination in different studies.

Dr. Meiri explains that there are at least 144 unique Phytocannabinoids within the cannabis plant aside from the more commonly known THC and CBD compounds. Dr. Meiri and his team have developed new analytical tools to identify these 144 cannabinoids and have been working diligently to isolate and categorise these cannabinoids so that their therapeutic effects can be further understood.

During the research over the past two years, Dr. Meiri has already successfully identified a unique cannabis strain that in-vitro and in-vivo has shown to not only slow down the progression of MS but in some cases actually reverses the damage caused by the disease. (For further information see the information in the Appendix to this announcement and also please view the 'Webinar With Dr Dedi Meiri "Science Of The Plant"- Cannabis Research Now by following this link <https://www.youtube.com/watch?v=KSCsTUqO-t4> (from minutes 18:20 to 20).

During the course of the research, the Technion has also established an in-vitro screening for various immune cells such as T cells (CD-4 cells) where it was found that different cannabis chemovars had different killing abilities on human CD4 cells. CFSE cell proliferation assays have shown that proliferating human CD4 cells were more sensitive to treatment with cannabis chemovars than resting-activated human CD4 T Cells. As a result, based on the latest progress report, the Technion has found a chemovar that can effectively kill CD4 T cells that have become destructive instead of productive. This process is essential to controlling harmful autoimmune responses.

Please refer to Appendix 1 for more details on the Research performed to date.

The Company is very encouraged with these results to date, and the possibility of a product being developed that may assist the millions of people suffering from this debilitating disease.



## POTENTIAL SIGNIFICANCE OF THESE RESULTS ON AUTOIMMUNE RESPONSES IN OTHER DISEASES

Based on the significance of the results to date on Medical Cannabis as a potential therapy for the malfunctioning of the immune system in multiple sclerosis which The Technion has successfully been investigating over the past two years, the research team has suggested that it is worth investigating if the same strain of cannabis may also be effective in helping patients avoid or recover from the serious harm a malfunctioning immune system may cause in other diseases.

The malfunction of the immune system where the immune system attacks the body instead of defending the body is the basis of auto-immune diseases including Multiple Sclerosis, (MS). *"Multiple sclerosis (MS) is a complex disease with many different immune cells involved in its pathogenesis, and in particular T cells as the most recognized cell type [...] Today, we know that MS is a chronic inflammatory, autoimmune, demyelinating and degenerative disease of the CNS."*<sup>1</sup> Dr Meiri believes it is worth researching if the same compounds already identified in our research to control the immune response in MS, may assist in blocking the progression of the attack on healthy tissues caused by harmful immune responses in other diseases.

As stated above, during the research performed for our Company on MS, the Technion has found a chemovar that can effectively kill CD4 T cells that have become destructive instead of productive. As put forward by researchers in the scientific community, the stabilisation of CD4 T cells should be looked at as a potential therapy for controlling other diseases.<sup>2</sup>

CD4 T cells play a central role in immune protection. Commonly known as helper cells, they carry out different roles, such as activating other immune cells, B-lymphocytes to produce antibodies and play a critical role in activating and regulating the immune reaction. However, in autoimmune diseases such as MS, and other diseases involving the immune response, the T cells can become the enemy causing the body to attack its own tissues.<sup>3</sup>

The Company is currently in discussions with the Technion to prepare the parameters of new concurrent intended clinical trials to investigate how this particular Cannabis strain can have an impact on other patients whose immune system is causing damage to healthy cells. The Technion and Dr. Meiri have the capability, in conjunction with leading hospitals in Israel, to perform these necessary clinical trials over the coming months.

<sup>1</sup> Høglund RA, Maghazachi AA. Multiple sclerosis and the role of immune cells. World J Exp Med. 2014;4(3):27-37. Published 2014 Aug 20. doi:10.5493/wjem.v4.i3.27

<sup>2</sup> Corthay A. How do regulatory T cells work?. Scand J Immunol. 2009;70(4):326-336. doi:10.1111/j.1365-3083.2009.02308.x (<https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-3083.2009.02308.x>)

<sup>3</sup> Chiappelli F, Khakshooy A, Greenberg G. CoViD-19 Immunopathology and Immunotherapy. Bioinformatics. 2020;16(3):219-222. Published 2020 Mar 31. doi:10.6026/97320630016219 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7147500/>)



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As Cannabis and the identified specific cannabis strains are already authorised to be given to patients in Israel under health care and physician prescription, it is important to note that clinical trials using whole Cannabis extracts are very different from traditional pharmaceutical clinical trials and do not require phase 1 of safety and toxicity. Phase 1 in clinical trials evaluate the drug's safety and toxicity at different dose levels which is not required when doing trials on natural cannabis products which have already been approved for use. Therefore, significant results on efficacy may be possible in relatively short periods of time.

This also means that a legal product can already be produced and distributed wherever cannabis can be legally prescribed.

The Company will inform the market once parameters of any clinical trials are finalised.

We believe this is a great opportunity for CGB to be part of further research which may be of significant benefit to global health. Scientists around the world have suggested different potential therapies to try to treat the immune system malfunction in auto immune diseases, but to date most suggested therapies have potential harmful side effect. The Company is proud to be backing up a clinical trial that will be focusing on a more natural compound which is proven to be safer to give to patients due to its extremely low levels of toxicity or side effects.

Since Dr. Meiri and his team have been exceedingly successful to date in their Medical Cannabis research, CGB hopes that the success of the research that the Technion is undertaking will continue for the benefit of both world health and added value for CGB's shareholders.

#### **DR MEIRI COMMENTS:**

"In parallel with working on the whole Cannabis extract for the clinical trials and the development of our product, we are also working on isolating specific Phytocannabinoids of the Cannabis strain that we have found to be the most effective in our research for MS treatment (which forms part of creating our IP) and further elucidating their mechanism of action.

"We believe that we will create strong Intellectual Property relating to cannabis-based treatments of MS, and we will have preclinical results from isolated molecules with a better understanding of the dosing and mechanism of action. This additional research in isolating the molecules from the whole strains should ready us for the next development stage of 'on shelf' pharmaceutical grade products and an option to licence the molecules to the 'real' pharma industry.

"It is important to mention again that the clinical trials currently being planned with whole Cannabis extract for immediate therapeutic treatment, are very different from regular clinical trials and do not demand phase 1 of safety. We are sufficiently experienced to begin these trials quickly."

## PNINA FELDMAN-EXECUTIVE CHAIRPERSON-SAYS:

"We were very excited to hear that the Israeli government has now approved Cannabis export. It means that if clinical trials are successful, this will allow CGB to facilitate the distribution of a Cannabis product as a potential remedy for MS and potentially other auto immune diseases in Australia and beyond. We are looking forward to the exciting times ahead and are thrilled to be a part of the ground-breaking research in the use of Medical Cannabis as a potential treatment for autoimmune diseases. Our research has provided us with consistent achievements along the way and we feel we are reaching our research goals quicker than initially anticipated.

"The Company is now actively seeking large pharmaceutical investors or companies to partner with us in developing and commercialising this research.

"We thank Professor Meiri and his team for their brilliant work and thank all the shareholders who have the confidence that despite difficult times we will achieve our goals."

## EXPLANATORY NOTES:

We have gathered below some further information based on empirical data to assist in understanding why we believe these clinical trials are important and why they are warranted.

## CYTOKINES

"Cytokines are a diverse group of small proteins that are secreted by cells for the purpose of intercellular signaling and communication. Specific cytokines have autocrine, paracrine, and/or endocrine activity and, through receptor binding, can elicit a variety of responses, depending upon the cytokine and the target cell. Among the many functions of cytokines are the control of cell proliferation and differentiation and the regulation of angiogenesis and immune and inflammatory responses."<sup>4</sup>

TABLE 1	
Major types and actions of cytokines	
TYPE	ACTIONS
Interferons	Regulation of innate immunity, activation of antiviral properties, antiproliferative effects
Interleukins	Growth and differentiation of leukocytes; many are proinflammatory
Chemokines	Control of chemotaxis, leukocyte recruitment; many are proinflammatory
Colony-stimulating factors	Stimulation of hematopoietic progenitor cell proliferation and differentiation
Tumor necrosis factor	Proinflammatory, activates cytotoxic T lymphocytes

Cytokines are involved in regulating immunity and inflammation.

<sup>4</sup> Tisoncik JR, Korth MJ, Simmons CP, et al. Into the eye of the cytokine storm. Microbiology and Molecular Biology Reviews : MMBR. 2012 Mar;76(1):16-32. DOI: 10.1128/mmbr.05015-11.

## CYTOKINE STORM

"Cytokine storms are associated with a wide variety of infectious and non-infectious diseases. [...]"

The term "cytokine storm" calls up vivid images of an immune system gone awry and an inflammatory response flaring out of control. The term has captured the attention of the public and the scientific community alike and is increasingly being used in both the popular media and the scientific literature."<sup>5</sup>

For the purpose of this document, the term cytokine storm will be used to describe a severe immune reaction in which the body releases too many cytokines into the blood too quickly. Cytokines play an important role in normal immune responses, but having a large amount of them released in the body all at once can be harmful. A cytokine storm can occur as a result of an infection, autoimmune condition, or other disease.

## CYTOKINE STORM PRESENT IN MS PATIENTS

Multiple sclerosis (MS) is the inflammatory disease of the central nervous system (CNS). An important body of evidence gathered from MS animal models such as experimental autoimmune encephalomyelitis (EAE), points to the central contribution of CD4 T lymphocytes in disease pathogenesis. As reported by the Multiple Sclerosis journal: "MS is associated with a cytokine storm characterized by the parallel upregulation of proinflammatory (IFN-g, TNF-a, and b, and IL-12) and immune response-down-regulating (TGF-b, IL-10) cytokines".<sup>6</sup>

## CYTOKINE STORM PRESENT IN PATIENTS WITH A SEVERE AUTOIMMUNE RESPONSE

*"Whenever a healthy body is fighting an infection, there's a natural immune system response that occurs [...] part of this response involves releasing cytokines[...] These cytokines, according to the American Cancer Society, essentially signal the immune system to start doing its job."*<sup>7</sup>

Cytokine storm is considered to be one of the major causes of [...] multiple-organ failure. It plays an important role in the process of disease aggravation.<sup>8</sup>

CD4 T cells play a central role in immune protection. Commonly known as helper cells, they carry out different roles, such as activating other immune cells, B-lymphocytes to produce antibodies and play a critical role in activating and regulating the immune reaction. However, in autoimmune diseases, the T cells can become the enemy causing the body to attack its own tissues.

<sup>5</sup> Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the eye of the cytokine storm. Microbiol Mol Biol Rev. 2012;76(1):16-32. doi:10.1128/MMBR.05015-11

<sup>6</sup> Link H. The cytokine storm in multiple sclerosis. Mult Scler. 1998;4(1):12-15. doi:10.1177/135245859800400104

<sup>7</sup> <https://www.health.com/condition/infectious-diseases/coronavirus/cytokine-storm>

<sup>8</sup> Ye Q, Wang B, Mao J. J Infect. 2020;80(6):607-613. doi:10.1016/j.jinf.2020.03.037

In order to better understand this, we need to look into another substance produced by T helper cells which is the cytokine called Interleukin-17A (IL-17A). "IL-17A is a pro-inflammatory cytokine known to play a role in host defense and pathologic inflammation in murine models of lung injury."<sup>9</sup>

IL-17 inhibition has been adopted as a common and successful strategy to reduce the injury associated with inflammatory autoimmune disease."<sup>10</sup>

Controlling T-Cells therefore seems to not only be a key to controlling auto immune diseases, but could play a crucial role in controlling the damage caused by autoimmune responses in other diseases.

CGB intends to support testings in clinical trials of the same cannabis strain that has been successful in vitro and in vivo in the MS trials to control the T-Cells, to see if it will be successful in helping prevent or reduce the harmful immune response in other diseases, and if so, to what statistical success.



Pnina Feldman  
Executive Chairperson

## ABOUT CANN GLOBAL:

Cann Global Limited (ASX:CGB) is a driving force in the hemp and medical Cannabis industries. Our strength comes from our team's core competencies and expertise, and our solid and strategic partnerships with experts in Australia, USA, Israel, Asia, Africa and Canada. We are working under the relevant legislation to ensure that the future in Medical Cannabis and Natural Foods will allow medical practitioners, patients, and consumers to gain access to the right information, as well as the safest, most effective and sustainable products.

## AUTHORITY AND CONTACT DETAILS

This announcement has been authorised for release by Pnina Feldman, Executive Chairperson.

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<sup>9</sup> Mikacenic C, Hansen EE, Radella F, Gharib SA, Stapleton RD, Wurfel MM. Crit Care Med. 2016;44(3):496-502. doi:10.1097/CCM.0000000000001409

<sup>10</sup> Pacha O, Sallman MA, Evans SE. Nat Rev Immunol. 2020;1-2. doi:10.1038/s41577-020-0328-z <https://www.nature.com/articles/s41577-020-0328-z>

## BACKGROUND - TECHNION ISRAEL INSTITUTE OF TECHNOLOGY

Technion - Israel Institute of Technology was founded in 1912 in Haifa and is the oldest and ranked as the leading university in Israel. The Institute is currently rated #85 in the World's Top 500 Universities and is the only Israeli university in the top 100 of the list  
<http://www.shanghairanking.com/ARWU2019.html>

Technion researchers, including three Nobel laureates in chemistry, have won many international awards for their research in the fields of Microbiology, Microbial Diagnostics, Genomics, Antibiotics to name just a few. Technion people, ideas and inventions make immeasurable contributions to the world including life-saving medicine, sustainable energy, computer science, water conservation and nanotechnology.

Technion is widely recognized as a global leader in Medical Cannabis Research. Israel has been leading the world since the early 1960's in Medical Cannabis research.

In November 2017, Times Higher Education ranked Technion as the world's leading academic institution in teaching digital skills to students, ahead of the prestigious American M.I.T. University.



**BACKGROUND -  
DR. DAVID (DEDI) MEIRI**

Assistant Professor, Heads the "Laboratory of Cancer Biology and Cannabinoid Research", Technion, Israel Institute of Technology, Israel.

**David (Dedi) Meiri, PhD**, is an Assistant Professor at the Faculty of Biology at the Technion Israel Institute of Technology and a member of the Technion Integrated Cancer Center (TICC). Dr. Meiri's scientific background is highly diverse. He holds a M.Sc. in biochemistry and a Ph.D. in plant biotechnology from Tel Aviv University. Dr. Meiri conducted his post-doctoral fellowship at the Ontario Cancer Institute where he expanded his knowledge in human biology and cancer pathogenesis and focused on the role of the GEF-H1 protein in tumor invasion and metastasis. Upon completion of his post-doctoral fellowship, Dr. Meiri took a position at the Technion Israel Institute of Technology, where he heads the "Laboratory of Cancer Biology and Cannabinoid Research".



Presently, his lab investigates the therapeutic potential of phytocannabinoids, the unique active compounds of the Cannabis sativa plant. On top of other research being conducted in the lab, the main focus of his research is to determine the antitumor effects of cannabinoids, including the anti-metastatic and pro-apoptotic effects of phytocannabinoids.

From the establishment of his lab it has grown significantly, and it is now comprised of 38 scientists; a highly trained team of skilled plant biologists, chemists, cancer experts and neuroscientists who work together in synergy to complement one another in order to achieve the highest level of results. Dr. Meiri's lab, is one of the leading laboratories in the world that is exceptionally equipped with the capabilities and resources to ask and answer almost any question in the field of medical cannabis.

Dr. Meiri operates the "Cannabis Database Project" and his lab is currently involved in eight clinical trials covering diverse aspects of cannabis treatment, such as: colon disease, pain prevention, cancer treatment and epilepsy. He collaborates with cannabis growers, clinicians and major manufacturers and distributors of medical cannabis, including Andrew Kavasilas from QBL: MCL in the purpose of revolutionizing cannabis treatment.

Dr. Meiri is also highly involved in governmental regulations and is a residing member in several Israeli Ministry of Health committees which seek to advance the fundamental understanding of optimal cannabis usage and minimization of adverse side effects.



**FURTHER INFORMATION ON THE RESEARCH REPORTED ABOVE – APPENDIX 1**

***Cannabis Extract as a Potential Therapy for Multiple Sclerosis***

Millions of people worldwide are afflicted with multiple sclerosis (MS). MS is a chronic disease of the brain and spinal cord and is a common cause of serious physical disability in young adults, especially women. MS has a heterogeneous presentation that can include sensory and visual disturbances, motor impairments, fatigue, pain and cognitive deficits.

The variation in its clinical manifestations correlates with the spatiotemporal dissemination of lesional sites of pathology within the central nervous system (CNS). Early lesions show invading peripheral immune cells and leakage of these immune cells into the blood-brain barrier. As these lesions are a hallmark of MS and are caused by immune cell infiltration across the blood-brain barrier (BBB), this promotes inflammation, demyelination, gliosis, and neuroaxonal degeneration: all leading to disrupted neuronal signaling. Inflammation is present at all stages of MS but is more pronounced in the acute phases than in chronic phases. Macrophages dominate the composition of the immune cell leakage infiltrate, followed by CD8+ T cells, and lower numbers of CD4+ T cells, B cells, and plasma cells (Frischer et al., 2009). Although generalized brain atrophy has been noted, in initial stages of the disease there is little overt damage to the brain and spinal cord in the areas outside the focal lesions (Chard, et al., 2002).

**The overall objective** of this study is to match effective Cannabis extracts that regulate/modulate immune function, specifically, autoimmunity in MS in order to optimize treatment for MS patients.

***Innovation***

We established the novel ability to analyze the metabolomics and the specific chemical composition of Cannabis plants (principally of phytocannabinoids and terpenes). We intended to identify the effects of different cannabinoids and terpenes both individually and in combination on the function of immune cells, specifically on MS-derived autoimmune and regulatory cells in vitro. We evaluated the immunomodulatory properties of specific cannabinoid extracts in in vivo rodent models of MS. This will enable us to detect the most effective Cannabis extract and cannabinoid profiles for regulating immunopathology in MS.

## **Results:**

### *1. Characterize clinically-used Cannabis chemovar using comprehensive mass-spectrometry based metabolomics.*

We have identified 110 cannabinoids in over then 100 different Cannabis chemovar, and established LC/MS/MS spectral mass library of cannabinoids. According to our preliminary findings, Cannabis chemovar vary greatly in their cannabinoid profiles. Thus, we hypothesized that different chemovar (each with a distinct cannabinoid composition) will have differential effects on the immune cells which regulates the chronic inflammation in MS. For starting this study, we analyzed 25 different cannabis chemovar that we suspect can have an effect on T-cells and the immune system.

### *2. Screen for the immunoregulatory properties of different Cannabis chemovars on different immune cell function and MS immunopathology*

For this purpose, we have established an in-vitro screening system for various immune cells such as T cells, monocytes and microglia. Multiple lines of evidence implicate that T cells (specifically CD4 cells) play a central role in both mediating and regulating MS pathophysiology. Therefore, cannabinoids that can regulates these cells function have a high therapeutic potential. We started by evaluating the killing properties of five cannabis chemovar on human CD4 T cells. Purified human CD4 T cells were treated with different cannabis chemovars for 1 hour in the indicated concentrations and analysed . Note that not all chemovars were found to have the same killing properties.

### *3. Testing the effect of different Cannabis chemovars on with proliferating human CD4 T cells*

Following the findings that different Cannabis chemovars had different killing abilities on human CD4 T cells, we went on to compare between resting human CD4 T cells and proliferating human CD4 T cells. CFSE proliferation assays showed that proliferating human CD4 T cells were more sensitive to treatment with Cannabis chemovars than resting non-activated human CD4 T cells.

## **Impact:**

We found that specific cannabis chemovars can kill human CD4 T cells while others had no effect in the tested concentrations. Importantly, we have a chemovar that can kill CD4 T cell in a very low concertation (Cann5), and in contrast, a chemovar that does not kill CD4 T cells even in the highest concentration that was tested. Moreover, we found that proliferating human CD4 T cells were more sensitive to killing by different Cannabis chemovars, as seen in the percentage of dead cells in resting cells Vs. proliferating cells. Harvesting the potential of cannabinoids as part of the arsenal for MS therapy may open new treatment possibilities for the immunopathology in MS.

Next:

- a. We screened for the immunoregulatory properties of many other Cannabis strains/extracts that are available to us on immune cell function in different subset of T cells (such as regulatory T cells and Th17) and MS immunopathology by applying our above developed in vitro screening system.
- b. We isolated the specific compounds from the strains that were effective and we are working to prove that these compounds are responsible for the observed effect (create an IP)
- c. We looked to understand the molecular mechanisms in which these compounds affect the Tcells and the MS disease.

#### *4. The killing properties of Cannabis extracts on murine CD4 T cells were tested*

From the screen of different Cannabis extracts on human CD4 T cells, we chose two extracts that showed different sensitivity to Cannabis and tested those on murine CD4 T cells. The viability assays shown in Figure 3, demonstrate higher sensitivity to the different Cannabis chemovars than human CD4 T cells.

#### *5. The effect of Cann1 and Cann2 on CD4+CD25+FoxP3+ mouse Tregs viability was tested.*

We started looking at different subsets of CD4 T cells, to evaluate whether different chemovar can specifically affect key subsets of T cells. We chose to start with regulatory T cells (Tregs) as they regulate the entire immune response. In patients with organ-specific autoimmune diseases, Tregs cells are compromised. Approaches to strengthen Treg cell function have being suggested as a potential treatment for autoimmune disease and some have entered clinical trials. These cells have specific markers, CD4+CD25+FoxP3+, that defines the subset amongst other T cells. We saw that one extract down regulated the subset. While the other extract did not.



*6. Effect of Cann1 and Cann2 on CD4+CD25+FoxP3+ mouse Tregs differentiation in-vitro*

In addition to viability of Tregs, we tested whether the two different extracts can influence the differentiation of Tregs in-vitro. We were able to get very high differentiated cells. From ~11% to ~80%. The cannabis extracts did not seem to affect significantly the number of polarized cells. Next, we evaluated the effect on the activity of Tregs. It was shown that in MS patients, the number of Tregs was similar to healthy individuals, but their activity was impaired. In order to be able to evaluate Tregs from EAE mice and compare to healthy mice or mice with EAE treated with the Cannabis extracts, we optimized the sorting method. We were able to get >90% FoxP3+ cs.

*Impact:*

As a result of the above testing and analyses we looked at Tregs, which are the key cell subsets that regulates immune responses. We tested the effect of the two cannabis chemovars (Cann1 and Cann2) on the viability of Tregs, the differentiation and established the system to test their activity. One of the chemovars down regulated FoxP3+ Tregs cells, whereas the other did not. There was no effect on Tregs differentiation. We were able to achieve highly purified CD4+CD25+FoxP3+ T cells from the sorter and planned to next use the sorting system to sort Tregs and test their activity.

*Next:*

We continued to validate our data on Tregs using FoxP3-GFP mice. In addition, we used the sorting system that was established and tested the activity of Tregs in Tregs sorted from EAE mice and compared with Tregs sorted from EAE mice treated with the two chemovars and Tregs sorted from healthy controls. Putting together the effect of the chemovars on the viability, differentiation and activity of Tregs, is essential to unveil the mechanism by which specific chemovars have the ability to improve the clinical score of EAE mice, while others can not, or even worsen (Figure 1).

In addition we looked at Th17 cells, which are the main pathogenic CD4 T cells in MS and EAE.

*7. Demonstration of the neuroprotective properties of specific Cannabis extracts on MS rodent models.*

We have finished establishing in our lab an acute rodent model for MS, experimental autoimmune encephalomyelitis (EAE). Over 150 mice were used in these trials. EAE was induced with vaccination of C57BL/6 mice with MOG(35-55). After the first sign of paralysis cannabis extract was injected I.P (5 mg/kg) every other day till the end of the experiments. To date we have managed to evaluate the effect of four chemovars on the progression of EAE (Figure 1). Note that three chemovars have the ability to reduce paralysis (lower EAE score) compared to control mice while one chemovar was detrimental and inhibited the natural recovery potential.

**Impact:**

We found that specific cannabis chemovars have a potential to inhibit progression of EAE while other might have harmful effects on recovery. These results show that although cannabis has a great potential to improve EAE symptoms, not all chemovars have the same therapeutic properties.

**Next:**

- a. We isolated specific compounds from the strains that were effective and intend to prove that these compounds are responsible for the observed effect (create an IP)
- b. We will look to understand the molecular mechanisms in which these compounds affect the progression of EAE.

**Figure 1**

**Evaluating the neuroprotective potential of cannabinoids in autoimmune encephalomyelitis Cannabis-treated EAE-injured mice display body motor function recovery after cannabis treatment.** C57BL/6 mice were immunized with MOG35–55/CFA and pertussis toxin (days 0 and +2). One group received cannabis extract (5 mg/kg) on day 10 and every other day thereafter. The control group received vehicle. Disease scores were assigned on the basis of motor function as follows: 0, no abnormality; 1, limp tail or hind limb weakness (waddling gait); 2, limp tail and hind limb weakness; 3, partial hind limb paralysis; 4, complete hind limb paralysis; and 5, moribund. Measure of disease degree is the evaluation of disability score.

