Glyphosate Proof-of-Concept Trial

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INTRODUCTION

We all know that glyphosate is toxic to humans in many ways but this information is downplayed extensively by numerous sources and as such will be the major push back on this or any clinical trial that shows a reduction of glyphosate by using any means. The Detox Project and The Organic Effect in Sweden shows us that simply changing to a 100% organic diet will significantly reduce glyphosate levels in the body. Here in lies the problem. Not everybody can eat organic due to regional availability, deceptive suppliers and financial reasons. If glyphosate is as ubiquitous as it is in our current food sources, i.e. it's here to stay, then we should have a solution to decrease the glyphosate levels in our bodies since we are forced to continue eating glyphosate contaminated foods. In fact, let's go one step further and repair the damage that it is causing in our bodies. To do this we need to look at several different biomarkers that reflect this damage at different functional levels. This proof of concept study will look not only at absolute glyphosate levels but more importantly will look at the first site of attack from this compound. The gut and its delicate biome.

The gut biome is made up of a precise balance of commensal and probiotic intestinal bacterial strains inhabiting the fingerlike villi of the single layer of intestinal epithelial cells that separates the intestinal lumen from the rest of the body. The space between these cells is sealed by tight junctions that regulate the permeability of the intestinal barrier. These tight junctions are complex structures that maintain the integrity of the gut barrier and are one of the main sites of damage in a leaky gut. The intestinal bacteria change the expression and distribution of tight junction proteins which regulates the intestinal barrier function. Various friendly bacterial strains lead to an increase in tight junction proteins at the gap junctions between the cells and can prevent or reverse the damage caused by various pathogens and toxins. Increased intestinal permeability can cause autoimmune, inflammatory and other diseases that are expressed both locally as in inflammatory bowel disease and celiac disease and systemically which can cause a whole body inflammatory state that effect all the organs in the body.

Maintaining the integrity of the gut biome is essential for many reasons. The gut is where most of our nutrients are absorbed. The gut is the entry point for most of the body's toxins and therefore the primary site for detoxification. 70-80% of our immune system is in our gut so

even small levels of gut dysfunction can have a huge impact on our immune function which is needed to prevent infection, fight cancer and maintain overall health. The markers we chose for this study will evaluate different aspects of gut health including gut inflammation, gut permeability and gut immune function.

Because we are dealing with human test subjects we want to humanize the study and correlate our objective lab data with subjective clinical data by looking at detailed clinical health questions that assess the person's mood, energy levels, sleep quality, bowel elimination quality and other signs and symptoms of a compromised gut biome. Remember, we are dealing with people; mothers, fathers, husbands, wives, friends and co-workers. At the end of the day we are in the business of saving lives, improving lives and helping families. This above all else is our ultimate goal and we will use sophisticated science to achieve overall health and wellness that translates to everyday life.

STUDY DESIGN

In order to test if the new Purium Product Biomedic has any effectiveness at reducing glyphosate levels compared to controls, we will need to recruit individuals with high normal levels of urine glyphosate levels. Half of those will receive Biomedic and half will receive a similar placebo product for a period of six weeks. After their initial assessment and randomization, follow-up visits will occur every two weeks. Blood and urine will be obtained during these visits.

Potential male subjects will be screened for higher ranges of glyphosate levels. Those in the high normal range to elevated levels will be included. The exact number of subjects needed to screen for ten subjects is unknown since we have no information on these levels in South Florida individuals. Presumably, those eating a fast food, non-organic food lifestyle will offer us the best chance to obtain these individuals.

Biomedic is designed to improve the gut biome by providing a prebiotic in the form of Prebiosure 350mg, a probiotic Lactospore 15mg (bacillus coagulans 15 billion cfu/gm) and Humic and Fulvic compounds (Humisure 5mg/FOS 135mg). As such, functional testing of gut health will be performed by measuring inflammation, intestinal permeability and intestinal immune function both before and after treatment. Controls will receive a placebo containing similar levels of both a pre and probiotic.

Laboratory testing will be done to assess glyphosate levels, highly sensitive inflammatory markers, highly specific gut biome surrogate markers of intestinal permeability and food sensitivity testing to assess gut immune function. This multi-factorial study will assess both the detection and removal of glyphosate from the body while at the same time assessing inflammation, intestinal permeability and intestinal immune function by measuring the

functional improvement of the gut as a result of repairing, re-inoculating and restoring the gut biome.

This proof-of-concept research will have Institutional Research Board (IRB) approval.

The goal of this pilot study is to have results by mid-August that will lead into a full clinical trial. Preliminary data may be available prior to mid-August.

LABORATORY TESTING

- **Glyphosate Levels (Quantitative Levels - ELISA).**

  All eight subjects, four from each group, will receive testing at each office visit.

- **Intestinal Permeability Assessment (Lactulose and Mannitol test).**

  The concept of this test is to ingest a specific amount of these two sugars, one large and the other small in size. The ratio in urine is measured pre and post treatment and the difference in these ratios will determine the extent of the intestinal permeability, or gut health, of the individual. This is not a direct method of measuring specific bacterial populations, but is a good surrogate test because it is recognized that the gut permeability is greatly influenced by the gut biome. Under the assumption that an improved ratio of "good" to "bad" bacteria will decrease the level of the "leaky gut" or intestinal permeability, this test will allow us to conclude that this product did or did not alter the gut biome. The advantage of this test is that it is easily collected compared to stool sampling and significantly less expensive compared to growing and qualitatively assessing thirty or forty different bacterial strains. To keep costs down, only three subjects from each group will undergo these tests.

- **hsCRP Levels – High Sensitivity C-Reactive Protein.**

  hsCRP is a highly sensitive, non-specific inflammatory marker. The High Sensitivity (hs) component is used as a cardiac marker. This serum marker is well recognized as an inflammatory marker. All eight subjects will receive testing every two weeks.

- **Gut Biome Markers – LPS (Lipopolysaccharide) and D-lactate.**

  These two gut biome markers are generally accepted as indicative of mucosal wall breakdown related to biome changes, among other reasons. LPS and D-lactate are metabolic by-products of the commensal bacteria in the gut. D-lactate is produced during fermentation. Levels in normal healthy individuals are typically extremely low but are elevated in small intestinal bacterial overgrowth (SIBO), intestinal infections, mesenteric ischemia, and other intestinal inflammatory conditions. LPS is the major component of the outer membrane of Gram-negative bacteria. LPS levels are elevated during phases of bacterial replication or death and are correlated to an impaired

mucosal barrier. To keep costs down, only two subjects from each group will receive LPS testing and only three subjects from each group will receive D-lactate testing.

- **Food Sensitivity Testing – IgG antibody testing.**

  The number of foods that are reactive will comprise the assessment for this series. Subjects will not be given their baseline test results as this might affect the study if they eliminate the reactive foods as this might affect the glyphosate levels as well as the inflammatory and gut biome surrogate markers. If the gut is helped by the Purium product, the number and severity of the reactive foods should decrease. This will serve as yet another functional test to assess the improvement in the gut biome as a result of removing glyphosate from the body and repairing, re-inoculating and restoring the gut biome. To keep costs down, only two subjects from each group will receive this testing.

NOTE: There are numerous other inflammatory and gut biome markers. The study design markers were chosen because they accurately represent dysbiosis of the gut biome as it relates to gut permeability and functionality. Adding several gut biome markers as clinical endpoints takes this study from a simple measurement of glyphosate elimination and correlates it with a functional improvement in the gut biome as evidenced in a multi-factorial assessment of gut inflammation, permeability and immune function.

PROOF-of-CONCEPT SUMMARY

Process

- **Agree to a study design, testing protocol and subsequent budget**
  - **Test subjects selection (age, sex, food habits)**
  - **Set all inclusionary and exclusionary criteria**
  - **Establish a time frame for results**
  - **Determine laboratory testing markers**

- **Secure IRB approval to increase validity of results and allow a quick and seamless transition to a full clinical trial if desired. Also, remember we are dealing with a Monsanto product so we want to stand up to any scrutiny**

- **Secure IRB approval from Western IRB (2-3 weeks)**

- **Meanwhile, put 2 of Dr. Popkin's patients through the protocol to work out the study design process (these two patients can't be used in reporting)**

- **Once IRB approval is obtained the following can occur**
  - **Begin recruitment of appropriate subjects and complete signing the consent forms**
  - **Complete baseline testing**

- **For those with elevated glyphosate levels:**
    - **Randomize**
    - **Complete Follow-Up testing according to the study protocol**
    - **Quickly complete analysis and write report**

CONTROLS:

This group will receive a placebo-based product. They will take this product on the same schedule as those taking the Purium product.

This will allow us appropriately compare the effects of the Purium product against a placebo product.

STUDY END-POINTS

Laboratory Testing

- **Glyphosate levels (determined by ELISA)**
- **Intestinal Permeability Assessment (Lactulose & Mannitol test)**
- **hs-CRP levels (High Sensitivity C-Reactive Protein)**
- **Gut Biome Markers: LPS (Lipopolysaccharide) and D-lactate**
- **Food Sensitivity Testing – IgG antibody testing.**

Clinical Observation Assessment

- **Changes in physiological parameters, i.e., blood pressure, weight, etc.**
- **Disease state parameters, i.e., IBS, arthritis, diabetes, obesity, etc.**
- **Overall Well-being: i.e., mood, energy levels, sleep quality, bowel elimination and other signs and symptoms of a compromised gut biome.**

Compliance, relating to taking the supplements and making appointments

Adverse events (required by the IRB)

CLINICAL TRIAL POPULATION

Exclusion Criteria:

Subjects who meet any of the following exclusion criteria are not eligible for participation in this proof-of-concept trial:

- **Are unwilling to follow the procedures of the trial, such as making visits or taking the supplements when asked to;**

- **Are unable to tolerate the ingredients in any of the botanical supplements or who have a propensity to allergic reaction;**

- **Have unintentionally lost or gained 10 or more pounds of body weight in the last 3 months;**

- **Have an acute illness (such as a severe cold or flu) or have been hospitalized within the past month for certain conditions;**

- **Have severe co-morbid disease including cardiac, pulmonary, renal, hepatic, carotid, peripheral vascular disease, stroke, neurological, clotting disorders or active cancer;**

- **Abuse alcohol or illicit drugs.**

- **Take anticoagulants other than aspirin or take MAO inhibitors**

- **Are uncontrolled or insulin-dependent diabetics (IDDM)**

- **Have uncontrolled hypertension;**
  - **Systolic blood pressure (SBP) > 180 mm Hg or diastolic blood pressure (DBP) > 100 mm Hg, upon two of three repeated measures, and not on medications for hypertension.**
  - **Systolic blood pressure (SBP) > 150 mm Hg or diastolic blood pressure (DBP) > 90 mm Hg, upon two of three repeated measures, and not on medications for hypertension;**

- **Have had a recent cardiovascular event (past 36 months), or a family history of sudden death or heart attacks before the age of 55;**

- **Have a Body Mass Index (BMI) of less than 16 or greater than 38 m/kg2;**

- **The anticipated need for surgery of any type during the entire study;**

- **Subjects who plan to donate blood or blood products during the study or for thirty (30) days following the study;**

- **Subjects with evidence of active peptic ulcer disease, or who have a reliable history of gastrointestinal bleeding within the past five (5) years;**

- **Subjects with recurrent or a history of intestinal disorders that may interfere with the absorption;**

- **Have any disease or condition that in the investigator's opinion compromises the integrity of the clinical trial or the safety of the subject;**

Severe co-morbid disease is defined as any condition that would cause severe limitations or inability to carry out usual activities of daily living.

The exclusion criteria identified above are based upon general safety concerns identified with the condition and/or product from recommendations made by the study physician, confounders identified by the biostatistician, or information identified in product ingredients' research.

Proof-of-Concept Trial Population Summary

A total of 8 male subjects are needed to complete this proof-of-concept clinical trial, requiring the enrollment of 16-20 subjects. Subjects will be enrolled at Dr. Popkin's office locations.

This section will provide details of the subjects.

As an example the following parameters need to be provided:

Age range and sex

Obviously in a proof-of-concept, the age is crucial. I suspect that the ideal age should be between 30-60 years of age. We will limit the study to just one sex to minimize variations. Reading published articles supports the use of one sex over another.

Diet and shopping habits

Individuals who eat organic –vs- those who rarely eat organic. Clearly, we do not want to choose organic as these individuals most likely will have lower levels of the target compound glyphosate.

Healthy eaters -vs- fast-food junkies. Presumably, those who eat more fast food will have higher levels of glyphosate.

Payment for test subjects

We will reimburse our subjects (those who ultimately become randomized) to ensure higher compliance.

We need individuals who will be compliant throughout the trial period.

Purium Study Product

Biomedic active ingredients:

1) **Prebiotic in the form of Prebiosure 350mg**
2) **Probiotic Lactospore 15mg (bacillus coagulans 15 billion cfu/gm)**
3) **Humic and Fulvic compounds (Humisure 5mg/FOS 135mg)**

****** A detailed list of ingredients of the placebo will be included.**

****** A short paragraph describing how the given ingredients in the Biomedic study product might produce the desired effects and why the ingredients of the placebo will act as an appropriate placebo will also be included.**

****** References will also be included.**

Product Administration

Capsules will be taken twice daily, morning and evening.

Product will be taken for six weeks

Memorandum of Understanding

This trial requires Institutional Review Board (IRB) approval. This is the FDA's arm for approving all research and for the protection of the patient's rights. Violations are taken

extremely seriously. They do not distinguish between small pilot studies and full-blown clinical trials.

Normally, all results from research studies are sent directly to the company's lawyers so they may guide the company on marketing claims. Dr. Popkin and Dr. Blum have agreed to by-pass this process for the purpose of this proof-of-concept trial. However, any statements as to the performance of this study product, written, oral, or otherwise, must be approved by Dr. Popkin. This study is too small in terms of numbers to make any general conclusions, even if the results are extremely positive.

This study has been designed for maximum potential of results by adding an inflammatory clinical endpoint as well as several surrogate gut biome and functional markers. If the results trend positive, a second, complete clinical trial will need to be designed and conducted in order to create claims. Studies of this nature cost significantly more than this study because the subject numbers are larger and additional labs will need to be run and independent monitors and scientists will need to be brought in for verification and validation purposes. Obviously having the data from a clinical trial opens the doors for marketing campaigns that can lead to a significant increase in revenue. These larger clinical studies will be appropriate for peer-reviewed medical publications and presentations in front of physicians at large, national meetings.

Our budget covers only the cost of the proof of concept trial and its reporting. Post-result representation of the trial is not included in the budget. This includes publications, speaking engagements, etc.

Due to the truncated time table we will be working under the understanding that "time is of the essence". As a result, we will need timely responses from the Purium Corporation when requested.

We will start this process next week since the Preliminary Budget is approved and the agreed upon initial payments are to be initiated and/or received today June 2, 2017.