Keys to understanding clinical studies on nutrition and the immune system

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Biothera, the immune health company

Why Do Clinical Studies on Immune Products?

The Regulatory and Immune Product Landscape
1. Increased scrutiny of the FTC and FDA over immune health claims.
2. The existing regulatory frameworks are not coordinated and differ among geographies.
3. Evolving regulatory framework includes a shared perspective among leading geographies on health claim regulatory requirements.
4. Foods & beverages can be delivery vehicles for immune health ingredients.

Topics to be Covered

- Laying the foundation for success
- Types of studies
- Elements of study design
- Understanding outcomes
- Examples (our studies and others)
- Summary

Before We Begin: Steps to Approval

Timeline (multiple years)
Laying the Foundation

- There are standard methods used for many of these studies.
- We will use studies completed with a particular strain of beta 1,3/1,6 glucan as examples.
- Multiple studies are often required to achieve the technical objective, we present a summary.

Lay the Foundation: Safety & Tox

- Published safety & toxicology data
  (Food & Chem. Tox., 45:1719-1730 2007)
- Two 90-day subchronic rat toxicology studies
- Acute toxicity study (1,000 times standard dose, completed in Japan)
- Geno-toxicology studies
- Safety demonstrated in multiple human clinical studies.

Lay the Foundation: Know the MOA

1) Taken orally, the specific beta 1,3/1,6 glucan is taken up into the body via the Peyer’s Patches in the intestines.
2) Immune cells called macrophages with the beta 1,3/1,6 glucan travel to the immune organs throughout the body.
3) Macrophages break down the beta 1,3/1,6 glucan into smaller fragments that bind to neutrophils, the most abundant immune cell in the body.
4) Neutrophils more quickly recognize and kill foreign challenges.

Lay the Foundation: Pre-clinical research Example

The lethal anthrax model used is the well-known mouse (Balb/c) model previously described by Welkos et al. (Infect. Immun. 51:795-800, 1986)

Day -8 -1 0 1 2 3 4 5 6 7 8 9 10 11

B. anthracis Challenge (strain Vollum 1B) 300

Oral Administration WGP Glucan (2 & 20 mg/Kg)

Survival Monitored

Day 0 1 2 3 4 5 6 7 8 9 10 11

* p<0.05
Types of Studies

- Two basics types of clinical studies exist:
  1) Observational
  2) Intervention- randomized, controlled trial (RCT)
- RCT is a managed study that can provide compelling evidence that a study treatment (intervention) causes an expected outcome
- Observational studies are less convincing because they observe people & correlate observations to human health

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Types of Study & Design Questions

- Study design is critical to eliminating unintentional bias.
- Single blind, open label, double blind, and crossover study designs all have merits.
- Double-blind study is considered to be the best.
- A crossover design reduces variations between treatment groups.

Suggested flow of clinical objectives

- Clinical studies to show basic product efficacy
- Explore, confirm, evaluate
- Clinical support for correlative biomarkers
- Properly powered, well-designed studies

Assess the Clinical Research

- Consistent results build credibility
- Multiple studies replicate benefit
- Multiple P.I.’s for independent verification
  - A key objective is to achieve significant scientific agreement...
- Use validated methods & surveys

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Elements of RCT Design

**Randomized**
- Subjects are randomly assigned to treatment or placebo
- Use a statistically valid random number generator

**Blinded**
- Single-subjects do not know if they are receiving placebo or treatment
- Double-subjects and investigators do not know...

**Placebo-controlled**
- Placebo is an inert substance lacking the effect of the treatment
- True “pharma” studies often use “standard of care” rather than placebo

Requisite Elements of a Clinical Study Protocol

- **Statement of objective**
- **Primary & secondary outcome(s)**
- **Design** (dbl-blind, crossover, etc.)
- **Subject inclusion & exclusion criteria**
  - Informed consent
- **Study Product, dose, placebo, study duration**
- **Study plan** (Varied format)

Statement of Objective

- “...To determine the effects of 10 days of specific strain of beta 1,3/1,6 glucan supplementation on Leukocytes, Cytokines, and Salivary Immunoglobulins following up to 120-min of walking/jogging in a warm, humid environment in subjects with below average ‘fitness.’”
- “...to conduct a randomized, double-blind, placebo-controlled clinical trial in young children (1-4 years old) to investigate the effects of yeast beta-glucan on common childhood infections in young children.

Primary and Secondary Outcomes

**One Primary Outcome**
- Cumulative days with infectious symptoms

**Secondary Outcomes**
- Proportion of subjects with at least one of the following: confirmed bacterial or viral infections
- Proportion of subjects that required, and average duration: a) antiviral treatment, b) antibiotic treatment, c) hospitalization
- Adverse Events
Study Design

- Open label
- Single blind
- Double blind
- Crossover
- Specialty studies
  - Safety evaluation
  - Dose titration
  - Others

Subject Inclusion and Exclusion Criteria

**Inclusion criteria**
- Age 18-70
- Frequent traveler
- Generally healthy
- Agree to study visits, procedures and compliance, informed consent signature

**Exclusion criteria**
- Current symptoms of illness
- Liver or kidney disease
- Allergy or other inflammatory disease
- Cigarette smoker
- >20 lb. weight loss or gain in past 3 months
- Unable to understand protocol

Investigation Study Product

- "...Investigational study or control will be provided in numbered bottles. When randomized, subjects will be given the lowest available randomization number."
- Randomization code held by Sponsor, not shared with P.I.
- Known serving size (or dose)
- Placebo should closely match color, flavor, physical characteristics of study product.

Typical Study Plan-Format

<table>
<thead>
<tr>
<th>Study plan:</th>
<th>V0</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
<th>V5</th>
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<tbody>
<tr>
<td>Study days</td>
<td>1</td>
<td>15</td>
<td>30</td>
<td>60</td>
<td>90</td>
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<td>Subjects' data and medical history</td>
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<tr>
<td>Clinical examination</td>
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<td>Eligibility criteria</td>
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<td>Randomization</td>
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<tr>
<td>Supplementation start</td>
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<tr>
<td>Innate immunity (Blood and saliva collection)</td>
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<tr>
<td>Symptoms of URTI</td>
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<tr>
<td>Mortality</td>
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<td>Medical confirmation of symptoms</td>
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</table>
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Understanding Outcomes

What kind of outcomes were employed in the study?

- Because of the complexity of immunology, the two major forms of outcomes that are of scientific and regulatory interest:
  - Biomarker outcomes
  - Physical health outcomes
- Many outcomes can be misinterpreted or are surrogates for the clinical endpoint. The outcome used in a human clinical study must have a demonstrable positive benefit for humans.
- The Institute of Medicine recommends a three-step biomarker selection process:
  - Analytical validation of biomarkers
  - Qualification that the evidence-based role of selected biomarkers has been confirmed with clinical endpoints
  - Use of a strong decision process on the evidence and specific context for the use of a biomarker as a surrogate endpoint exists.

Grading the Evidence

<table>
<thead>
<tr>
<th>Strength of Evidence</th>
<th>Grade I: Good/Strong</th>
<th>Grade II: Fair</th>
<th>Grade III: Limited/Weak</th>
<th>Grade IV: Expert Opinion</th>
<th>Grade V: Not Assignable</th>
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</thead>
<tbody>
<tr>
<td>Quality</td>
<td>Studies of strong design for question</td>
<td>Studies of strong design for question</td>
<td>Studies of weak design for question OR Inconsistent evidence due to execution problems</td>
<td>No research studies available; based on usual practice, expert opinion, clinical experience, or extrapolation from basic research</td>
<td>No evidence that pertains to question being addressed</td>
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<tr>
<td>Scientific validity</td>
<td>Studies of strong design for question</td>
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<td>No research studies available; based on usual practice, expert opinion, clinical experience, or extrapolation from basic research</td>
<td>No evidence that pertains to question being addressed</td>
</tr>
<tr>
<td>Consistency of findings across studies</td>
<td>Inconsistency among studies with strong design, OR Inconsistency among studies with weaker design</td>
<td>Repeatedly inconsistent among studies OR Inconsistency among studies OR Inconsistent evidence across studies</td>
<td>Inconsistent evidence with minor exceptions of weak</td>
<td>Conclusion supported solely by statements of informed experts or medical commentators</td>
<td>NA</td>
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</tbody>
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Understanding Outcomes—Review the Research

What is the quality and quantity of the clinical research?

- Look at the number of studies conducted, whether they were peer-review published and the quality of the studies. When reviewing the entire body of research, look for consistent outcomes from multiple studies.
- Peer-reviewed, double-blinded, placebo-controlled clinical studies are the gold standard, but other forms of human clinical study can be valid and provide support of efficacy.
- In vitro (test tube studies) may be interesting but insufficient to demonstrate clinical efficacy.
- Preclinical studies may demonstrate interesting health benefits, but are not directly applicable to humans.
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<tr>
<td><strong>Good/ Strong</strong></td>
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<tr>
<td><strong>Fair</strong></td>
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<tr>
<td><strong>Expert Opinion</strong></td>
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**Quantity**
- Number of studies
- Number of subjects in studies
- One to several good quality studies
- Large number of subjects studied

**Clinical Impact**
- Size of effect
- Some doubt about the statistical or clinical significance of the effect
- Studied outcome is an intermediate outcome or surrogate for the true outcome of interest

**Generializability**
- Studied population, intervention, and outcomes are similar to expected population of interest
- Some doubt about generalizability
- Serious doubt about generalizability due to narrow or different study population, intervention, or outcomes studied

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**Are the Outcomes Specific?**

- Was the research conducted with the specific ingredient or borrowed from other products?
  - The regulatory position that research supporting the safety and efficacy of probiotics must be strain-specific has implications for other immune-enhancing ingredients.
  - It is critical to ask whether the research was conducted with the specific ingredient that the study allegedly supports.
  - Borrowed (aka hijacked) science is junk science.
  - The specific biological strain or chemical structure matters as has been demonstrated repeatedly with, for example, probiotics, vitamin E tocopherols and various carotenoid sources.

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**Vaccine** 27 (2009) 5877-5884

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Nutritional Formula Enhanced Immune Function and Reduced Days of Symptoms of Upper Respiratory Tract Infection in Seniors


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**NUTRITION**

Oral intake of *Lactobacillus fermentum* CECT5716 enhances the effects of influenza vaccination.

Mónica Olivanero, Ph.D., Mónica Par Díez-Ropero, M.B.S., Salina Serra, Ph.D., Federico Laro-Villalba, Ph.D., Juan J. Ruíz-Ruiz, Ph.D., Ismael Navas, M.D., Juan Miguel Rodríguez, Ph.D., and Jordi Suné, Ph.D.

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- **N = 50:** Placebo vs. $10^{10}$ *L. fermentum* for 28 d
- **Flu vaccination at d 14**
- **At d 28 vaccine-specific IgA higher in probiotic group (but not specific IgG or IgM)**

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- **66 older subjects:** Placebo vs. formula providing antioxidants, Zn, Se, structured lipids and fructooligosaccharide for 183 d
- **Flu vaccination at d 15**
- **At d 57 vaccine-specific Ab to one flu strain higher in probiotic group**
Multiple Studies Replicate Benefit

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Presented</th>
<th>Published</th>
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<tbody>
<tr>
<td>3) Firefighters</td>
<td>2008 American Society of Sport Medicine</td>
<td>Under consideration</td>
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<tr>
<td>6) Medical Students</td>
<td>2010 British Society of Immunology Annual Meeting</td>
<td>Nutrition, 2012</td>
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<tr>
<td>7) Allergy</td>
<td>2011 FASEB (Exptl Biology) Annual Meeting</td>
<td>Manuscript in preparation</td>
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Medical Students Stress Study

**Study Protocol**
- U.K. study – 4th year medical students – Southampton University Hospital
- 90-day study – randomized, double-blinded, placebo controlled
- 250 mg Wellmune WGP or placebo
- Medically verified cold-flu symptoms-validated survey (WURSS-21 survey)
- Blood biomarker evaluation included cytokines and WBC profile

Presented: British Society for Immunology, December 2010. Accepted by Nutrition for publication.

- There was a 22% reduction in the total number of days with URTI symptoms in Wellmune group vs. placebo (198 days vs. 241 days, p=0.06).
- Wellmune WGP did not induce inflammatory cytokines.
Study Protocol

- 182 subjects completed the 2011 Austin TX Marathon
- Double-blinded, placebo-controlled
- 250 mg for four weeks post-race
- Placebo, Wellmune WGP Soluble and Dispersible
- Health questionnaire (WURSS-21) focusing on (1) upper respiratory tract infection (URTI) Symptoms & (2) daily health log of overall physical health

Dr. Brian McFarlin, University of Houston Department of Health and Human Performance

Texas Marathon Stress Study

Average Symptom Days per Subject

>34% Reduction in URTI Symptoms vs Control: * p<0.05%

Summary

- Safety first (published data).
- Peer-reviewed research of RCT’s is the standard for evidence of safety and efficacy
- Proper design of clinical studies with ultimate objective of demonstrating physical health benefit and correlating biomarker
- Product-specific data (no borrowed science) on a well-characterized product composition (known active components)

Questions & Answers