

Clinical Trial Of *Immune-Assist*™ as an Adjunct for Chemo- and Radiation Therapy

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ABSTRACT: This trial was designed to assess the efficacy of Immune Assist™ dietary supplement as an adjunct therapy for improving the immune function and reduce the side effects of conventional therapies. Immune Assist™ is a dietary supplement ingredient manufactured in the United States of America by Aloha Medicinals Inc. of Carson City, Nevada USA, and marketed worldwide as a raw material and included in many proprietary formulas. Immune Assist™ is an orally active, immune-modulating beta-glucan/heteropolysaccharide formulation, derived from six species of fungi regularly used in Traditional Chinese Medicine (TCM) for patients undergoing cancer therapy using conventional treatments. The Immune Assist™ formulation is a complex blend of immune-active polysaccharides of several categories. This includes high molecular weight (HMW) non-linear Heteropolysaccharides, which tend to be insoluble or only slightly soluble in water, as well as a significant percentage (>39%) of 1,3-1,6 beta glucans with a triple right hand helix tertiary conformation, which is the form shown in nearly all previous research to be most active in triggering mammalian immune response. These 1,3-1,6 beta-glucans have a range of molecular weight from less than 20 kD to over 2,000 kD, and are thought to be effective in activating a wide range of immune responses. A tremendous body of reference work exists indicating over 800 different species of higher basidiomycetes fungi (mushrooms) contain immune modulating and anti-tumor polysaccharide compounds with this tertiary structure. This has led to the development of a number of drugs of the Lentinan class. These drugs are widely used (primarily in Asia) as adjuncts in the treatment of cancer. Prior to the introduction of the Immune Assist™ formula, all the mushroom-derived polysaccharide immune modulators brought to market have been derived from a single species of fungi, consisting of only a single molecular weight polysaccharide with single tertiary structure, which indicates the probable attachment to only single immune triggering receptors at the cellular level. However, recent advances in immune science have revealed a wide range of different cellular receptors, each of which attach to different structural types and sizes of polysaccharide molecules. This multi-receptor, multi-structure binding indicates multiple mechanism of action in the triggering of immune responses, which is much more complex than was previously thought. While it has been known since the 1970's that Natural Killer (NK) cells were activated by polysaccharide triggers, it is now believed that most, if not all, of the different classes of immune cells are activated or triggered by the attachment of either pure polysaccharides or protein-bound polysaccharide molecules to receptors found at the cell surface such as CR3, LacCer, Dectin-1 and other activation receptors. It is this multi-receptor activation pathway that led to the development of Immune Assist™, which contains over 200 different immune-active polysaccharide structures. This complex polysaccharide formulation is thought to activate the full range of immune response in the human body. The species of fungi from which these polysaccharide immune compounds are

derived are: Agaricus blazei, Lentinula edodes, Grifola frondosa, Ganoderma lucidum, Trametes (Coriolus) versicolor, and hybrid Cordyceps sinensis.

The Immune Assist[®] composition for this trial was tableted at 500 mg per tablet, and co-administered along with chemotherapy or radiation therapy at a dose of 2000 mg., three times daily. This trial was designed as an active comparator study, comparing the effectiveness of Immune Assist[®] to Polyactin-A[®], rather than a placebo controlled trial, since active treatment in this group of subjects is obviously preferable to placebo. The trial was conducted among 56 subjects, 30 chosen to receive the Immune Assist[®] and another 26 comparable patients receiving the accepted pharmaceutical drug Polyactin-A[®] as a control group. All patients were in the middle-to-late stages (Stage 3 and 4) of cancer. The Polyactin-A[®] used in this trial was manufactured by Taixing Medicine Company, Ltd., Chengdu, PRC, lot number 20000327.

Results: Significant differences between the experimental group and the control group were shown in both disease progression and Quality of Life measurements. **Discussion:** The Immune Assist[®] formulation as tested in this trial showed greater effectiveness than the Polyactin-A[®] in both inhibiting disease progression and in limiting the severity of side effects. Immune Assist[®] shows great potential as an adjunct for the improvement of immunity and reduction of side effects in cancer patients undergoing conventional therapy.

Key words: Immune Assist[®], Beta-Glucans, heteropolysaccharides, Immune function.

INTRODUCTION

It has been found that fungal-derived polysaccharides such as beta-glucans and the HMW heteropolysaccharides are bioactive in many ways. Their use in stimulating and/or modulating immune response is well known, and is the basis for many immune modulator drugs such as Lentinan, PSK, Grifola, Schizophyllan and others. Extensive research into the mechanisms of action has been conducted since the introduction of this class of medication in the mid-1970s. The key structural element of the polysaccharide immune modulators leading to absorption in the human gut and resulting immunological activity seems to be determined by the presence of a 1,3 beta-bound, triple right hand helix tertiary structure of the polysaccharide molecule. To this triple main chain formation, various degrees and positions of branching, and the additions of other sugars such as five and six carbon sugars other than glucose, attached in side branches, vary receptivity to different binding receptors on the cell surface, and therefore the class of immune cells which are activated by the different structures of polysaccharides. In other words, when a 1,3 beta bound main chain of the correct shape is present, it will be absorbed through the gut wall via the M-cell transport pathway, and immunological activity conferred. But exactly which class of immune cell is activated appears to be determined more by the side branching of the molecule and by what other sugars are attached, and their positions. With over 250 different classes of immune cells identified to date, and with more than a dozen polysaccharide receptor types identified, it is assumed there are many different polysaccharide structures which are involved in the activation, or triggering, of the immune system function. For example some of the simple 1,3-1,6 beta-glucans will active NK cell activity with no effect on macrophage activity, while structurally related polysaccharides may trigger only macrophage activity with no effect on NK cells. Since it

is impossible at our present state of scientific understanding to determine exactly what aspects of immune dysfunction was responsible for the onset and progression of any particular instance of disease, it is difficult-to-impossible currently to determine exactly which polysaccharide immune compound out of the many types available, would be the best choice for treating any particular patient. In TCM, many types of medicinal mushrooms are commonly used for enhancing immune function, and in many instances multiple types are used concurrently. It is generally well understood in TCM that multiple mushrooms are more efficacious in activating immune function than any single type used alone. In researching the most commonly used medicinal mushrooms used in TCM for immune enhancement application, it was found that the ones most highly regarded are Royal Sun Agaricus mushroom (*A. blazei*), Maitake (*G. frondosa*), Shiitake (*L. edodes*), Reishi (*G. lucidum*), Turkey Tails (*C. versicolor*), and Caterpillar fungus (*C. sinensis*). With this historical usage in mind, the formulators of Immune Assist[®] developed proprietary methods for concentration and purification of the polysaccharide compounds from these sources. This research group found apparent roles of the polysaccharides among the subjects during these trials. All subjects were being treated concurrently with radiotherapy or chemotherapy.

MATERIALS AND METHODS

1. Study subject selection criterion:

This trial was conducted with volunteer subjects who had given their informed consent, and was overseen by an ethics committee ensuring compliance with the International Conference of Harmonisation Guidelines for Good Clinical Practice. Subject selection was based upon the following criteria.

- 1) The patients all had apparent pathological diagnoses.
- 2) The patients have had no surgery before, or have had surgery but had a relapse, or have new transference of tumor about focus. All subjects had clinical focus of the observation markers by X-ray and CT in order to estimate the disease extent and progression.
- 3) The patients have normal function of liver and kidneys before treatment, a Kafnofsky score greater than 60, and could be expected to live more than 3 months.
- 4) According to the international standard of TNM by stages, the middle-late cases are chosen; American measure of stage 3 and stage 4.
- 5) White Blood cell count $> 4.0 \times 10^9/L$. and blood platelet count $> 100 \times 10^9/L$.
- 6) The patients were all being treated concurrently by radiotherapy or chemotherapy.
- 7) Complete randomization of subjects between the two groups was maintained, ensuring accurate and unbiased trial results.

The subjects chosen were all middle-late stage patients with malignant tumors in all 56 subjects. Among the 56 subjects, 30 were in the experimental group, and 26 were in the control group. All subjects for the two groups came as referrals for this trial from co-operative hospitals. The subjects had similar conditions, such as physical condition, total number of white blood cell count and granular leukocyte count, appetite, and the clinical treatment plans of radiotherapy or chemotherapy were comparable in each subject. The white blood cell count in the two groups had no significant differences through comparison by statistical treatment before trials. The details of the two groups can be seen from tables 1 and 2.

Table 1. The common comparison between two groups

	number	Gastric carcinoma	Liver carcinoma	Lung carcinoma	Large intestine carcinoma	Naso-pharyngeal carcinoma
Experimental group	30	6	10	4	5	5
Control group	26	6	9	4	6	1

Table 2. The comparison of total number of white blood cells between the two groups before trials

	Total number of white blood cells ($\times 10^9/L$)			
	number	<3.5-4.0	4.0-5.0	>5.0
Experimental group	30	3 (10%)	21 (70%)	6 (20%)
Control group	26	2 (7%)	19 (73%)	5 (19%)

2) Experimental methods:

The two groups had basically the same radiotherapy and chemotherapy plans, procedures, and similar anti-nausea drug therapy (Shudan[®]). The subjects in the experimental group begin to take the Immune Assist[®] tablets for one week before radiotherapy and chemotherapy, 3 x day, 4 tablets each time (total 6 grams/day). The subjects in the control group begin to take the tablets of Polyactin-A[®] for one week before radiotherapy and chemotherapy, 3 x day, 10 mg each time (total 30 mg/day). Both groups continued to take the tablets during the course of treatment and afterwards for a total of 2 months.

3) Observation markers:

- 1) Vomiting episodes were recorded, with four classes of vomiting categorized: 0 x/day, 1-2 x/day, 3-4 x/day, 5 or more x/day;
- 2) Three categories of appetite were recorded: No food or less than half of usual food servings consumed, half of usual food servings consumed, usual food servings consumed.
- 3) Alteration of hemogram noted, total CBC was conducted every 3 days before and after radiotherapy and chemotherapy.
- 4) Observation of other adverse reactions: i.e. hair loss, changes in organ function; such as stomach, intestines, heart, liver, kidney; sleep loss, Vomiting, etc.
- 5) Statistical determination of QOL condition: Divide the conditions of markers 1-4 for the assessment of quality of life: According to the KPS, adding 10 points after treatment is

considered improvement, increasing or decreasing by 4 points is stable, and reducing 10 points or more is considered a decline in condition.

RESULTS

After radiotherapy or chemotherapy, comparisons in the various trial parameters were made between the two groups. These can be seen from Tables 3, 4, 5 and 6.

Table 3. Alteration in blood count of the two groups after chemotherapy

		Total number of white blood cells (x10 ⁹ /L)			Number of granular leukocyte	
	number	<3.0	3.0-4.0	>4.0	<50%	>50%
Experimental group	30	4 (13%)	5 (17%)	21 (70%)	11 (37%)	19 (63%)
Control group	26	5 (19%)	7 (27%)	14 (54%)	16 (62%)	10 (38%)

P<0.1

Table 4. Comparison of vomiting after treatment

	number	0 x/day	1-2 x/day	3-4 x/day	5+ x/day
Experimental group	30	24 (80%)	2 (7%)	2 (7%)	2 (7%)
Control group	26	18 (69%)	3 (12%)	3 (12%)	2 (8%)

P<0.01

Table 5. Appetite comparison after treatment

	number	< Half servings food	Half servings food	Full servings food
Experimental group	30	4 (13%)	17 (57%)	9 (30%)
Control group	26	6 (23%)	12 (46%)	8 (30%)

P<0.01

Table 6. Comparison of KPS value between the two groups

	<i>number</i>	<i>Improvement or stable (%)</i>	<i>Decline (%)</i>
Experimental group	30	23 (78%)	2 (22%)
Control group	26	19 (73%)	7 (27%)

The result indicates the score of the experimental group is higher than that of the control group.

Adverse Reactions:

The reaction of the digestive tract (i.e. nausea) is very light for both groups: the Immune Assist[®] group is 30% (9/30), and the control group is 50% (13/26); Hair loss was 8.3% in the experimental group and 10% in the control group. Both groups showed no abnormalities of the heart, liver and kidneys. There were two cases of lung infection in the control group, which were brought under control after treatment with antibiotics. No deaths occurred in either group during the course of these trials.

Typical examples:

Example 1: Female, 58 years old with fluid retention in left thoracic cavity. About 600 ml of fluid with blood was extracted on the second day after entering the hospital. The adenocarcinoma could be seen from testing, and the disease was determined to be membrana pleuralis transference of carcinoma of liver. Immune Assist[®] tablets were added to the treatment plan, and continued administration after chemotherapy. The respiratory tract symptoms were relieved, and the water retention in the thoracic cavity was controlled. The patient's condition was stable and only a loss of hair was noted as a side effect of the chemotherapy. She gained 1 kg to her body weight and her KPS score increased by 60. A followup CBC after chemotherapy showed white blood cell count was $3.8 \times 10^9/L$, and the functions of liver and kidneys were normal.

Example 2: Male, 69 years old with carcinoma of right lung. He entered the hospital for abdominal pain following chemotherapy treatments for two months and radiotherapy of 1 month. The disease was very aggressive with anemia, and his KPS score was 40. Immune Assist[®] tablets were added to the treatment plan, and the conventional therapy was continued. Subject's abdominal pain was relieved, his appetite improved, and overall condition was much improved.

DISCUSSION

- 1) Immune Assist[®] tablets: It was shown in this trial that the mixed immune-active polysaccharides in Immune Assist[®] can inhibit the protein synthesis of cancer cells, change the physiological condition of cancer cells, inhibit the growth and transference of cancer cells, relieve the side effects of the chemotherapy drugs, improved the patient's sleep and appetite and resulted in overall improvement of the symptoms.
- 2) The Immune Assist[®] has an apparent role in controlling and improving the immunity. After taking the Immune Assist[®] tablets, nonspecific immunity of the body was enhanced, improvements in the secretion of IGA, increase in the function of monocyte-macrophage and in the activity of NK cells, and in maintaining the immunological balance of the body.
- 3) The Immune Assist[®] has antagonistic action for the complications caused by the use of the anti-tumor drugs, and the drop in WBC caused by the conventional therapy that is usually seen was reduced.
- 4) The compounds in Polyactin-A[®] used by the control group are immune enhancing glucomannan peptides. This is a highly effective, pharmaceutically available immune enhancement drug. It enhances immunity and increases the activity of phagocytes and other white blood cells. It is used for reducing the drop in white blood cells commonly seen during cancer treatment using radio- and chemotherapy.
- 5) The results shown during this trial indicate that the Immune Assist[®] has a greater effect than the commonly used adjunct Polyactin-A[®]. This presents a potential for its use as an adjuvant in the treatment of various types of cancer, for protecting the haematopoiesis function of the bone marrow, inducing the action of the digestive tract, increasing the cellular immunity, increasing the activity of the NK cells, the LAK cell and the ratio of the T_H/T_S cells. The effects of the Immune Assist[®] formulation is greater than that of Polyactin-A[®], and presents a potentially excellent role for use adjunctively in chemo- and radiation therapy.

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