NON-CONFIDENTIAL

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Application for the Approval of Lentinex®, derived from the Mushroom *Lentinus edodes*, as a dietary supplement.

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients

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Introduction

Lentinex® is a liquid product obtained through a fermentation process with *Lentinus edodes* (the Shiitake mushroom) and is manufactured by GlycaNova A/S, a Danish food company. It is a clear, light brown liquid containing glucose, other sugars, protein and polysaccharides. All of these compound classes have already received GRAS status. *Lentinus edodes* produces lentinan, a well known polysaccharide which has a long history of use as a dietary supplement. The fruiting body of the mushroom has been marketed extensively in the EU while aqueous extracts of the mushroom have also been marketed either as capsules or liquid extracts. GlycaNova intend to market Lentinex® for use as a dietary supplement to companies within the EU as an alternative form of the lentinan from *L. edodes*. This will present a standardised lentinan product that is free from fungal cellular debris, has a long shelf life and offers consumers an increased choice when selecting lentinan containing products.

Approval of this product is being requested under EC Regulation No. 258/97 which is conceived with the introduction of novel foods and ingredients into the EU and ensures that the novel food in question has been assessed for its safety prior to its introduction to the general public. Lentinex® is derived from plant (mushroom) material obtained from non-GM sources and the classification under section 4, “Scientific Classification of Novel Foods for the Assessment of Wholesomeness” which facilitates the nutritional and safety of the novel foods, is applicable. Lentinex® is classified as class 2 “Complex NF from non-GM Sources” and is also applicable to sub-heading 2.1 “The source of the NF has a history of food safety”.

I. SPECIFICATIONS OF THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to the specifications of the novel food:

"…is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?"

"Is the information representative of the novel food when produced on a commercial scale?"

"Is there an appropriate specification (including species, taxon *etc*. for living organisms) to ensure that the novel food marketed is the same as that evaluated?"

I.1 SAFETY INFORMATION

According to the literature, *Lentinus edodes* has been consumed for more that 3000 years. Thus, the safety of the mushroom must be regarded as proven and the mushroom is not known to produce, or contain harmful toxicants. The ingredients GlycaNova use in the production process are all GRAS listed so there will be no external contaminants or nutrient.

I.2 CHEMISTRY OF LENTINAN

In the literature, the term “lentinan” is often used as a general term for *Lentinus edodes* polysaccharides. As polysaccharides in general comprise a wide variety of polymers made from one or more types of monosaccharide, connected by different kinds of glycosidic bonds, with different degrees of branching, possible chemical substituents and ternary structures, there is no straightforward method to quantify and characterise polysaccharides.

Polysaccharides from *L. edodes* are often quantified by ethanol precipitation, a method that GlycaNova has implemented for use in product definition and QC. This method, in particular, quantifies the beta-glucans that are of special interest from a DS point of view. Beta-glucan from *L. edodes* is equivalent to “lentinan” as this term is used in the DS market.

The term “lentinan” is also defined very specifically by IUPAC as a β-(1-3) β-(1-6)-D glucan that has a molecular weight of approximately 5×10^5 Daltons, a degree of branching of 2/5 and a triple helical tertiary structure. Lentinan’s 2006 Medical Subject Headings Descriptor Data are: Tree: DO9.698.365.089.500 (National Library of Medicine) and its Registry Number is 9051-97. However, as there is no analytical method available that corresponds to this definition, it is not applicable for product QC in the DS market.

I.3 SPECIFICATION OF THE LENTINEX® PRODUCT

The specification of Lentinex® is given in Table I.3

Table I.3 Specifications for Lentinex®

Parameter	Method	Spec.
Appearance	Visual inspection	Light brown, slightly turbid
Microbiological data	Total viability count on PCA and MDSA	Sterile
Lentinan	GNm-003 (Ethanol precipitation)	1 mg/ml ± 0.2
Residual glucose	Enzymatic	< 20 mg/ml
Total protein	GNm-005 (Bradford)	< 100 μg/ml
pH	pH-meter	3.0 - 4.0
Protein including free amino acids	Kjeldahl (external method)	< 10 mg/ml
Pesticides	SLV Method 200 (external method)	Not detectable

A Certificate of Analysis (CoA) will follow each batch of Lentinex® product. An example in CoA is given in Appendix E.

I.4 ENERGY CONTENT

Lentinex® contains 14.4 kcal per 100 ml (= 100 grams).

I.5 LEVEL OF UNDESIRABLE TRACE METALS

Lentinex® is manufactured using drinking water from the local waterworks. This waterworks supply water in accordance with Norwegian legislation and directives. The levels of selected heavy metals in Lentinex® are given in Table I.4.

Table I.5: Heavy metals content in Lentinex®

Cd	<10ug/l
Cr	<50ug/l
Mn	<280ug/l
Pb	<70ug/l
Ni	<100ug/l
Hg	< 20μg/l
Cu	<550ug/l

I.6 LEVEL OF PESTICIDES

Analysis has shown that Lentinex® does not contain detectable amounts of pesticides.

Analysed by *AnalyCen*, Moss, Norway (an accredited laboratory) according to the Norwegian Medicines Agency Method no. 200

I.7 FREE AMINO ACIDS

Earlier total protein was determined by the Bradford method. To be in accordance with the common method for food in Norway, the Kjeldahl method is implemented.

The free amino acids level in Lentinex® has been determined by an independent analytical laboratory in Norway (*AnalyCen*, Moss, Norway - an accredited laboratory).

Table I.7 Amino acid profile

Amino acid	AA (g/kg solid)
Asparagine	0.52
Treonine	0.55
Serine	0.50
Glutamic acid	1.39
Proline	0.10
Glycine	0.51
Alanine	0.65
Valine	0.59
Cystine	<0.05
Methionine	<0.05
Isoleucine	0.44
Leucine	0.69
Tyrosine	0.21
Phenylanine	0.50
Histidine	0.11
Lysine	0.41
Arginine	0.25
Glutamine	<0.05

I.8 FATS

The fat content in Lentinex® has been determined to < 40 mg/L by an independent analytical laboratory in Norway.

Analysed by *AnalyCen*, Moss, Norway (an accredited laboratory).

I.9 PRODUCT STERILITY

As described in section II.2 Lentinex® is heat sterilised as part of the production process. Sterility is tested by plating the product on PCA and MDSA agar plates. The acceptance criterion is zero cfu (colony forming units) per ml, denoted as “OK” on the CoA.

I.10 STABILITY OF LENTINEX®

There is no published information of stability of lentinan based products from mushrooms. Even though traditional mushroom products are generally regarded as stable products, GlycaNova undertook stability studies on Lentinex®. Included in the test were important parameters such as content of lentinan, free sugar, protein, pH and product appearance. It was found that Lentinex® can be sold with a 12 month claim for stability.

I.11 QUALITY ASSURANCE

The data provided in this application is representative for the commercial product. The production process is stable and not affected by scale provided the ratio of starting material to final volume is constant. Five production batches are compared in Table I.11.

Table I.11

	Lentinan		Free glucose	Protein (Bradford)	Protein (Kjeldahl)
Batch	**mg/ml**	**pH**	**mg/ml**	**ug/ml**	**mg/ml**
10-071002	1,0	3,5	15,6	39,2	
10-070921	1,2	3,5	13,5	61,8	<3
10-070511	0,8	3,6	16,5	22,2	6
10-050530	1,1	3,5	21,0	41,8	
10-061110	1,1	3,5	15,3	29,6	8
Average	1,0	3,5	16,4	38,9	7,0
Stdav	0,2	0,0	2,8	15,0	1,4

These data are the basis for the specifications for Lentinex ® given in section I.3, and on the CoA's in Appendix F.

II. EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

"Does the novel food undergo a production process?"

"Is there a history of use of the production process for the food?"

"Does the process result in a significant change in the composition or structure of the NF compared to its traditional counterpart?"

II.1 CURRENT METHODS FOR PRODUCING DIETARY SUPPLEMENTS (DSs) FROM MUSHROOMS SUCH AS *Lentinus edodes*

There are presently several approaches predominantly used in the Far East for producing DSs from *L. edodes* and other mushrooms:

1. Dried and pulverised naturally growing mushroom fruit-bodies in the form of capsules or tablets.
2. Artificially cultivated fruit-bodies dried and pulverised, hot water or alcohol extracts from them, or the same extracts concentrated and their mixtures.
3. Dried and pulverised preparations of the combined substrate, mycelium and mushroom primordia following inoculation of edible semi-solid medium (usually grains).
4. More recently, biomass, extracts of mycelium or the broth harvested from submerged liquid mycelial cultures grown in bioreactors are being used.

There are currently no standard protocols for guaranteeing edible mushroom DSs for product quality and efficacy. Many mushrooms, including *L. edodes*, have been used for

traditional health benefits for long periods of time, in some cases for thousands of years. There are few documented examples of adverse effects to man and as such could be considered as 'safe'.

The main advantage of using mushroom-based DSs with respect to safety includes the following:

1. The overwhelming majority of mushrooms, such as *L.edodes,* used for the production of DSs is pure cultures and is cultivated artificially. Proper culture maintenance ensures genetic uniformity and stability.
2. Mushrooms are easily propagated vegetative and thus keep to one clone. The mycelium can be stored for long periods of time and demonstrate genetic and biochemical consistency.
3. Many mushrooms, such as *L. edodes*, are capable of growing in the form of mycelial biomass in submerged controlled fermenter (bioreactor) culture. From a safety aspect, this will undoubtedly be the major forward direction for many aspects of mushroom DSs.

Marketed DSs from *L. edodes* are currently mostly being derived from whole fresh or dried mushrooms. Such products normally give no indication of the exact amount of lentinan present. Thus, the current use of the complex mushroom fruit-body does imply that the standardisation of the DS from such mushrooms is problematic.

The use of pure culture mycelial cultivation in defined liquid fermentation conditions will offer several advantages over traditional whole mushroom methods:

1. Speed of growth with reduction in production time;
2. Optimisation of culture medium composition;
3. Optimisation of physico-chemical conditions to allow regulation of mushroom metabolism; and
4. Improved yield of specific products.

Increasingly in other parts of the world (such as Japan), producers of DSs such as lentinan and others are moving or have already moved over to fermenter produced

products. Clearly, this will increasingly become the mode of choice for the production of most mushroom DSs. Such fermenter methods will give increased levels of reproducibility and known concentration and consistency of the main products. Lentinex® is an example of such a product.

II.2 DESCRIPTION OF GLYCANOVA PROCESS FOR PRODUCING LENTINEX®

GlycaNova has developed a sophisticated production technology based on the cultivation of mushrooms in aseptic submerged liquid cultivation which allows the manufacture of a lentinan ingredient - Lentinex®. This method uses stable, pure cultures of the fungus, defined and commercial available ingredients as nutrients, and carefully controlled fermentation conditions, e.g. temperature, aeration rate, pH etc.

Lentinus edodes is grown in the liquid system that contains sterilised medium components: 30g/l glucose (separately sterilised), 6 g/l malt extract, 10 g/l soy peptone and 6 g/l yeast extract.

The biomass is removed by filtration and the remaining cell free liquid is the raw material for the lentinan-based products. Final concentrations of lentinan are adjusted with water to the required concentration.

The GlycaNova process cultivates *L. edodes* mycelium in a liquid aerobic fermentation process that ensures complete control of the growth and physiology of the organism ensuring a constant supply of a clear, product of defined composition.

Post fermentation, Lentinex® is heat sterilized, using the traditional 20 minutes at 115 ^{o}C procedure.

Due to market demand 0.1% of sodium benzoate (E211) may be added to preserve the product and support long term storage even after the customer has opened the product.

Process controls:

For 2L and 3L bioreactors a daily log (pH and oxygen content) is recorded automatically, and results are checked manually daily and recorded in a log book.

For the 50L bioreactor samples for pH and sterility are taken at the start, in the middle and at the end of the fermentation. Results are recorded in a log book.

For the 750L bioreactor, samples (sterility) are taken daily from start of fermentation and recorded in a log book. Oxygen content and pH are automatically monitored and recorded daily.

Lentinex® is produced according to GMP (Good Manufacturing Practice). Written operating procedures (SOPs), Quality Control and Quality Assurance is established at the manufacture. The most relevant SOPs are included in Appendix H.

GlycaNova is guided by the guideline laid down in the ICH Harmonised Tripartite Guideline, "GOOD MANUFACTURING PRACTICE GUIDE FOR ACTIVE PHARMACEUTICAL INGREDIENTS, Q7, Current version".

Although this is cGMP for production of API for the pharmaceutical industry GlycaNova has chosen to follow this guideline. This is to reach a quality level that will ensure Lentinex ® is produced under the best practices obtainable.

II.3 COMPARISON OF LENTINEX® TO TRADITIONAL COUNTERPARTS

The GlycaNova production process relies on the physiological export of lentinan from *L.edodes* mycelia growing in a liquid fermentation process. Traditional counterparts in the DS market produce lentinan by different extraction processes, based on mushroom fruiting bodies or harvested mycelia that subject the lentinan to different chemical treatments and agents. Thus, while lentinan produced by the GlycaNova process must be considered native, traditional counterparts will only retain native lentinan to some extent, depending on their particular production process. However, lentinan from the GlycaNova process is thus very similar to the lentinan present in fresh Shiitake mushroom marketed as a normal food ingredient.

III. HISTORY OF THE ORGANISM USED AS THE SOURCE

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the history of the source organism:

"Is the novel food obtained from a biological source (*i.e.*, a plant, animal or micro-organism)?"

"Is the organism applied a GM organism?"

"Is the organism characterised?"

"Is there information that shows the organism and the Novel Food are not harmful to humans?"

III.1 HISTORY OF *Lentinus edodes* AS A SOURCE OF LENTINAN

Lentinus edodes, taxonomic classification: *Lentinus edodes* (Berk.) Sing. (Agaricomycetideae) is a mushroom fungus (known widely as the Shiitake mushroom), indigenous to Japan, China and other Asian countries with temperate climates, growing on fallen deciduous trees. However, it has been grown artificially for several centuries on cut logs and, more recently, worldwide mass cultivation is predominantly achieved by enriched sawdust culture technology. Fresh mushrooms are now produced widely in the UK and Europe, and are available in most supermarkets and fresh food stores. In the USA, the fresh mushroom is approaching the sales level of the common white button mushroom. In Japan and China it has long been a regular part of dietary intake in fresh and dried form.

This mushroom has been renowned in Japan and China for thousands of years both as a food and as a component of traditional Chinese medicine. Furthermore, the exotic and delicious taste of this mushroom is a central part of many Oriental dishes which are increasingly being adopted in the West mainly due to the greatly increased availability of these mushrooms. In the UK, the major grower of the fresh mushroom is in Lancashire.

L. edodes is now established as the leading edible mushroom worldwide that can be used both as a nutritious and tasty food, and as a rich source of β-glucans. While the natural growing process utilises complex, lignocellulolytic substrates of undefined chemical composition, the fungus can also be grown in a mycelial form in controlled, nutrient defined liquid fermentation (bioreactor) and it is from this source that Lentinex® is prepared, and is the subject of this application. By means of batch liquid fermentation, a pure, uniform and reproducible biomass can be generated with a more easily defined organic composition.

Commercial DS concentrates from several mushrooms, including *L. edodes*, are available as tablets, capsules or elixirs and are widely on sale in most Oriental countries, and in the USA and increasingly in Europe in natural food/health stores.

Consequently, *L.edodes* must be considered safe and not harmful to humans.

III.2 ORIGIN AND OCCURRENCE OF LENTINAN IN NATURE

Lentinan and, more specifically, β-glucans belong to a structurally diverse group of biological macromolecules of widespread occurrence in nature and have been part of the food chain for millennia. Lentinan isolated from edible mushrooms in general (fruit-body, submerged cultured mycelial biomass, or liquid culture broth) mainly consists of water soluble β-glucans.

III.3 CLASSIFICATION OF THE ORGANISM

Lentinex® is produced using the Basidiomycete *Lentinus edodes*. The International Depositary Authority accession number is IHEM18992. It is not a GM organism.

III.4 LENTINEX® IS NOT HARMFUL TO HUMANS

As described in section XIII.3 and Appendix B, the safety of Lentinex® was tested in a clinical study, concluding that Lentinex® is not harmful to humans.

IX. ANTICIPATED INTAKE/EXTENT OF USE OF NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

“Is there information on the anticipated uses of the novel food based on its properties?”

“Is there information to show anticipated intakes for groups predicted to be at risk?”

“Will introduction of the novel food be restricted geographically?”

“Will the novel food replace other foods in the diet?”

IX.1 INTENDED USE

Lentinex® is intended for use as a food supplement or dietary supplement. Lentinex® is intended for ingestion in the form of a capsule, a liquid, powder, gel or in dairy products, e.g. yoghurts and drinking yoghurts, fruit and vegetable products, e.g. fruit drinks, nectars, soft drinks, soups, etc, confectionary, e.g. chocolate bars, candy, chewing gums, etc. or in cooked or processed foods. The food categories also include baked goods and baking mixes; beverages and beverage bases, breakfast cereals; grain products and pastas; processed fruits and fruit juices.

Lentinex® may be added to a variety of food categories that will be eaten by all segments of the population.

GlycaNova proposes to market Lentinex®, as a DS with a recommended daily intake of 1 – 2.5 mg of lentinan, corresponding to 1-2.5 ml of Lentinex®. This is way below levels used by other suppliers of β-glucan.

Lentinex® has been tested in bread, yoghurts, fruit drinks and soft drinks, sweets, chewing gum and food oils. The purpose was to evaluate if Lentinex® was suited to be included in these products and to determine the effect of the processing conditions on the bioactive material. In all the foods described above, mixing Lentinex® into the food material had no impact on organoleptic characteristics of the products. The lentinan was not affected by the processing of the final product.

These tests were carried out on behalf of potential customers. We have been given permission to include data in our application that refers to the fate of Lentinex® in fruit juices and yoghurt. This is included in Appendix G.

No groups are predicted to be at risk after intake of the recommended dosage of Lentinex®. The distribution of Lentinex® will not be restricted geographically.

IX.2 LABELLING

GlycaNova will produce Lentinex® as an ingredient for e.g. functional food as described in the application. This will be done through a business to business cooperation. Since GlycaNova for the moment only have customers in USA the discussion of the presentation of the Lentinex® content has not been initiated in EU.

GlycaNova will propose the following labelling for Lentinex® to our business cooperation partners:

Lentinex®, a standardised lentinan product

Calories	<144 cal/ml
Protein	<1.0%
Lentinan	1 mg/ml
Fat (total)	Not detected
Cholesterol (mg)	Not detected
Sugars	<2.5%
Ash	<0.2%
Sodium (Salt)	<0.035%

Also the addition of 0.1% sodium benzoate (E211) will be declared if required. Some customers have indicated that they require addition of sodium benzoate as a preservative.

X. INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE

X.1 HUMAN EXPERIENCES WITH CONSUMPTION OF LENTINAN

There are many natural sources of β-glucans which can vary in molecular structure. Beta-glucans from oats and other cereals are predominantly composed of (1-3), (1-4), β-D-glucose. Yeasts and mushrooms are he best known non-green sources and are mainly composed of (1-3), (1-6) β-D-glucose.

While oats β-glucans have been shown to have many health benefits they are particularly recognised for their cardiovascular properties. This is believed to be due to their high water solubility and ability to build up viscosity in the gastrointestinal tract with resultant health benefits, e.g. lowering cholesterol, controlling blood glucose, improved bowel function and weight control.

The health benefit of *Lentinus edodes* is well accepted in certain societies. These societies typically recommend a daily dose of 6 to 16 grams of dried mushroom. This corresponds to 50 to 140 grams of fresh mushroom, so an average of 90 grams of fresh *L. edodes* would be recommended to obtain the health effect. At about 20 grams per mushroom, 4.5 fresh L edodes would contain about 1.8 grams of lentinan.

There have been over 1,000 research papers written about β-glucans in general since the 1960's, and at least 400 of these deal directly with lentinan from *L. edodes*.

The consensus of the scientific community is that Lentinan in appropriate amounts and by the oral route of administration is safe for human consumption.

X.2 LENTINEX® AND CONSUMPTION BY HUMANS

GlycaNova has carried out a study in healthy volunteers (see Appendix B). This was a double-cross over blind study where 2.5 ml Lentinex® were administered to the participants.

On the safety point of view, there was no difference in the incidence of adverse events (including side effects and all type of disease and accidents occurring during the clinical study) between both types of supplementation. Moreover, Lentinex® was not affecting negatively any of the safety variables measured in blood tests.

XI. NUTRITIONAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following question must be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

"Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?"

XI.1 NUTRITIONAL EQUIVALENCE

Lentinex® will be marketed as a DS, and is not meant to replace existing food in the diet. However, containing lentinan, Lentinex® is nutritionally equivalent to other marketed products based on the Shiitake mushroom. One bottle of Lentinex® containing 100ml liquid with 1 mg/ml lentinan contains the same amount of lentinan as approx. ¼ fresh Shiitake mushroom, according to figures for lentinan content given in the literature (see section X.1).

The content of free glucose and protein in Lentinex® is of less nutritional significance. As described in section IX.1 the maximum recommended daily dose is 2.5 mg lentinan per day, corresponding to 2.5 ml Lentinex® per day. This dose contains < 50 mg free glucose and < 25 mg protein. Based on the Dietary Reference Intake recommendations from the Institute of Medicine of the USA National Academy, this corresponds to <0.04% and <0.04% for carbohydrates and protein, resp. (calculated for a male in the age group 19-30 years).

XII. MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following question must be addressed to ensure sufficient microbiological information on the novel food:

“Is the presence of any micro-organisms or their metabolites due to the novelty of the product/process?”

XII.1 MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Lentinex ® is a sterile product and contains no living micro-organisms, due to the fact that it is heat sterilised as an integral part of the production process, as described in II.2.

XIII. TOXICOLOGICAL ASSESSMENT OF THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient toxicological information pertaining to the novel food:

“Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?”

“Compared to the traditional counterpart, does the Novel Food contain any new toxicants or changed levels of existing toxicants?”

“Are there any allergy risks associated with consumption of the Novel Food?”

XIII.1 TOXICOLOGY AND SAFETY INFORMATION (METABOLISM)

As described in sections I.1 and II.3 traditional counterparts have been consumed by humans for many centuries, thus effectively establishing a baseline of safety and non-harmful use.

Lentinex® has been extensively studied with several animal species, *viz*. mouse, rat and pig (see Appendix C) and there is sound evidence that Lentinex® does not cause any significant or harmful changes in metabolism of these species, under the intended conditions of planned use.

All rats were monitored for toxic effects. In addition to the immunological variables and haematology, all rats were observed for weight loss, lethargy and hind limbs were monitored for paralyses. In addition, the Lentinex® group was monitored for toxic effects, ataxia and behavioural changes. No abnormalities were observed.

A cross-over placebo, controlled human study with Lentinex® demonstrated the safety of this product in a group of healthy elderly humans (Appendix B).

To better understand and compare the dosage levels, doses are presented as mg/kg/day of lentinan. In the BN rat study 3 dose levels were used, 7.8 mg/kg, 8.3 mg/kg and 12 mg/kg

In the BNML model studies, doses ranged from 0.5 mg/kg to 20 mg/kg.

In comparison the daily dose given in the human study was 2.5 mg corresponding to 0.04 mg/kg.

Thus the toxicological dose ranges between 15 to 560 times higher than the human dose, with no safety concerns.

XIII.2 ALLERGENICITY

To our knowledge allergic reactions to orally taken Shiitake mushroom, is very seldom, taken the high consumption into consideration.

By a PubMed search, and search in a couple of governmental ADR-databases, only one case was found. A generalized exanthem developed in a patient after eating raw shiitake mushrooms. Reactions to prick and patch tests with shiitake mushrooms were negative. The skin eruption in this patient corresponded to the previously reported shiitake-induced toxicodermia reported in mushroom workers.

Due to the production method, Lentinex® does not contain spores, or derivatives thereof.

In a study in 40 healthy elderly, ingesting 2.5 ml Lentinex® a day, for a period of 6 weeks, no allergic reaction was observed. A summary of the study is given below, with the complete study given in Appendix B:

XIII.3 SUMMARY OF A STUDY ON LENTINEX® IN HUMANS

Lentinex® clinical study (Norway)

In the present study, Lentinex® has been tested for its ability to stimulate the immune response and/or restore the immune response of healthy elderly over the age of 65 and for its safety. It is known that immune response increases in the first years of life to reach its maximum in the adult period, prior to reduce progressively with ageing.

The study involved 42 elderly subjects (Caucasian) from the surrounding of Bergen in Norway. They received randomly either Lentinex® or placebo for a first period of 6 weeks, followed by a 4 weeks wash-out period with no supplementation. The participants were then receiving the cross supplementation for a second period of 6 weeks, either placebo (if they received primarily Lentinex®) or Lentinex® (if they received primarily placebo). The study was performed double blind, meaning that none of the participants neither the medical staff knew who received what. The study was performed according to the principles of the good clinical practise as defined in the International Conference of Harmonization guidelines. The advantage of using such a study design was to let each participant his/her own control in order to reduce the possible bias of the high individual variation of immune response.

There was no difference in the incidence of adverse events between both types of supplementation. Moreover Lentinex® was not affecting negatively any of the safety variables measured in blood tests.

In conclusion, Lentinex® given orally for 6 weeks to elderly subjects stimulate the immune response (B-cells) and appears to be a safe supplement, not causing unwanted adverse reactions.

Conclusions

Lentinex® is an aqueous system produced by a proprietary fermentation process with the Basidiomycete *Lentinus edodes* (the Shiitake mushroom). Lentinex® is a clear, light brown liquid containing lentinan, protein and free glucose. The physical properties of Lentinex® have been characterized, and product specifications have been established.

In the production process, *L. edodes* mycelium is cultivated in a bioreactor that is rigorously controlled. The production technology itself is similar to processes used in the production of a range of food products such as bakers yeast, food acids, beer, etc. The Lentinex® GMP production is carefully monitored and Quality Assurance programs have been established to assure that the product meets release specifications. No toxicants (e.g. fertilizers, pesticides) are added to the aqueous system.

The safety of orally administered lentinan is well established. A detailed summary of previously published acute, subacute/subchronic, chronic, fertility and reproduction toxicology and teratology safety data and human safety data has been presented in the Appendixes of the present Novel Food Application.

GlycaNova has conducted animal and human studies with Lentinex®. The evaluation of Lentinex® in mice, rats, chickens, and pigs showed that Lentinex® was found to be safe at all tested doses. These results were consistent with other lentinan-related publications. Finally, a cross-over, placebo-controlled human trial was conducted. Forty-two elderly, healthy individuals of both genders were administered Lentinex® (2.5 mg of lentinan per day), or a cellulose placebo, for 6 weeks. The results of this study indicated that the ingestion of Lentinex® for six weeks was safe for human consumption.

The producer organism, *Lentinus edodes* has been classified. It is a non-GM organism. Detailed analysis of the products has been provided and stability tests demonstrate the long term stability of the product.

APPENDIX A – FIVE MONTHS INTERIM STABILITY REPORT

Five months interim stability report

MM-10-001-x (Lentinex®)

Method:

Stability testing is done in accordance with ICH guidelines (stability testing of new drug substances and products q1a(r2) Current *Step 4* version dated 6 February 2003). For long term testing, the storage condition chosen was 25°C ± 2°C/60% RH ± 5% RH. Also Accelerated storage condition were tested, 40°C ± 2°C/75% RH ± 5% RH.

Storage was performed using two separate BINDER climatic chambers for constant condition with program control. One was set for long term storage condition, the other for accelerated condition. Temperature and humidity were logged every hour.

The following variables were to be followed:

Appearance (Lentinex® is a light brown, opaque liquid).
Microbiological data: sterile
pH: start value ± 0.3 units
Bioactive ingredients, EtOH (Ethanol precipitation): start value ± 15%
Free glucose : < 20 mg/ml
Total protein : < 60 μg/ml

Results:

Results from the lower concentration are given below.

Table I

Long-term 25C / 60% RH		**Lentinex®**			
Time	start	1 month	2 month	3 month	5 month
Identifier	ID 174	ID 197	ID 245	ID 273	ID 368
Appearance	Approved	Approved	Approved	Approved	Approved
Sterility	OK	OK	OK	OK	OK
Protein μg/ml	21,1	23,5	15,1	16,6	23,9
pH	3,9	4,2	4,2	4,2	4,2
EtOH mg/ml	0,9	**0,6**	0,9	**0,7**	0,8
Free glucose mg/ml	3,8	4,1	4,1	3,9	2,4

There is a statistically ($p<0.001$) significant higher pH after 1 month compared to start value.

However, as the later values are completely stable, and inside the specification, the starting value is probably to low.

There are no other significant changes. Even if there is greater variability in the EtOH precipitation, this is probably due to the method. The starting value of 0.88 is included in the 95% confidence interval for the mean of the following months, (0.543 - 0.951) indicating a not statistically significant difference from the starting value (p=0.13) This is also true for the accelerated data, 95% confidence interval being (0.585 – 1,007) There are no differences between the long term EtOH and accelerated storage EtOH, fig 2

Fig 2 Variation in EtOH determination over time. Accelerated storage does not differ from long term storage conditions.

Table II. Due to technical errors, some results are missing. ND = not done

Accelerated 40C /75%		**Lentinex®**			
Time	start	1 month	2 month	3 month	5 month
Identifier	ID 174		ID 249	ID 277	ID 372
Appearance	Approved		Approved	Approved	Approved
Sterility	OK		OK	OK	OK
Protein μg/ml	21,1	ND	missing	10,4	22,6
pH	3,9	ND	4,2	3,6	3,6
EtOH mg/ml	0,9	ND	0,9	0,7	0,8
Free gluc mg/ml	3,8	ND	missing	4,2	missing

Conclusion:

The following variables were recorded:

Appearance, should be light brown, slightly turbid. …….	Approved
Microbiological data: sterile……	OK
pH: start value ± 0.3 units ……..	Mainly fulfilled
Bioactive ingredients, EtOH (Ethanol precipitation): start value ± 15%	Stable
Free glucose : < 20 mg/ml	Fulfilled
Total protein : < 60 μg/ml	Fulfilled

There were no negative trends observed during 5 months of storage, neither in room temperature (25°C) nor in an accelerated condition, 40°C and 75% relative humidity.

We will suggest 1 year stability and a one year re-test period.

Sarpsborg 19.10.07

T. Albrektsen
Medical Director

APPENDIX B – CLINICAL STUDY REPORT NON- CONFIDENTIAL VERSION

CLINICAL STUDY REPORT

STUDY TITLE:

Efficacy and safety of Lentinex® as an immune stimulant in healthy elderly humans. A crossover, placebo, controlled study.

STUDY NUMBER:
10601

SPONSOR:
MediMush AS, Denmark

STUDY TEAM LEADER:
Dr.philos. Bjørn Kristiansen
MediMush AS
Agern Allè 3
DK-2970 Hørsholm
DENMARK
Telephone: + 47 69338691

This study was conducted in compliance with Good Clinical Practice, according to the ICH- Tripartite Guideline E6.

Confidentiality Statement

The information contained in this document is provided in confidence. It is understood that this information will not be disclosed to others without prior agreement with the sponsor.

APPROVALS

For MediMush AS:

Bjørn Kristiansen, PhD
Clinical Research Director

13.09.06
Date

For LINK Medical Research AS:

Ola Gudmundsen, PhD
Project Leader

06 Sept 2006
Date

Jean-Michel Gaullier, PhD
Project Manager

06 Sep 2006
Date

Minna Nurminiemi, PhD
Study Statistician

06 SEP 2006
Date

Jowita Sleboda, PhD
Medical Writer

06 SEP 2006
Date

For the Investigational Site:

ØFJORD

Erik Snorre Øfjord, MD, PhD
Principal Investigator

06 SEP 2006
Date

SYNOPSIS

<table>
<tr><td colspan="3">Name of sponsor: MediMush AS</td></tr>
<tr><td colspan="3">Name of investigational product: Lentinex®</td></tr>
<tr><td colspan="3">Name of the active ingredient: lentinan</td></tr>
<tr><td colspan="3">Title of study: Efficacy and safety of Lentinex® as an immune stimulant in healthy elderly humans. A crossover, placebo, controlled study.</td></tr>
<tr><td colspan="3">Study centres: Cenclin, Bergen, Norway</td></tr>
<tr><td>Publication reference:
None</td><td>Subject Inclusion period:
October-November 2005</td><td>Phase of development:
II</td></tr>
<tr><td colspan="3">Objectives:
Primary objective:
The primary objective was to evaluate the efficacy of Lentinex® to stimulate/restore the cellular immune response by affecting the number of helper and cytotoxic T cells in healthy elderly humans as compared to placebo for 6 weeks.

Secondary objectives:
- To evaluate the efficacy of Lentinex® to stimulate the cellular immune response by affecting the total number of T cells as compared to placebo for 6 weeks.
- To evaluate the efficacy of Lentinex® to stimulate cellular immune response by affecting the number of NK cells and B cells as compared to placebo for 6 weeks
- To evaluate the efficacy of Lentinex® to stimulate the nonspecific immune response by affecting the number of cytokines, inflammation factors and immunoglobulins as compared to placebo for 6 weeks.
- To evaluate the frequency of infectious disease as compared to placebo occurring during the study.
- To evaluate the safety of daily supplementation of Lentinex® by measuring safety blood parameters, adverse events and vital signs.</td></tr>
<tr><td colspan="3">Study Design: Randomized, double-blind, cross-over study.</td></tr>
<tr><td colspan="3">Number of subjects: It was planned to randomize 40 subjects. By the end of the trial 42 subjects received study medication and were evaluated for safety; 1 subject dropped out of the study. 33 subjects were included in the per protocol (PP) efficacy population and 41 subjects were included in the intention to treat (ITT) population.</td></tr>
</table>

Main eligibility criteria:
Inclusion Criteria
Healthy subjects of both genders
Subject that are 65 years of age or older

Exclusion Criteria
Subjects who fail to give written informed consent.
Subjects with BMI over or equal to 30 kg/m^2.
Subjects using corticosteroids or NSAIDs anti-inflammatory drugs.
Subjects with uncontrolled hypertension (diastolic blood pressure>95 mmHg).
Subjects with ongoing allergy or history of anaphylactic reaction.
Subjects with ongoing allergen specific immunotherapy.
Subjects immunocompromised (HIV infected, cancer and other disease affecting the basal immune response).
Subjects with chronic inflammatory disease.
Subjects with diabetes (type 1 and 2).
Subjects with chronic severe renal disease (creatinine outside normal range).
Subjects with chronic severe liver disease (ASAT and/or ALAT more than two times the upper limit of the normal range).
Subjects with cardiac failure (New York Heart Association Class III and IV).
Subjects with acute or chronic pancreatitis, impaired pancreatic function, or history of pancreatitis.
Subjects with any clinical condition that renders the subject unfit to participate.
Subjects with known/suspected drug or alcohol abuse
Subject with ongoing vaccinations

Investigational product, dose and mode of administration, batch number: Lentinex® tablets were administered orally in a dose of two capsules á 540mg (1.25mg lentinan) a day. Batch 5101102 was used**.**

Duration of treatment: Subjects were included 2 weeks prior to start of treatment. They received treatment number one for 6 weeks followed by a wash-out period of 4 weeks. At week 10 they received treatment number two for 6 weeks. Treatment duration was 2 x 6 weeks and the subjects were participating in the study for a total of 18 weeks.

Reference therapy, dose and mode of administration, batch number: Placebo tablets were administered orally in a dose of two capsules á 535mg a day. Batch 5101101 was used**.**

Criteria for evaluation:
Primary endpoint
Helper and cytotoxic T cells::
- helper T cells (CD4+) count
- cytotoxic T cells (CD8+) count
- CD4+/CD8+ ratio

Secondary endpoints
Efficacy - cell mediated immune response:
- total T cells ($CD3^+$) count
- NK cells ($CD56^+$) count
- B cells ($CD19^+$) count

Efficacy - humoral immune response:
- immunoglobulins IgM, IgG, IgA
- complements C3, C4

Efficacy - inflammatory markers
- S-CRP
- cytokines: IL-8, IL-10, IL-12 and TNFα

Efficacy: frequency and duration of infectious diseases
Safety: adverse events, SBP, DBP, heart rate
Safety: laboratory variables

- haematology: B-haemoglobin, B-leukocytes, B-platelets, B-basophiles, B-eosinophiles, B-monocytes, B-lymphocytes, B-neutrophils
- biochemistry: S-ASAT, S-ALAT, S-γGT, S-creatinine, S-bilirubin
- lipids: S-total cholesterol, S-HDL cholesterol, S-LDL cholesterol, S-TG

Statistical methods:
A linear ANCOVA was applied using SAS PROC GLM, with sequence group, period (I or II), and treatment drug as fixed effects, subject as a random effect, and baseline value, gender, and age as covariates.
The end value (i.e. the last observation within each period) was used as the dependent variable (the 'left' side, Y) in the model.
A simplified ANCOVA model with fewer variables (reduced model), ANOVA and Wilcoxon Rank Sum test were also applied as apropriate.
Assumptions on normality were investigated by testing if the residuals from the ANCOVA model were normally distributed by using Shapiro-Wilk test. If the residuals were not normally distributed non-parametric methods of Wilcoxon Rank Sum test were used to test the 1) treatment sequence effect = carry-over effect, 2) treatment effect, and 3) period effect on immuno- and inflammation factors.
The occurrence of infectious diseases was tested with Mc Nemar's test, and the duration of infectious diseases with ANCOVA.

Summary of results:
There were no differences between treatments regarding the helper and cytotoxic T cells. A decrease in the number of helper T cells was observed within the placebo group, but not within Lentinex® group.
Among the different analyses performed a significant difference between treatments in the number of B-cells was found. This was clearly significant when analyses were performed in a subpopulation of subjects with starting CD19+ values lower than the median.
Effects within treatment groups were observed for several parameters in both placebo and Lentinex® groups. Lentinex® treatment gave an increase in CD56+, CRP and a decrease in C3, while subjects in placebo group showed an increase in CD56+ and a decrease in CD4+, CD3+ and C3 at the end of the study.

Unfortunately, period effects were observed for CD56+, C3, C4, IgG, IgA and IgM. Furthermore, carry-over effect was detected for IgA. These findings could contribute to masking of potential treatment effects.

There were no differences between treatments regarding the incidence and duration of infectious diseases and
there was no difference between treatments regarding the incidence of Adverse Events, Adverse Drug Reactions and Serious Adverse Events.

Conclusions:
Lentinex® given orally for 6 weeks to elderly subjects shows a potential to increase the number of circulating B-cells, but does not seem to affect the other immunological and inflammatory markers in this population. It could, however, be possible that longer treatment time or higher dose would reveal additional effects. Furthermore, Lentinex® appears to be a safe supplement, not causing unwanted adverse events.

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ABBREVIATIONS

Abbreviation	Explanation of Term
ADR	Adverse drug reaction
AE	Adverse event
ALAT	Alanine amino transferase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ASAT	Aspartate amino transferase
BMI	Body Mass Index
BP	Blood pressure
C	Complement
CD	Cluster differentiation
CI	Confidence interval
CRA	Clinical research associate
CRC	Clinical research coordinator
CRF	Case report form
CRP	C-reactive protein
DBP	Diastolic blood pressure
DTH	Delay type hypersensitivity
γGT	gamma glutamyl transferase
GCP	Good clinical practice
Hb	Hemoglobin
HbAlc	Glycohemoglobin
HDL	High-density lipoprotein
ICH	International conference on harmonization
IEC	Independent ethics committee
IFN□	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
ITT	Intention to treat
LDL	Low-density lipoprotein
LEM	Lentinus edodes mycelium
LINK	LINK Medical Research AS
LVCF	Last value carry forward
MedDRA	Medical Dictionary for Regulatory Activities
NK	Natural killer
NSAID	Non-steroidal anti-inflammatory drug
PP	Per protocol
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Statistical Analysis System
SBP	Systolic blood pressure
SD	Standard deviation

SOP	Standard operational procedure
TG	Triglyceride
TNF□	Tumor necrosis factor alpha

ETHICS

Independent Ethics Committee

Before inclusion of subjects, an Independent Ethics Committee (IEC) assessed the protocol and gave a written statement expressing that they had no objections to the project as it was presented. The coordinating IEC was Regional committee for medical research ethics, East-Norway (REK Øst).
Chair of the committee was professor Knut Engedal, MD, PhD

Ethical Conduct of the Study

This study was conducted in full accordance with the current revision of the Declaration of Helsinki, the *Good Clinical Practice (GCP): Consolidated Guideline* approved by the International Conference on Harmonisation (ICH) and any other applicable national and local laws and regulations. The investigators were responsible for performing the study in accordance with the protocol and the ICH-GCP guidelines.

Subject Information and Informed Consent

Written informed consent was obtained from each subject before any study procedures or assessments were done and after the aims, methods, anticipated benefits, and potential hazards were explained. It was explained to the subjects that they were free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment.

The subjects' willingness to participate in the study was documented in writing in a consent form, which was signed and dated by the subject. The investigator kept the original consent forms and copies were given to the subjects.

Written and oral information about the study, in a language understandable by the subject, was given to all subjects. The information provided included an adequate explanation of the aims, methods, anticipated benefits, potential hazards and insurance arrangements in force.

Copies of the subject information, including the informed consent form, provided to the subjects is found in Appendix 13.3

INVESTIGATORS AND STUDY ADMINISTRATION

The study was sponsored by MediMush AS, Hørsholm, Denmark. Monitoring, statistical analyses and data management were performed by LINK Medical Research AS (LINK), PO Box 135, NO-2027 Kjeller, Norway. The principal investigator in this study was Erik Snorre Øfjord, Cenclin, Bergen, Norway.

Blood samples were analysed at Haukeland Hospital, Bergen, Norway at the central laboratory (safety parameters) and in flowcytometry research laboratory (T-cells, B-cells, NK-cells and immunoglobulins) and at Bioceros BV, Utrecht, The Netherlands (IL-8, IL-10, IL-12, TNFα).
Study medication was produced by Faun Pharma AS (Drøbak, Norway) and distributed to the investigational site by LINK. Unused medicines were returned to the delivery site and sent for destruction. Return and destruction were registered in accountability logs.

INTRODUCTION

Edible Mushroom – Lentinus edodes

A mushroom contains many different bioactive compounds (proteins, fatty acids essentially polyunsaturated, carbohydrates, vitamins and minerals) with diverse biological activity, and the content and bioactivity of these compounds depend on how the mushroom is prepared and consumed. It is estimated that approximately 50% of the annual 5 million metric tons of cultivated edible mushrooms contain functional "nutraceutical" or medicinal properties (Chang et al, 1996).

Edible mushrooms may have important salutary effects on health or even in treating disease and have often been subject to a long traditional use. This is the case with shiitake mushroom (*Lentinus edodes*), the second most prominent edible mushroom on the market and used for centuries in Asia (Jong et al., 1993). The shiitake mushroom has been shown to lower blood cholesterol and inhibit aggregation of platelets (anti-thrombotic activity). It has also been shown that shiitake mushroom had antibiotic activity, anti-viral activity and anti-tumour effect (Jong et al., 1993).

The main biologic active substance, lentinan, in shiitake mushroom has been characterized as a high molecular weight polysaccharide organized in a triple helix (Chihara et al., 1970; Bluhm et al., 1977). Other biologic substances are described as KS-2, a peptidomannan (mannose based polymer containing peptides) (Fujii et al., 1978) and LEM (Lentinus edodes mycelium), a water soluble extract from the mycelium of cultivated shiitake mushroom.

Mechanisms of Action

These extracts of shiitake mushroom share the anti-viral and anti-tumour properties as shown in cellular experimental models and animal studies (Jong et al., 1993). The effects seem to occur by stimulating the maturation, differentiation or proliferation of immune cells involved in host defence mechanisms against cancer or infection (Chihara et al., 1989).

Lentinan activates NK-cells and stimulates T helper cells. Whereas lentinan inhibits synthesis of prostaglandins (that can slow the differentiation of T cells and affect the

suppressor T-cell activity), lentinan can also stimulate peripheral blood lymphocytes *in vitro* to increase IL-2-mediated lymphokine-activated killer cell and NK-cell activity. Increased productions of interleukins IL-1□, IL-1□, TNF□, IL-3 and IFN□ have also been observed.

In summary, lentinan has the capacity to act as T-cell immune adjuvant, to restore and potentiate helper T-cell functions, but not to stimulate B-cells or T-suppressor cells, or to stimulate production of cytokines.

Clinical Studies

Most of the clinical trials performed with lentinan have been done with Asiatic patients suffering gastric, stomach, lung or prostate cancer (Tari et al., 1994; Hazama et al., 1995; Takeshita et al., 1996; Matsuoka et al., 1997; Mio et al., 1997; Nakano et al., 1999; Yoshino et al., 2000; Kagawa et al., 2002; Kimura et al., 2003; Piao et al., 2004). In these studies, lentinan has been injected intravenously and at different dose regimen. However, all trials reported some significant effect on the cancer proliferation, improvement of quality of life during chemotherapy and some gain in lifetime.

The few trials that have been performed with oral supplementation of lentinan to patients suffering cancer give conflicting results. In one case, the Shiitake mushroom extract lentinan improved the quality of life of patients suffering of breast, ovarian and small lung cancer with a significant reduction in the number of side effects (Piao et al., 2004). However, in another study it showed no effect on prostate cancer suffering patients (deVere et al., 2002).
In a study, where 4g powder of the whole shiitake mushroom was given daily for 10 weeks to healthy subjects, there was found an increase in blood eosinophils in 5 of the 10 subjects, an increase in eosinophile granule proteins in serum and stools, and an increase in gastrointestinal symptoms in the same subjects (Levy et al., 1998).

In another study, lentinan has been given intravenously to HIV-positive patients with immune deficiency (Gordon et al., 1998). This study showed that lentinan was well tolerated in Caucasian patients as described previously in an Asiatic population, and an increase in CD4 cells (T-cell helper) was observed but not the difference was not significant due to the low number of patients in the study.

Rationale: Clinical experience indicates strongly that lentinan acts as an immune stimulator in patients suffering cancer, and may also have the potential to restore the immune function in HIV patients.
Lentinex®, which is an extract containing lentinan designed to be taken orally, may stimulate the immune response and/or restore the immune response of healthy immune-compromised subjects such as elderly.

STUDY OBJECTIVES

Primary Objective

The primary objective was to evaluate the efficacy of Lentinex® to stimulate/restore the cellular immune response by affecting the number of helper and cytotoxic T cells in healthy elderly humans as compared to placebo for 6 weeks.

Secondary Objectives

Secondary objectives were:

- To evaluate the efficacy of Lentinex® to stimulate the cellular immune response by affecting the total number of T cells as compared to placebo for 6 weeks.
- To evaluate the efficacy of Lentinex® to stimulate cellular immune response by affecting the number of NK cells and B cells as compared to placebo for 6 weeks
- To evaluate the efficacy of Lentinex® to stimulate the nonspecific immune response by affecting the number of cytokines, inflammation factors and immunoglobulins as compared to placebo for 6 weeks.
- To evaluate the frequency of infectious disease as compared to placebo occurring during the study.
- To evaluate the safety of daily supplementation of Lentinex® by measuring safety blood parameters, adverse events and vital signs.

STUDY PROCEDURES

Study Overview

Study protocol can be found in Appendix 13.1 and a sample CRF can be found in Appendix 13.2

An overview of the study procedures is presented in table 1.

Table 1. Overview of Study Procedures

		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
		Week -2	Day 0	Week 6	Week 10	Week 16
Eligibility criteria		x	x	x	x	
Demographic data		x				
Medical history		x				
Informed consent		x				
Randomization			x			
Vital signs[1]		x		x[2]	x[2]	x[2]
Blood samples	Efficacy[3]		x	x	x	X
	Safety[4]	x		x	x	X
Study medication	Dispensing		x		x	
	Capsule counting			x		X

Adverse events		x	x	x	X

[1] BP, heart rate, weight and height
[2] BP and heart rate only
[3] T cells CD4+, CD8+, CD3+, B cells CD19+, NK cells CD56+, IL-8, IL-10, IL-12, TNFα, CRP, IgG, IgA, IgM, C3, C4
[4] B-haemoglobin, B-leukocytes, B-platelets, B-basophils, B-eosinophils, B-monocytes, B-lymphocytes, B-neutrophils, S-ASAT, S-ALAT, S-γGT, S-creatinine, S-bilirubin, S-total cholesterol, S-HDL cholesterol, S-LDL cholesterol, S-TG

Description of Study Procedures and Discussion of Study Design

Description of Study Design

Methodology

The present study was a randomized, double-blind, cross-over study with two treatment arms comparing Lentinex® 1.08g daily and placebo for 6 weeks

Subjects were included 2 weeks prior to start of treatment. They received treatment number one for 6 weeks followed by a wash-out period of 4 weeks. At week 10 they received treatment number two for 6 weeks. Treatment duration was 2 x 6 weeks and the subjects were participating in the study for a total of 18 weeks.

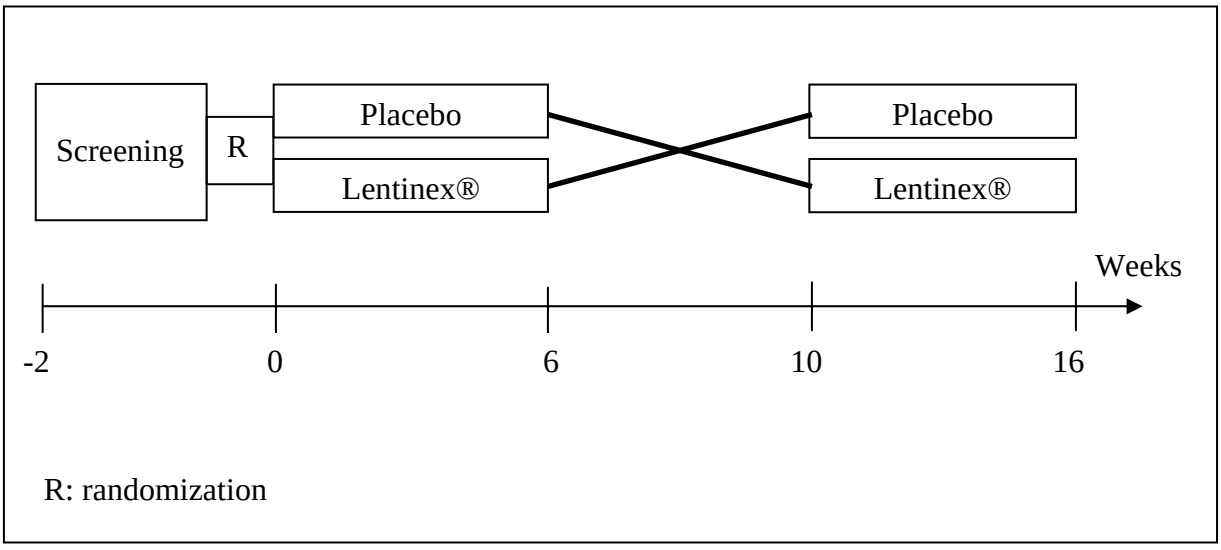

Figure 1. Treatment Regimen

Blinding

The study was performed double-blind. Placebo tablets used were physically indistinguishable from Lentinex® tablets. All patients took 2 tablets (active/placebo) once daily.

Information about the study treatment for each individual (as identified by the subject number) was available to LINK and the principal investigator in sealed emergency envelopes. Unblinding could only occur in emergencies where the knowledge of treatment was considered necessary for the proper clinical care of the subject or after all subjects had completed the study period and all data had been entered into the database and a clean file declared. Unblinding could also be used in the event of any AE that was serious, unexpected and of possible relation to the study medication.
No emergency unblinding was necessary in this study.

Randomization

Random lot numbers were given to all investigational product boxes. The eligibility of the subjects was checked before randomization and the eligible subjects were randomized to a treatment sequence by simple block randomization method.

Discussion of Study Design and Appropriateness of Measurements

Selection of Study Population

Immune functions fluctuate within fixed limits and are influenced by endogenous factors such as aging and stress, and by exogenous factors such as treatment associated diseases, malnutrition and undesirable lifestyle. The elderly are less immune competent than the young with a decrease in delayed type hypersensitivity, a decrease in antigen-specific antibody production, a reduced proliferative response of T cells, and a decline of the proportion of T cells with aging (Sansoni et al., 1993; Stulning et al., 1995; Fagnoni et al., 1996; Mariani et al., 1999; Fagnoni et al., 2000; Ravaglia et al., 2000). The elderly are thus more susceptible to infection than the young.

This study evaluates therefore the potential of Lentinex® to stimulate the immune response and/or restore the immune response of elderly aged $\geq$ 65 years from both genders.

All relevant medical and non-medical conditions were taken into consideration when deciding whether this protocol was suitable for a particular subject.

Choice of Dose and Reference Therapy

Shiitake is a mushroom that has been used traditionally in Asia for centuries. The only side effects reported has been some skin rash of some workers working with the cultivation of the mushroom (Jong et al., 1993). Toxicity studies performed in rats and mice showed that the oral LD_{50} of lentinan was > 2.5g/kg (Ishii et al., 1980; Moriyuki et al., 1980; Shimazu et al., 1980). Other toxicity studies performed on rhesus monkeys (Sortwell et al., 1981) and beagle dogs (Chesterman et al., 1981) showed that a daily dose of 0.5mg/kg was without adverse effects.

Intravenously administered is lentinan given to human patients in doses up to 2mg/day without toxic effects. Since the oral absorption of lentinan is far below the absorption obtained intravenously, it was decided to give the subjects a 2.5mg/day dose of lentinan. This dose corresponds to 2 tablets Lentinex® (1tablet = 540mg =1.25mg lentinan).

Efficacy Assessments

Efficacy assessments were selected on the background of the planned primary and secondary objectives. The most appropriate laboratory tests and measurements were chosen.

Statistical Considerations

Sample size estimation was based on a previous study performed using the same methodology, but with other supplements (Meydani et al., 1990; Wu et al., 1999). A difference of $\geq$ 30% was expected between Lentinex® and placebo groups for CD4/CD8. A two-sided t-test with significance level 5%, and test power 90% gave n=18 for each treatment group. When adjusted for 10% drop out rate, 2 x 20= 40 subjects were required.

Eligibility Criteria

Inclusion Criteria

1. Healthy subjects of both genders
2. Subject that are 65 years of age or older

Exclusion Criteria

1. Subjects who fail to give written informed consent.
2. Subjects with BMI over or equal to 30 kg/m^2.
3. Subjects using corticosteroids or NSAIDs anti-inflammatory drugs.
4. Subjects with uncontrolled hypertension (diastolic blood pressure>95 mmHg).
5. Subjects with ongoing allergy or history of anaphylactic reaction.
6. Subjects with ongoing allergen specific immunotherapy.
7. Subjects immunocompromised (HIV infected, cancer and other disease affecting the basal immune response).
8. Subjects with chronic inflammatory disease.
9. Subjects with diabetes (type 1 and 2).
10. Subjects with chronic severe renal disease (creatinine outside normal range).
11. Subjects with chronic severe liver disease (ASAT and/or ALAT more than two times the upper limit of the normal range).
12. Subjects with cardiac failure (New York Heart Association Class III and IV).
13. Subjects with acute or chronic pancreatitis, impaired pancreatic function, or history of pancreatitis.

14. Subjects with any clinical condition that renders the subject unfit to participate.
15. Subjects with known/suspected drug or alcohol abuse
16. Subject with ongoing vaccinations

Withdrawal of Subjects from the Study

A subject could be removed from the study treatment based on their own decision or if the investigator would find it medically necessary. When a subject decided to discontinue participation in the study, he/she was contacted in order to obtain information about the reason(s) for discontinuation, while fully respecting that the subject was not obliged to give her/his reason(s) for withdrawing. Whenever possible, the subject returned to the investigator for an unscheduled visit at the time of or soon after discontinuation.

Subjects could also be removed from the study due to protocol violations. These subjects would not be included in the analysis and would be permanently dismissed from the study.

Reasons for withdrawal were recorded on the CRF.

Investigational Product

Identity of the Investigational Product

The investigational product Lentinex® (batch 5101102) and placebo (batch 510101) were supplied in tablets with identical appearance. Their physical/chemical characteristics are presented in table 2.

Table 2. Content of tablets

	Lentinex®	**Placebo**
Colour	Light beige	Light beige
Weight per tablet	540mg	535mg
Dimension (diameter)	11mm	11mm
Disintegration time in water	ca.4min	<3min
Active compound (lentinan)	1.25mg	-
Cellulose (E460 and E466)	ca.88%	ca.91%
Sugar	ca.6%	ca.6%
Protein	ca.0.02%	ca.0.02%
Heavy metals	<10ppm	<10ppm
Microbiological data	<1000CFU/g	<1000CFU/g

Dose of the Investigational Product

A total of 2 tablets (2.5mg lentinan) a day was taken with meals.

Administration of the Investigational Product

All medication was administered orally.

Handling of the Investigational Product

The investigational product was manufactured, packed and released by Faun Pharma AS. The investigation product was shipped to Cenclin which functioned as delivery site for the patients. Unused medicines were returned to the delivery site and sent for destruction. All returned and destructed investigational product is registered in accountability logs.
The study medication was stored at room temperature in a secure area with restricted access.

Efficacy and Safety Assessments

Overview of Endpoints

Table 3 presents the objectives with the corresponding endpoints.

Table 3. Corresponding Objectives and Endpoints

Primary objective To evaluate the efficacy of Lentinex® to stimulate/ restore the cellular immune response by affecting the number of helper and cytotoxic T cells in healthy elderly humans as compared to placebo for 6 weeks.	***Primary endpoints*** The primary endpoint is the effect of Lentinex® on: ▪ helper T cells (CD4+) count ▪ cytotoxic T cells (CD8+) count ▪ CD4+/CD8+ ratio as measured by flow cytometry
Secondary objectives ▪ To evaluate the efficacy of Lentinex® to stimulate the cellular immune response by affecting the total number of T cells as compared to placebo for 6 weeks. ▪ To evaluate the efficacy of Lentinex® to stimulate cellular immune response by affecting the number of NK cells and B cells as compared to placebo for 6 weeks ▪ To evaluate the efficacy of Lentinex® to stimulate the nonspecific immune response by affecting the number of cytokines, inflammation factors and immunoglobulins as compared to placebo for 6 weeks. ▪ To evaluate the frequency of infectious disease as compared to placebo occurring during the study. ▪ To evaluate the safety of daily supplementation of Lentinex® by measuring safety blood parameters, adverse events and vital signs.	***Secondary endpoints*** ▪ Efficacy – cell mediated immune response: o total T cells ($CD3^+$) count o NK cells ($CD56^+$) count o B cells ($CD19^+$) count ▪ Efficacy – humoral immune response: o immunoglobulins IgM, IgG, IgA o complements C3, C4 ▪ Efficacy – inflammatory markers o S-CRP o cytokines: IL-8, IL-10, IL-12 and TNFα ▪ Efficacy: frequency and duration of infectious diseases ▪ Safety: adverse events, SBP, DBP, heart rate ▪ Safety: laboratory variables o haematology: B-haemoglobin, B-leukocytes, B-platelets, B-basophils, B-eosinophils, B-monocytes, B-lymphocytes, B-neutrophils o biochemistry: S-ASAT, S-ALAT, S-γGT, S-creatinine, S-bilirubin o lipids: S-total cholesterol, S-HDL cholesterol, S-LDL cholesterol, S-TG

Efficacy Assessments

Primary Endpoint – Helper T Cells and Cytotoxic T Cells

These analyses were performed in order to study the effect of Lentinex® on the cellular immune response. The number of helper T cells (CD4+) and cytotoxic T cells (CD8+), as well as the ratio CD4+/CD8+ were measured by flow cytometry at visit 2, 3, 4 and 5.

Secondary Endpoint – Cell Mediated Immune Response

These analyses were performed in order to study the effect of Lentinex® on the cellular immune response. The number of T cells (CD3+), B cells (CD19+) and NK cells (CD56+) were measured by flow cytometry at visit 2, 3, 4 and 5.

Secondary Endpoint - Humoral Immune Response

These analyses were performed in order to study the effect of Lentinex® on the nonspecific immune response. The number of IgM, IgG, IgA, C3 and C4 were measured at visit 2, 3, 4 and 5.

Secondary Endpoint - Inflammatory Markers

These analyses were performed in order to study the effect of Lentinex® on inflammatory markers. The amount of CRP, IL-8, IL-10, IL-12 and TNFα were measured at visit 2, 3, 4 and 5.

Secondary Endpoint - Frequency and Duration of Infectious Diseases

These analyses were performed in order to study the effect of Lentinex® on the frequency and duration of infectious diseases. All occurrences of infectious disease were registered at visit 2, 3, 4 and 5.

Safety Assessments

Secondary Endpoint - Adverse Events

These analyses were performed in order to study the effect of Lentinex® on the occurrence of adverse events.
An adverse event is any unfavourable, unintended event (symptom) reported by a subject or observed by the investigator during the study and which does not necessarily have a causal relationship with the study product. An adverse event (AE) can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of the investigational product, whether or not related to the investigational product.
All adverse events were recorded on the adverse event form with information about the nature, severity, frequency, date of onset, duration, and action taken regarding study products. In addition, the investigator recorded the relation between the adverse event and the study product and the subject outcome. Follow-up of any adverse event reported on the last day of treatment was performed as medically indicated.

The AE reporting period began upon receiving the first dose of study treatment and ended at the day of stopping trial medication.

Secondary Endpoint – Safety Laboratory Analyses

These analyses were performed in order to study the effect of Lentinex® on selected safety blood parameters. Following laboratory tests were performed:
Haematology: B-haemoglobin (Hb), B-leukocytes, B-platelets, B-basophils, B-eosinophils, B-monocytes, B-lymphocytes, B-neutrophils
Biochemistry: S-ALAT, S-ASAT, S-γGT, S-creatinine, S-bilirubin
Lipids: S-total cholesterol, S-HDL cholesterol, S-LDL cholesterol, S-TG
Samples were taken at visit 1, 3, 4 and 5 and analysed at Haukeland Hospital and Bioceros BV.

Secondary Endpoint - Vital Signs

These analyses were performed in order to study the effect of Lentinex® on blood pressure and heart rate.
Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate were measured at visits 1, 3, 4 and 5.

Data Management

LINK was responsible for data processing and control of data quality. Data management was carried out as described in the LINK's SOPs and working instructions for clinical studies and complied with regulatory requirements (i.e. ICH-GCP guidelines).

Completed CRFs were checked visually by the monitor for completeness (source data verification), consistency, and legibility. Before the data were entered electronically into LINK's clinical database, the CRFs were reviewed for completeness of pages, mandatory data, legibility and completeness.

When entering the data into LINK's clinical database (iVal Data Management System), the system automatically noted false types of data, values outside of defined range and invalid values. Verification between data entry and CRFs was performed by the means of double entry. All inconsistencies were corrected in the database.

All AE and Medical History terms recorded on the CRF were encoded according to MedDRA and all concomitant medications were encoded according to ATC system. All coded entries were checked, reviewed and corrected as appropriate.

Edit checks have been executed to verify and validate records for study specific details.

Data queries for further clarification of data recorded on CRFs were answered, dated and signed by the investigator. Changes were implemented in LINK's database, and the validation procedures were repeated until non-resolvable errors remained.
After all the quality control steps were completed the database was locked, the randomisation code broken and all data released for reporting and statistical evaluation.

Statistical Methods and Determination of Sample Size

Statistical Evaluation

A statistical report with SAS printouts, p-values and short statistical conclusions are available in Appendix 13.5. LINK performed the statistical analyses. All analyses and tabulations were performed using SAS® for Windows Version 8.2. Appropriate descriptive statistics were presented for each variable. Statistical tests were performed using 5 % as the nominal level of significance and interval estimates were constructed using 95 % as the level of confidence.

Statistical Methods

All continuously distributed variables described as primary or secondary efficacy variables are summarised by the treatment and by the sequence of the treatment (L-P or P-L) during the cross-over study at all relevant time points with number of subjects, mean, median, standard deviation, minimum, maximum. Changes from the end to the baseline within each treatment period are summarized with number of subjects, mean, median, standard deviation, minimum, maximum. All categorical (discrete including ordinal) variables are presented in contingency tables showing counts and percentages for each treatment sequence at all time points. The primary variables are presented with 95% confidence intervals.

A linear ANCOVA model (full model) was applied using SAS PROC GLM, with sequence group, period (I or II), and treatment drug as fixed effects, subject as a random effect, and baseline value, gender, and age as covariates.
The end value (i.e. the last observation within each period) was used as the dependent variable (the 'left' side, Y) in the model.
$Y_{ijkl} = Y_{0l(j)} + \alpha_i + \beta_j + S_k + G + Z + e_{ijkl}$,
Where $Y_{0l(j)}$ is the baseline value of the variable 'Y' in period 'j', α_i is the treatment effect, β_j is the period 'j' (I or II) effect, S_k is the sequence effect 'k' (L-P, or P-L), G is the gender effect, Z is the age effect for the subject 'l', and e_{ijkl} is an error term for subject 'l' having treatment 'i' at period 'j' in sequence 'k'.
A simplified ANCOVA model with fewer variables (reduced model), ANOVA and Wilcoxon Rank Sum test were also applied when appropriate.
The carry-over effect was tested for each subject by summarising the periods I and II, and comparing the sequences L-P and P-L. If the sequence effect was significant then the carry-over effect existed, and follow-up analysis was considered. Treatment effect was estimated by taking the differences between periods I and II, and comparing the sequences L-P and P-L. Period effect was tested by testing the differences within

sequence over periods, e.g. difference for a subject in sequence L-P between periods I and II. Testing of treatment and period effects required an assumption of no carry-over effect.

Assumptions on normality were investigated by testing if the residuals from the ANCOVA model were normally distributed by using Shapiro-Wilk test. If the residuals were not normally distributed a non-parametric method of Wilcoxon Rank Sum test was used to test the 1) treatment sequence effect = carry-over effect, 2) treatment effect, and 3) period effect on immuno- and inflammation variables.

Changes within groups have been calculated by subtracting the baseline value from the end value, and they have been tested by using the Student's T-test or Wilcoxon Signed Rank test.

Analysis of Efficacy Endpoints

Primary Endpoints

The primary variables were compared and tested with the previously described tests according to the following hypotheses:

$H_{0:}$ Mean change in CD4 /CD8+ ratio from baseline to the end of a treatment period of 6 weeks is equal in Lentinex® and placebo groups,

vs.

$H_{1:}$ Mean change in CD4+/CD8+ ratio from baseline to the end of a treatment period of 6 weeks is not equal in Lentinex® and placebo groups.

Another analysis is based on change from baseline to the end in values of CD4+ / CD8+ ratio within treatment group.

H_0 : there is no change within treatment in CD4+/CD8+ ratio, and

H_1 : there is change within treatment in CD4+/CD8+ ratio.

The changes were tested two-sided, and no assumptions on size of change were made.

In-text tables present mean, SD and p-value form the appropriate statistical tests.

Secondary Endpoints

Testing of the secondary variables was exploratory and the results were used for exploratory purposes. Due to this, no adjustments for multiple testing was performed.

Total number of T cells, NK cells and B cells in addition to immunoglobulins and inflammation markers were tested using the ANCOVA models described earlier and a ANOVA / Wilcoxon Rank Sum test.
In-text tables present mean, SD and p-value from the appropriate statistical tests.

The occurrence of infectious diseases was tested with Mc Nemar's test, and the duration of infectious diseases with ANCOVA.

Analysis of Safety Variables

Adverse Events

Adverse events were tested by comparing the occurrence of AE between treatments at the end of each treatment period (I and II). Mc Nemar's test was used to analyse the 2x2 cross-table of Lentinex® vs. placebo.

Among the safety variables adverse events registered are tabulated by organ class. Subject was defined as having an AE if one or more AE occurred within a treatment period. The adverse events were coded using MedDRA dictionary.

Laboratory Variables

Laboratory safety variables were tested using a similar models and null hypothesis as for the primary and secondary efficacy parameters. All safety laboratory variables are given descriptively per treatment. In source tables blood and serum variables are tabulated with mean, median, SD, min-max at the time points measured, while only mean and SD are presented in in-text tables.

Vital Signs

In source tables SBP, DBP and heart rate are tabulated with mean, median, SD, and range (min-max) at the time points measured, while only mean and SD are presented in in-text tables.

Presentation of Demographics and Baseline Characteristics

In the source tables categorical demographics assessments and baseline characteristics data are presented descriptively with counts and rates, while continously variables are given descriptively with n, mean, median, SD, min-max. In in-text tables only mean, SD and min-max are presented for continuously variables.

Determination of Sample Size

Sample size estimation was based on a previous study performed using the same methodology, but with other supplements (Meydani et al., 1990; Wu et al., 1999). A difference of $\geq$ 30% was expected between Lentinex® and placebo groups for CD4/CD8. A two-sided t-test with significance level 5%, and test power 90% gave n=18 for each treatment group. When adjusted for 10% drop out rate, 2 x 20= 40 subjects were required.

Changes in the Conduct of the Study or Planned Analyses

Amendments

One amendment to the protocol was written. IEC approval was obtained 31OCT2005. In this amendment the following changes to protocol were made:

- Changes to a few of the exclusion criteria
  Reason: To describe more accurately the desired group of patients.
- Physical examination at last visit removed.
  Reason: Does not provide extra information
- DTH test will not be performed
  Reason: This test can be associated with side effects.
- Number of tablets reduced to 2 daily
  Reason: New production methods for tablets
- Changes in selected efficacy variables
  Reason: To more accurately describe the planned objectives.
- Changes in selected safety variables
  Reason: To more accurately describe the planned objectives.
- Publication no longer contingent on sponsor's approval
- Reason: Investigator is more free to publish study results.
- New flow chart
  Reason: To reflect the new changes

Other changes in the conduct of the study

The following variables were not measured:

- IL-4
- IL-5
- IFNγ
- Erythrocytes
- Erythrocyte sedimentation rate

Notes-to-file

TwoNotes-to-file have been written.

- Note-to-file 1: Norwegian Medicinal Agency does not require an EudraCT application for the present study
- Note-to-file 2: Subject no.115 is to be withdrawn from PP analyses due to a high number of concomitant medications.

Additional statistical analyses

In addition to analyses described in the statistical analysis plan the following analysis was performed:

1) Analysis of treatment differences, carry-over effects and period effects for CD4+, CD8+, CD19+ and CD56+ for a subpopulation of subjects starting with immunological values lower than the median.
2) Analysis of treatment differences for CRP and IgG for a subpopulation without subjects with high CRP values which were due to known infectious diseases.

SUBJECT DISPOSITION

Disposition of Subjects

42 subjects were randomized and 41 subjects completed the study. One patient withdrew from the study, the reason was withdrawal of consent.

Summary of subject disposition is presented in figure 2.

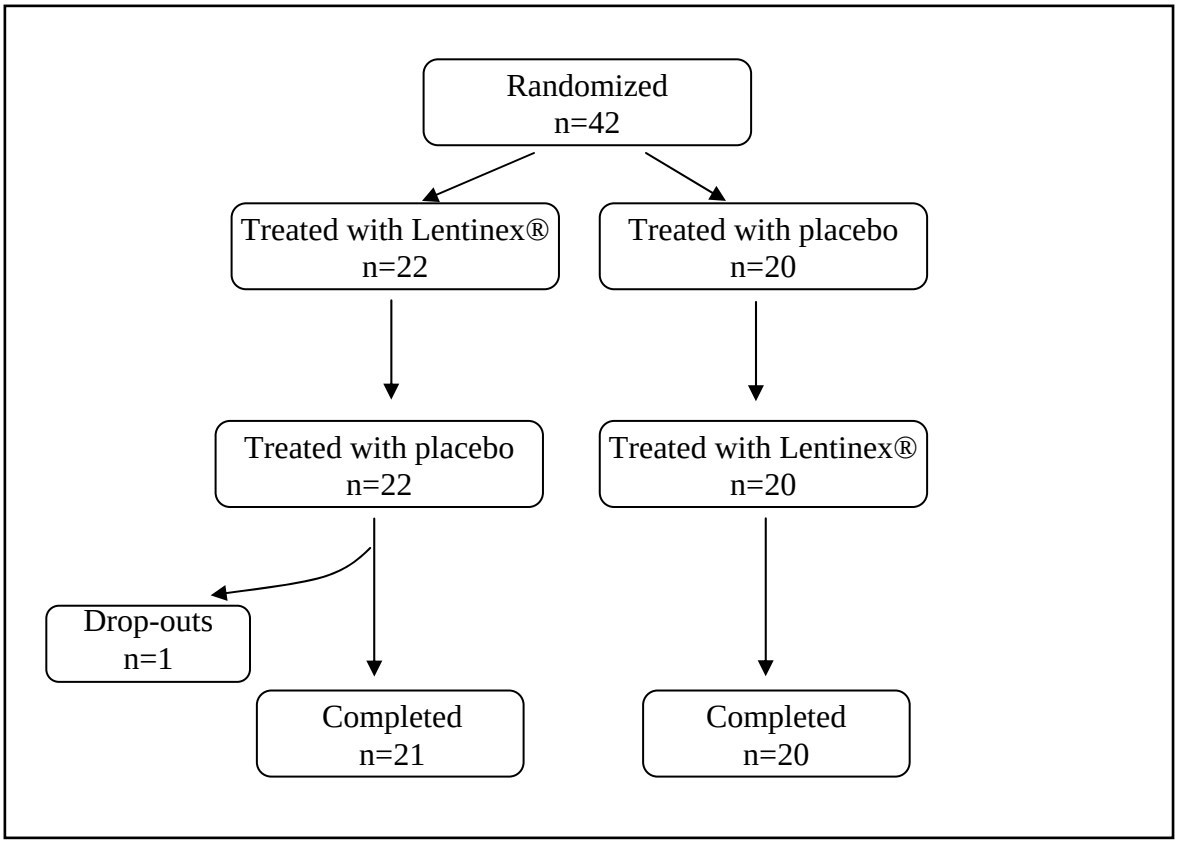

Figure 2. Subject Disposition

Protocol Deviations

There were 9 subjects with a protocol violation. These subjects were excluded from PP population.

Table 4. Protocol Violators

Specification of violation	Subject
Blood sample for V3 damaged, results uncertain	109
Blood sample for V3 and V5 damaged, results uncertain	110
Use of too many concomitant medications.	115
Compliance 49%	119
Missing data for V4 and V5	121
Blood sample for V3 damaged, results uncertain	125
Blood sample for V5 damaged, results missing	133
Blood sample for V3 and V5 damaged, results uncertain	134
Blood sample for V3 damaged, results uncertain	142

Data Sets Analyzed

The study team together with the investigator decided which subjects, or individual values belonging to a subject, were to be excluded from the analysis. Subjects were excluded from the efficacy analysis in case of protocol violation, or extensive loss of data.

The Safety Population (SP) included all patients who were treated at least once with study medication. This population was used to evaluate the safety laboratory variables and adverse events.
The intention-to-treat (ITT) population was defined as all subjects who were randomised and had at least one dose of study medication. ITT was equal to SP in this study.
The per protocol (PP) population was defined as a subset of ITT population which has completed the treatment period of 16 weeks as according to the protocol and did not deviate from the protocol in a manner which would influence the validity of the data, or would result in an extensive loss of information.
Immunological efficacy variables were summarised using the ITT and PP population. Inflammatory efficacy variables and safety variables were summarised using the ITT population.

Demographic and Other Baseline Characteristics

Demographic Characteristics and Body Measurements

Table 5 presents the main demographic characteristics and baseline body measurements in both populations. All the study subjects were of Caucasian ethnic origin. None of the subjects used snuff.

Table 5. Demographic Characteristics – ITT population

Variable		Value
Age (years)	Mean ± SD	71.0±5.4
	Range	64.9-84.0
Gender	Female n (%)	20 (48)
	Male n (%)	22 (52)
Weight (kg)	Mean ± SD	72.4±12.8
	Range	43-95
Height (cm)	Mean ± SD	171.7±9.0
	Range	157-192
BMI (kg/m^2)	Mean ± SD	24.6±2.6
	Range	17.0-29.0
Smoking (cigarettes/day)	0/day n (%)	36 (85.7)
	1-10/day n (%)	4 (9.5)
	11-19/day n (%)	2 (4.8)
	≥20/day n (%)	0
Alcohol consumption (units/day)	0/day n (%)	4 (9.5)
	1-7/day n (%)	36 (85.7)
	8-14/day n (%)	2 (4.8)
	15-25/day n (%)	0
	>25/day n (%)	0
Physical exercise (Yes/No)	No n (%)	10 (23.8)
	Yes n (%)	32 (76.2)
Physical exercise (hours/week)	Without sweating mean ± SD	2.57±3.07
	With sweating mean ± SD	1.12±2.45

Reference tables 13.2.1.1.a - 13.2.1.5.a

Table 6 presents medical history.

Table 6. Medical History - ITT Population

System Organ Class	N (%)
Cardiac disorders	6 (14.3)

Endocrine disorders	1 (2.4)
Eye disorders	1 (2.4)
Gastrointestinal disorders	1 (2.4)
Immune system disorders	1 (2.4)
Infections and infestations	1 (2.4)
Metabolism and nutrition disorders	4 (9.5)
Musculoskeletal and connective tissue disorders	3 (7.1)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (2.4)
Nervous system disorders	3 (7.1)
Psychiatric disorders	1 (2.4)
Reproductive system and breast disorders	1 (2.4)
Respiratory, thoracic and mediastinal disorders	1 (2.4)
Surgical and medical procedures	1 (2.4)
Vascular disorders	14 (33.3)

Reference table 13.2.8.1

Concomitant Medication

The use of concomitant medication is presented in table 7.

Table 7. Concomitant Medication - ITT Population

ATC code level 4	Drug name	N
Ace-Inhibitors, Plain	Triatec	1
Acetic Acid Derivatives And Related Substances	Diclofenac	2
Adrenergics And Other Anti-Asthmatics	Seretide	1
Alpha - Adrenoceptor Blocking Agents	Carduran	3
Angiotensin II Antagonists And Diuretics	Coaprovel	3
	Cozaar Comp	1
Angiotensin II Antagonists, Plain	Aprovel	1
	Cozaar	6
Anilides	Pinex	1
Antiallergic Agents, Excl Corticosteroids	Livostin	1
Antibiotics	Fucithalmic	1
Anticholinergics	Atrovent	1
Antiinfectives	Ciproxin	1
Ascorbic Acid (Vit C), Plain	Vitamin C	1
Benzodiazepine Derivatives	Mogadon	1
Benzothiazepine Derivatives	Cardizem	2

Beta Blocking Agents, Selective	Atenolol	1
	Selo-Zok	4
	Uniloc	1
Beta-Lactamase Sensitive Penicillins	Apocillin	2
Calcium, Combinations With Other Drugs	Ideos	1
Corticosteroids	Nasonex	1
Cyclopyrrolones	Imovane	1
Digitalis Glycosides	Digitoxin	1
Dihydropyridine Derivatives	Amlodipine	1
	Norvasc	1
	Zanidip	1
Ergot Alkaloids	Anervan	1
Heparin Group	Klexane	1
HMG CoA Reductase Inhibitors	Lipitor	3
	Pravachol	1
	Pravastatin	2
	Simvastatin	2
Influenza Vaccines	Influenza Virus Vaccine	1
Mucolytics	Bronkyl	1
Natural And Semisynthetic Estrogens, Plain	Progynova	2
Opium Derivatives And Expectorants	Solvipect Comp	1
Organic Nitrates	Nitromex	1
Other Antiinfl./Antirheumatic Agents, Non-Steroid	Glucosamine	1
Other Cholesterol And Triglyceride Reducers	Omega-3 Marine Triglycerides	1
Other Ophthalmologicals	Oftan	1
Other Opioids	Nobligan - Slow Release	1
Oxicams	Brexidol "Nycomed"	1
Phenylalkylamine Derivatives	Verakard	1
Piperazine Derivatives	Zyrtec	1
Platelet Aggregation Inhibitors Excl. Heparin	Albyl-E	7
	Persantin	2
Pneumococcal Vaccines	Pneumococcal Vaccine	1
Proton Pump Inhibitors	Lanzo	1
	Omeprazole	1
Selective 5ht1-Receptor Agonists	Imigran	1
Selective Beta-2-Adrenoceptor Agonists	Bricanyl	1
	Ventoline	1
Sympathomimetics, Plain	Rhinox	1
Tetracyclines	Tetracycline "Nm Pharma"	1

Thiazides And Potassium In Combination	Centyl Med Kaliumklorid	1
	Centyl mite w/potassium chloride	1
Vitamin B-Complex, Plain	Afi-B-Total Forte	1
Vitamin K Antagonists	Marevan	4

Reference table 13.2.6.a

Baseline Data for Efficacy Variables

Tables 8, 9 and 10 present a summary of the baseline efficacy variables in both populations.

Table 8. Cellular Immune Response - ITT Population

Type of cells		Period I		Period II	
		Lentinex®	Placebo	Lentinex®	Placebo
CD4+ (%)	Mean ± SD	51.723±9.724	50.520± 9.665	51.500± 9.804	54.148± 9.305
	Range	36.80-69.50	34.10-72.10	31.90-72.00	38.20-74.40
CD8+ (%)	Mean ± SD	25.241± 8.508	27.055± 11.107	26.840± 11.637	23.210± 8.608
	Range	13.80-46.60	12.10-61.60	10.40-63.80	4.50-38.60
CD4+/CD8+ ratio	Mean ± SD	2.365± 1.126	2.25±1.14	2.338± 1.261	3.154± 2.983
	Range	0.79-4.55	0.55-4.81	0.50-5.67	1.16-14.93
CD3+ (%)	Mean ± SD	72.127± 9.171	72.015± 9.624	73.445± 9.058	72.986± 8.689
	Range	45.30-83.50	56.10-91.60	60.40-90.20	46.90-86.40
CD19+ (%)	Mean ± SD	12.327± 3.827	12.285± 4.642	11.885± 5.347	13.176± 5.305
	Range	6.90-19.60	5.00-22.20	4.80-26.10	5.20-24.00
CD56+ (%)	Mean ± SD	11.782± 4.919	13.970± 7.180	12.045± 7.152	9.510± 4.315
	Range	5.20-21.40	3.30-30.60	3.20-27.90	2.80-18.10

Reference table 13.4.1.1.1.a - 13.4.1.1.1.f

Table 9. Humoral Immune Response - ITT Population

Type of cells		Period I		Period II	
		Lentinex®	Placebo	Lentinex®	Placebo
C3 (g/l)	Mean ± SD	1.380± 0.241	1.339± 1.375	1.085± 0.155	1.077± 1.050
	Range	0.93-1.75	0.90-1.64	0.71-1.35	0.79-1.40
C4 (g/l)	Mean ± SD	0.245±0.069	0.238±0.075	0.217±0.056	0.218±0.056
	Range	0.12-0.39	0.12-0.42	0.12-0.41	0.14-0.38
IgG (g/l)	Mean ± SD	10.408±2.217	10.480±1.405	9.259±1.683	9.130±2.083
	Range	7.00-15.70	7.80-13.00	6.06-12.90	5.83-14.10
IgA (g/l)	Mean ± SD	2.423±0.877	2.357±0.886	2.201±0.843	2.329±0.928
	Range	1.14-3.94	1.37-4.38	1.20-4.18	1.16-4.03
IgM (g/l)	Mean ± SD	0.866± 0.471	0.802± 0.409	0.741± 0.379	0.837± 0.445

	Range	0.22-1.97	0.23-1.79	0.19-1.59	0.23-1.96

Reference table 13.4.1.1.1.g - 13.4.1.1.1.k

Table 10. Inflammatory Markers - ITT Population

Type of cells		Period I		Period II	
		Lentinex®	Placebo	Lentinex®	Placebo
IL-8 (pg/ml)	Mean ± SD	7.693± 5.751	5.595± 3.815	6.976± 5.404	5.287± 2.311
	Range	3.03-25.00	2.11-14.83	2.07-25.00	2.84-12.79
IL-10 (pg/ml)	Mean ± SD	5.619± 22.857	1.373± 4.437	1.588± 4.160	0.403± 0.266
	Range	0.20-100.00	0.20-20.15	0.20-18.31	0.20-1.12
IL-12 (pg/ml)	Mean ± SD	61.463± 34.415	65.078± 37.002	74.362± 40.976	57.491± 28.837
	Range	28.77-136.49	16.17-191.14	21.07-199.28	23.46-131.35
TNFα (pg/ml)	Mean ± SD	1.419± 0.647	1.595± 0.569	1.632± 0.479	1.691± 0.516
	Range	0.10-2.23	0.26-2.45	0.57-2.53	0.85-2.64
CRP (mg/l)	Mean ± SD	1.69±1.40	1.80±1.36	2.200± 1.765	2.524± 3.829
	Range	1-7	1-5	1-7	1-18

Reference table 13.4.2.1.a – 13.4.2.4.a, 13.5.1.a

Baseline Data for Safety Variables

Baseline data for safety laboratory variables are presented in table 11, and for blood pressure/pulse in table 13. Table 12 presents reference ranges for laboratory values where such ranges exist.

Table 11. Laboratory Variables - ITT Population

Variable		Period I		Period II	
		Lentinex®	Placebo	Lentinex®	Placebo
Hb (g/dl)	Mean ± SD	14.57±1.06	14.19±0.98	13.955± 0.804	14.50±1.240
	Range	13.2-17.3	12.2-16.7	12.50-15.20	12.90-17.40
Basophils (10^9/l)	Mean ± SD	0.041±0.050	0.015±0.037	0.056 ±0.051	0.086± 0.048
	Range	0-0.10	0-0.10	0-0.10	0-0.20
Eosinophils (10^9/l)	Mean ± SD	0.118±0.101	0.14±0.037	0.133± 0.059	0.129± 0.085
	Range	0-0.40	0-0.10	0.10-0.30	0-0.40
Lymphocytes (10^9/l)	Mean ± SD	1.89±0.51	1.73±0.41	1.833± 0.388	2.081± 0.556
	Range	1.4-3.0	1.3-2.5	1.20-2.50	1.30-3.20
Monocytes (10^9/l)	Mean ± SD	0.386± 0.105	0.486± 0.189	0.523± 0.243	0.425± 0.160
	Range	0.20-0.64	0.20-0.90	0.20-1.00	0.20-0.94
Neutrophils (10^9/l)	Mean ± SD	2.991± 0.845	3.205± 0.835	3.094± 0.787	3.124± 1.007
	Range	1.8-5.3	1.60-4.90	2.00-4.6	1.60-6.0
Platelets	Mean ± SD	244.773± 53.280	241.350± 51.799	243.778± 60.508	236.190 ±56.642

(10^9/l)	Range	178-396	136-320	154-405	165-397
Leukocytes (10^9/l)	Mean ± SD	5.418± 1.094	5.590± 0.868	5.628± 0.989	5.819± 1.385
	Range	4.0-7.9	3.60-7.10	4.10-7.20	3.70-8.60
ALAT (U/l)	Mean ± SD	24.6±9.3	22.9±9.7	25.150± 12.351	26.524± 7.705
	Range	13-56	12-48	11-66	17-45
ASAT (U/l)	Mean ± SD	26.0±4.2	24.5±5.7	24.250± 5.884	25.714± 5.789
	Range	20-38	14-36	13-39	20-46
γGT (U/l)	Mean ± SD	30.8±20.9	31.5±23.5	34.450± 26.824	36.714± 35.801
	Range	10-95	13-118	11-112	11-134
Creatinine (μmol/l)	Mean ± SD	78.1±10.8	78.3±15.1	77.211± 16.130	74.762± 12.369
	Range	55-93	58-124	54-114	53-99
Bilirubin (μmol/l)	Mean ± SD	12.9±5.7	12.4±4.6	10.950± 5.236	10.714± 3.703
	Range	5-28	6-23	3-25	7-24
TG (mmol/l)	Mean ± SD	1.068± 0.393	1.092± 0.455	1.191± 0.498	1.207± 0.607
	Range	0.50-2.01	0.52-2.14	0.43-2.28	0.48-2.84
Total cholesterol (mmol/l)	Mean ± SD	5.973± 1.012	5.720± 1.315	5.720± 1.199	6.114± 1.144
	Range	4.0-8.5	3.9-8.4	4.0-8.5	4.50-9.40
LDL cholesterol (mmol/l)	Mean ± SD	3.773±0.933	3.825±1.126	3.730±1.011	3.829±1.082
	Range	2.40-5.70	2.5-6.1	2.40-6.20	2.60-6.90
HDL cholesterol (mmol/l)	Mean ± SD	1.95±0.48	1.63±0.33	1.700±0.376	2.005±0.448
	Range	1.3-3.0	1.1-2.4	0.90-2.40	1.30-2.90

Reference table 13.5.1.a

Table 12. Reference Ranges for Laboratory Variables

Variable		Range
Hemoglobin (g/dl)	female (until 13NOV2005)	11.6-16.0
	female (from 13NOV2005)	11.7-15.3
	male (until 13NOV2005)	13.2-16.6
	male (from 13NOV2005)	13.5-17.0
Complement C3 (g/l)		0.83-1.65
Complement C4 (g/l)		0.13-0.36
ALAT (U/l)	female	10-45
	male	10-70
ASAT (U/l)	female	15-35
	male	15-45
γGT (U/l)	female <40 years	10-45
	female >40 years	10-75
	male < 40years	10-80

	male >40 years	15-115
Creatinine (μmol/l)	female	45-90
	male	60-105
Bilirubin (μmol/l)		5-25
TG (mmol/l)		0.45-2.60
Total cholesterol (mmol/l)	>50 years	3.9-7.8
LDL cholesterol (mmol/l)		1.8-5.7
HDL cholesterol (mmol/l)	female	1.0-2.7
	male	0.8-2.1

Table 13. Blood Pressure and Heart Rate – ITT population

Variable		Period I		Period II	
		Lentinex®	Placebo	Lentinex®	Placebo
SBP (mmHg)	Mean ± SD	129.32±12.47	130.00±11.47	130.25± 8.81	125.81± 13.11
	Range	100-150	105-150	110-145	100-145
DBP (mmHg)	Mean ± SD	78.86±8.58	80.65±7.88	77.90± 7.13	80.29± 10.31
	Range	60-90	60-90	60-90	60-100
Heart rate (beats/min)	Mean ± SD	72.10±4.81	69.3±4.70	68.35±5.01	68.62±5.73
	Range	60-84	60-80	60-78	60-87

Reference tables 13.2.2.1.a, 13.2.2.3.a, 13.2.2.5.a

SAFETY RESULTS

Adverse Events

Overview of Adverse Events

All AE results are presented in Tables 13.6.1.1- 13.6.1.7 and in the Statistical Report and in Statistical Appendices.

Table 24 presents a short summary of adverse events.

Table 14. Summary of Adverse Events

Variable		Lentinex®	Placebo
Adverse Events	No of patients with AE	23 (55%)	20 (47%)
	Number of AEs	26	27

Adverse Drug Reactions	No of patients with ADR	2	1
	Number of ADRs	2	1
Serious Adverse Events	No of patients with SAE	0	0
	Number of SAEs	0	0
Withdrawals due to AEs SAEs		 0 0	 0 0

Reference tables 13.6.1.1, 13.6.1.2, 13.6.1.3
Reference data A10.6_AE_INF_McNemar.rtf
Abbreviations: AE- adverse events, ADR-adverse drug reactions, SAE- serious adverse events

The differences between number of patients with at least one AE in Lentinex® group as compared to placebo group were not statistically significant (p=0.5485).

Table 25 summarizes adverse events independent of their relationship to the treatment.

Table 15. Incidence of Adverse Events (AEs) – All Causalities

System Organ Class	Preferred term	Lentinex® Number of subjects n	Placebo Number of subjects n
Gastrointestinal disorders	Abdominal distension	1	0
	Dry mouth	1	0
	Flatulence	0	1
	Nausea	2	0
	Toothache	1	0
	Vomiting nos	1	0
General disorders and administration site conditions	Fatigue	0	3
Infections and infestations	Bronchitis nos	1	0
	Eyelid infection nos	0	1
	Gastroenteritis nos	0	3
	Influenza	3	1
	Nasopharyngitis	7	9
	Respiratory tract infection nos	1	1
	Upper respiratory tract infection nos	1	0
Injury, poisoning and procedural complications	Animal bite	0	1

Investigations	Blood cholesterol increased	1	0
	C-reactive protein increased	1	2
Musculoskeletal and connective tissue disorders	Osteoarthritis aggravated	1	0
Nervous system disorders	Dizziness	0	2
	Migraine nos	1	0
Respiratory, thoracic and mediastinal disorders	Asthma nos	1	0
	Epistaxis	0	1
	Nasal congestion	0	1
	Rhinorrhoea	0	1
Skin and subcutaneous tissue disorders	Paronychia	1	0
Vascular disorders	Phlebitis nos	1	0

Reference table 13.6.1.3.2

Table 26 presents adverse events which are possibly or probably related to one of the treatments.

Table 16. Incidence of Adverse Events (AEs) – Possibly or Probably Related to Treatment

System Organ Class	Preferred term	Lentinex®	Placebo
		Number of subjects n	Number of subjects n
Gastrointestinal disorders	Abdominal distension	1	0
	Dry mouth	1	0
General disorders and administration site conditions	Fatigue	0	1

Reference table 13.6.1.5.1

Specification of Serious Adverse Advents

There were no Serious Adverse Events

Laboratory Tests

Table 27 presents a short summary of laboratory variables. The only significant change was observed in placebo group eosinophils (increase; p=0.002). This change was however not clinically significant.

Table 17. Laboratory Variables Changes

Treatment difference	Lentinex® Mean ± SD	Placebo Mean ± SD
Hb (g/dl)	0.10±0.61	0.06±0.46
Basophils (10^9/l)	0.01±0.05	0.01±0.05
Eosinophils (10^9/l)	0.01±0.06	0.05±0.08
Lymphocytes (10^9/l)	-0.05±0.36	-0.09±0.38
Monocytes (10^9/l)	-0.02±0.15	-0.01±0.18
Neutrophils (10^9/l)	0.30±1.71	0.11±0.97
Platelets (10^9/l)	-2.5±42.1	4.2±28.8
Leukocytes (10^9/l)	0.28±1.63	0.08±1.14
ALAT (U/l)	0.7±8.9	1.0±7.1
ASAT (U/l)	-0.3±5.2	0.3±5.7
γGT (U/l)	2.5±21.5	-1.5±14.4
Creatinine (μmol/l)	0.1±6.5	-1.1±5.2
Bilirubin (μmol/l)	-0.5±4.2	-0.0±4.5
TG (mmol/l)	0.040±0.371	0.040±0.501
Total cholesterol (mmol/l)	0.10±0.73	0.02±0.75
LDL cholesterol (mmol/l)	0.02±0.56	-0.10±0.63
HDL cholesterol (mmol/l)	0.00±0.20	0.06±0.23

Reference data A10.4_chol_carry_wilc.rtf, A10.4_chol_carry_wilc.rtf, A10.4_chol_carry_wilc.rtf, A10.4_chol_carry_wilc.rtf, A10.4_HDL_carry_wilc.rtf, A10.4_LDL_carry_wilc.rtf, A10.4_TG_carry_wilc.rtf, A10.5_alat_carry_wilc.rtf, A10.5_asat_carry_wilc.rtf, A10.5_basof_carry_wilc.rtf, A10.5_bilir_carry_wilc.rtf, A10.5_cre_carry_wilc.rtf, A10.5_eosin_carry_wilc.rtf, A10.5_ggt_carry_wilc.rtf, A10.5_hemog_carry_wilc.rtf, A10.5_leuko_carry_wilc.rtf, A10.5_lymph_carry_wilc.rtf, A10.5_monoc_carry_wilc.rtf, A10.5_neutro_carry_wilc.rtf, A10.5_trombo_carry_wilc.rtf,

Pat. no. 143 had a clinically significant increase in cholesterol value from visit 4 to visit 5, i.e. during the Lentinex® treatment (specified in table 28). This patient had a very high cholesterol level already at the entry to the study. The increase was reported to be not related to study drug.

Table 18. Abnormal Cholesterol Values

Placebo		Lentinex®		Normal range
Visit 2 value	**Visit 3 value**	**Visit 4 value**	**Visit 5 value**	

7.9 mmol/l	7.3 mmol/l	7.5 mmol/l	9.7 mmol/	3.9-7.8 mmol/l

Reference subject listing

Vital Signs

Table 29 presents a short summary of changes in vital signs.

Table 19. Blood Pressure and Heart Rate - ITT Population

Treatment difference	Period I		Period II	
	Lentinex® Mean±SD	Placebo Mean±SD	Lentinex® Mean±SD	Placebo Mean±SD
SBP (mmHg)	-1.36±11.04	0.50±9.58	-2.25±7.86	1.10±10.99
DBP (mmHg)	-0.45±7.06	-1.90±8.71	-0.40±7.26	-1.95±4.54
Heart rate (beats/min)	0.00±6.43	0.90±5.67	0.10±4.80	2.71±5.16

Reference tables 13.2.2.2.a, 13.2.2.4.a, 13.2.2.6.a

Only small changes were observed.

Compliance

Table 30 presents an overview over compliance. The compliance was high for both placebo and Lentinex® group.

Table 20. Compliance - ITT Population

	Period I		Period II	
	Lentinex®	Placebo	Lentinex®	Placebo
Mean±SD	98.3±5.9	97.2±3.3	98.4±3.3	96.6±12.9
Min-max	79.8-104.8	88.6-101.2	88.1-104.3	48.4-116.7

Reference tables 13.3.3.1

Safety Conclusions

There was no significant difference between treatments regarding the incidence of Adverse Events, Adverse Drug Reactions and Serious Adverse Events.

One of the safety laboratory variables eosinophils increased in the placebo group, but not in the Lentinex group.
One patient showed changes in a safety laboratory value (cholesterol). This was reported as an AE.

DISCUSSION

The safety of Lentinex® supplementation was evaluated on the background of safety laboratory parameters and the registered AEs.

The present study also investigated the frequency and duration of infectious diseases that the subjects suffered from during the treatment period. No differences between tratment groups for these variables were found, but the number of infectious diseases was low and the study was not designed to assess this question.

The safety of the supplementation with Lentinex® has also been explored. No differences between the frequency of adverese events between groups have been found. Furthermore, no laboratory safety variables seemed to be negatively affected.

In summary: Lentinex® given orally for 6 weeks to elderly subjects appears to be a safe supplement, not causing adverse events.

List of source tables and statistical appendices

List of Source Tables

Table 21. Source Tables

Table Numbers start with 13, only Numbers Thereafter are Listed Below

Main content	Specified content		Table
Population disposition	Inclusion criteria	ITT population	1.1.a
		PP population	1.1.b
	Exclusion criteria	ITT population	1.2.a
		PP population	1.2.b
	End of study		1.3
	Primary reason for study termination		1.4
	Listing of subjects who discontinued		1.5
Demographics and Physical examination At baseline	Age, race and gender	ITT population PP population	2.1.1.a-b
	Smoking	ITT population PP population	2.1.2.a-b
	Snuff	S ITT population PP population	2.1.3.a-b
	Alcohol	ITT population PP population	2.1.4.a-b
	Physical exercise	ITT population PP population	2.1.5.a-b
Vital signs	Systolic BP, per visit	ITT population PP population	2.2.1.a-b
	Systolic BP, Cross over difference	ITT population PP population	2.2.2.a-b
	Diastolic BP, per visit	ITT population PP population	2.2.3.a-b
	Diastolic BP, Cross over difference	ITT population PP population	2.2.4.a-b
	Heart rate per visit	ITT population PP population	2.2.5.a-b
	Heart rate, Cross over difference	ITT population PP population	2.2.6.a-b
Body measurements At baseline	Weight	ITT population PP population	2.3.1.a-b
	Height	ITT population PP population	2.3.2.a-b
	BMI	ITT population PP population	2.3.3.a-b
Concomitant medication	Concomitant medication, by ATC code level 4	ITT population PP population	2.6.a-b
Medical history	Medical history, by system organ class (SOC)	ITT population	2.8.1
	Medical history, by preferred term	ITT population	2.8.2

	Medical history, status at baseline	ITT population	2.8.3
Drug accountability	Compliance for study period	ITT population	3.1
		PP population	3.2.
Efficacy			
Immunology	Per period, visit and treatment. ITT population	Ratio CD4/CD8	4.1.1.1.a
		CD4	4.1.1.1.b
		CD8	4.1.1.1.c
		CD3	4.1.1.1.d
		CD19	4.1.1.1.e
		CD56	4.1.1.1.f
		C3	4.1.1.1.g
		C4	4.1.1.1.h
		IgG	4.1.1.1.i
		IgA	4.1.1.1.j
		IgM	4.1.1.1.k
	Change per period, carry-over effect, treatment effect and period effect ITT population	Ratio CD4/CD8	4.1.1.2.a
		CD4	4.1.1.2.b
		CD8	4.1.1.2.c
		CD3	4.1.1.2.d
		CD19	4.1.1.2.e
		CD56	4.1.1.2.f
		C3	4.1.1.2.g
		C4	4.1.1.2.h
		IgG	4.1.1.2.i
		IgA	4.1.1.2.j
		IgM	4.1.1.2.k
	Per period, visit and treatment. PP population	Ratio CD4/CD8	4.1.2.1.a
		CD4	4.1.2.1.b
		CD8	4.1.2.1.c
		CD3	4.1.2.1.d
		CD19	4.1.2.1.e
		CD56	4.1.2.1.f
		C3	4.1.2.1.g
		C4	4.1.2.1.h
		IgG	4.1.2.1.i
		IgA	4.1.2.1.j
		IgM	4.1.2.1.k
	Change per period, carry-over effect, treatment effect and period effect PP population	Ratio CD4/CD8	4.1.2.2.a
		CD4	4.1.2.2.b
		CD8	4.1.2.2.c
		CD3	4.1.2.2.d
		CD19	4.1.2.2.e
		CD56	4.1.2.2.f
		C3	4.1.2.2.g
		C4	4.1.2.2.h
		IgG	4.1.2.2.i
		IgA	4.1.2.2.j
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	Interleukin 8	Per period, visit and treatment.	4.2.1.a
		Change per period, carry-over effect, treatment effect and period effect	4.2.1.b
	Interleukin 10	Per period, visit and treatment.	4.2.2.a

			Change per period, carry-over effect, treatment effect and period effect	4.2.3.b
	Interleukin 12		Per period, visit and treatment.	4.2.3.a
			Change per period, carry-over effect, treatment effect and period effect	4.2.3.b
	TNF alpha.		Per period, visit and treatment.	4.2.4.a
			Change per period, carry-over effect, treatment effect and period effect	4.2.4.b
Safety				
Laboratory values	Per visit and treatment		ITT population PP population	5.1.a-b
	Cross over difference		ITT population PP population	5.2.a-b
Adverse Events (all tables per treatment)				
	Adverse Events (all causalities)	Number of patients with AE, number of AE per patient	ITT population	6.1.1.a
			PP population	6.1.1.b
		Incident of AE	ITT population	6.1.2.a
			PP population	6.1.2.b
		AE per System Organ Class and Preferred Term	All AE	6.1.3.1-2
			Intensity	6.1.4.1-2
			Relationship to study drug	6.1.5.1-2
			Action taken	6.1.6.1
			Outcome at study end	6.1.7.1
			Duration at study end	6.1.7.2
	Adverse Events (Infectious disease)	Number of patients with Infectious disease, number of Infectious disease per patient	ITT population	6.2.1.a
			PP population	6.2.1.b
		Incident of Infectious disease	ITT population	6.2.2.a
			PP population	6.2.2.b
		Infectious disease per System Organ Class and Preferred Term	All Infectious disease	6.2.3.1-2
			Intensity	6.1.4.1-2
			Relationship to study drug	6.2.5.1-2
			Action taken	6.2.6.1
			Outcome at study end	6.2.7.1
			Duration at study end	6.2.7.2

List of Statistical Appendices

Table 22. Statistical Appendices

Main content	Specified content	Table
Primary endpoints	Analysis of primary endpoints, T-test, PP population	A10.1_prim_imm_ttest_within.rtf
	Analysis of primary endpoints, ANCOVA, PP population	A10.1_prim_immun_ancova.rtf
Primary and secondary endpoints. Immunological variables	Analysis of carry-over and period effects for immunological variables	A10.1_imm_carry_wilc.rtf
	Analysis of treatment, carry-over and period effects for a subpopulation of subjects with starting immunological values lower than median	A10.1_median_anova_wilc.rtf
Secondary endpoints.	Analysis of secondary endpoints, T-test, PP population	A10.1_sec_imm_ttest_within.rtf

Immunological variables	Analysis of secondary endpoints, ANCOVA, PP population	A10.1_sec_immun_ancova.rtf
Secondary endpoints. Inflammatory variables	Analysis of IL-8, ANCOVA, ITT population	A10.2_il8_ancova.rtf
	Analysis of IL-8, carry-over effects, period effects and T-test, ITT population	A10.2_il8_carry_wilc.rtf
	Analysis of IL-10, ANCOVA, ITT population	A10.2_il10_ancova.rtf
	Analysis of IL-10, carry-over effects, period effects and T-test, ITT population	A10.2_il10_carry_wilc.rtf
	Analysis of IL-12, ANCOVA, ITT population	A10.2_il12_ancova.rtf
	Analysis of IL-12, carry-over effects, period effects and T-test, ITT population	A10.2_il12_carry_wilc.rtf
	Analysis of TNFα, ANCOVA, ITT population	A10.2_tnf_ancova.rtf
	Analysis of TNFα, carry-over effects, period effects and T-test, ITT population	A10.2_tnf_carry_wilc.rtf
	Analysis of CRP, ANCOVA, ITT population	A10.3_crp_ancova.rtf
	Analysis of CRP, carry-over effects, period effects and T-test, ITT population	A10.3_crp_carry_wilc.rtf
Secondary endpoints. Laboratory safety parameters	Analysis of cholesterol, ANCOVA, ITT population	A10.4_chol_ancova.rtf
	Analysis of cholesterol, carry-over effects, period effects and T-test, ITT population	A10.4_chol_carry_wilc.rtf
	Analysis of HDL cholesterol, ANCOVA, ITT population	A10.4_hdl_ancova.rtf
	Analysis of HDL cholesterol, carry-over effects, period effects and T-test, ITT population	A10.4_HDL_carry_wilc.rtf
	Analysis of LDL cholesterol, ANCOVA, ITT population	A10.4_ldl_ancova.rtf
	Analysis of LDL cholesterol, carry-over effects, period effects and T-test, ITT population	A10.4_LDL_carry_wilc.rtf
	Analysis of triglycerides, ANCOVA, ITT population	A10.4_tg_ancova.rtf
	Analysis of triglycerides, carry-over effects, period effects and T-test, ITT population	A10.4_TG_carry_wilc.rtf
	Analysis of ALAT, ANCOVA, ITT population	A10.5_alat_ancova.rtf
	Analysis of ALAT, carry-over effects, period effects and T-test, ITT population	A10.5_alat_carry_wilc.rtf
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	Analysis of ASAT, carry-over effects, period effects and T-test, ITT population	A10.5_asat_carry_wilc.rtf
	Analysis of basophils, ANCOVA, ITT population	A10.5_basof_ancova.rtf
	Analysis of basophils, carry-over effects, period effects and T-test, ITT population	A10.5_basof_carry_wilc.rtf
	Analysis of blirubin, ANCOVA, ITT population	A10.5_bilir_ancova.rtf
	Analysis of bilirubin, carry-over effects, period effects and T-test, ITT population	A10.5_bilir_carry_wilc.rtf
	Analysis of creatinine, ANCOVA, ITT population	A10.5_cre_ancova.rtf
	Analysis of creatinine, carry-over effects, period effects and T-test, ITT population	A10.5_cre_carry_wilc.rtf
	Analysis of eosinophils, ANCOVA, ITT population	A10.5_eosin_ancova.rtf
	Analysis of eosinophils, carry-over effects, period effects and T-test, ITT population	A10.5_eosin_carry_wilc.rtf
	Analysis of γGT, ANCOVA, ITT population	A10.5_ggt_ancova.rtf
	Analysis of γGT, carry-over effects, period effects and T-test, ITT population	A10.5_ggt_carry_wilc.rtf
	Analysis of haemoglobin, ANCOVA, ITT population	A10.5_hemog_ancova.rtf

	Analysis of haemoglobin, carry-over effects, period effects and T-test, ITT population	A10.5_hemog_carry_wilc.rtf
	Analysis of leukocytes, ANCOVA, ITT population	A10.5_leukocytes_ancova.rtf
	Analysis of leukocytes, carry-over effects, period effects and T-test, ITT population	A10.5_leukocytes_carry_wilc.rtf
	Analysis of lymphocytes, ANCOVA, ITT population	A10.5_lymph_ancova.rtf
	Analysis of lymphocytes, carry-over effects, period effects and T-test, ITT population	A10.5_lymph_carry_wilc.rtf
	Analysis of monocytes, ANCOVA, ITT population	A10.5_monoc_ancova.rtf
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	Analysis of neutrophils, ANCOVA, ITT population	A10.5_neutro_ancova.rtf
	Analysis of neutrophils, carry-over effects, period effects and T-test, ITT population	A10.5_neutro_carry_wilc.rtf
	Analysis of thrombocytes, ANCOVA, ITT population	A10.5_trombo_ancova.rtf
	Analysis of thrombocytes, carry-over effects, period effects and T-test, ITT population	A10.5_trombo_carry_wilc.rtf
Secondary endpoints. Adverse Events	Analysis of frequency of adverse events	A10.6_AE_INF_McNemar.rtf
	Analysis of duration of infectious adverse events ANCOVA,	A10.6_infdis_ancova.rtf
	Analysis of duration of infectious adverse events, carry-over effects, period effects, ITT population	A10.6_infdis_carry_wilc.rtf

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APPENDIX C – SAFETY AND PHARMACOLOGICAL EFFECTS OF LENTINEX®

In an initial toxicity and efficacy study, Lentinex® was administered to 10 BN rats via oral lavage, 2.5 mL and 3.1 mL daily in 5 day cycles, with a 2 day rest between cycles. All rats were observed for weight loss, lethargy and for toxic effects, ataxia and behavioural changes. Initially (Wk 7), rats were administered 2.5 ml (0.5 mg/mL) for 3 cycles. This dose of Lentinex® was tolerated well with no ill-effects observed and regular weight gain as observed with untreated controls. However, after 3 cycles of treatment there was no observed change in any hematological component from basal levels. Thus, the dose of Lentinex® was increased to 3.1 mLs per day and 0.8 mg/mL (Wk 10). Again, animals displayed no toxic effects and continued to gain weight at the same rate as the untreated controls.

While after the initial period of administration (Wks 7-10) with 2.5 mL (0.5 mg/ml) of Lentinex®, there were no observed changes in haematological components, following administration of the first cycle of 3.1 mL (0.8 mg/ml) per day there was an observed increase in average platelet production ($P>0.05$, Wk 11) from basal levels which increased further ($P<0.05$) following 2 additional cycles (Wk 13). Subsequent to a total of 9 cycles of this dose of Lentinex®, average platelet levels have declined from these initially high values. Concurrently, average red blood cell counts (RBC) increased from basal levels by Wk 13 ($P<0.05$) and remained higher than basal levels up to Wk 19 ($P<0.05$), with a slight but statistically insignificant increase in hemoglobin levels by Wk 13 ($P>0.05$).White blood cells counts (WBC) decreased initially from basal levels by Wk 11 ($P<0.05$).However, increased levels were observed on Wk 13 in comparison to Wk 11 ($P<0.0001$) and basal levels (Wk 9; $P>0.05$) with levels dropping well below basal levels by Wk 19($P<0.0001$).While percentages of CD8 and CD4 positive cells remained relatively unchanged there was a considerable increase in both the B-cell ($P<0.0001$) and monocyte populations ($P<0.0001$) on Wk 13, consistent with the above observations of increases on Wk 13.

Interferon gamma increased significantly in comparison to controls by Wk 11 ($P<0.01$), with an apparent, consistent escalation following increasing length of Lentinex® administration. There were no significant alterations of tumour necrosis factor alpha, IL-1, IL-1 or IL-6. IL-2 exhibited a transient decrease at week 11. Following an initial increase in IL-10 by Wk 9, levels subsequently normalized up to and including Wk 19. GM-CSF remained unchanged until Wk 19 when levels increased, but with a spread of data points.

GlycaNova concluded “Animals have displayed no physiological toxic side-effects to Lentinex® at the doses described and continue to thrive following 34 weeks of administration. There are no indications of haematological toxicity on the subset of blood cells or parts of the immune system examined following Lentinex® administration.”

In a series of two rat studies Lentinex® was administered at 2.5 mL (0.5 mg/mL) in study (1), and 3.1 ml (0.8 mg/mL) in study (2), via oral lavage daily to rats that were previously injected with BNML leukemia. All rats were observed for weight loss, lethargy and hind limbs were monitored for paralyses. In addition, the Lentinex® group was monitored for toxic effects, ataxia and behavioural changes. While no adverse events were noted for any of the Lentinex® treated BNML rats, neither did the Lentinex® increase survival of these rats compared to controls. It was concluded that while Lentinex® was safe at these doses, the doses were not high enough to get a therapeutic effect.

Following these studies, two additional studies were conducted. In the first study Lentinex® was administered at 0.5 mg/kg, and Idarubicin (1.5 mg/kg) plus Lentinex® at 0.5 mg/kg were administered orally to rats that were then later injected with BNML leukemia. In the second study Lentinex® at 20 mg/kg and Idarubicin (1.5 mg/kg) plus Lentinex® at 20 mg/kg were administered orally to rats that were then later injected with BNML leukemia. Both studies had negative (saline) and positive (Idarubicin only) controls, as well. All rats were observed for weight loss, lethargy and hind limbs were monitored for paralyses. In addition, the Lentinex® groups were monitored for toxic effects, ataxia and behavioural changes. Survival of the BNML rats was the primary outcome variable. The rats that received the Lentinex® did not exhibit any adverse effects, and had survival times equal to the Idarubicin control groups. The combination of Lentinex® plus Idarubicin had slightly (not significant) lower survival times than the Idarubicin or Lentinex® only groups, by 1 day for the 0.5 mg/kg Lentinex® plus Idarubicin dose, and 1.5 days for the 20 mg/kg Lentinex® plus the Idarubicin dose.

In a study conducted at Department of Veterinary Medicine, Louisiana State University (sponsored by GlycaNova), twenty-four mice were divided into four groups. Group 1 received 2 mg/kg Lentinex® in 0.15 M NaCl, intraperitoneally (i.p.), Group 2 received an equal volume of saline i.p., Group 3 received 10 mg/kg Lentinex® in water by gastric gavage (oral) and group 4 received an equal volume of water orally. All mice received treatment once daily for 5 days. Twenty four hours after the last treatment the mice were bled and euthanized for cell isolation. It was concluded that Lentinex® had no effect on the health of the mice and no statistically significant effect on their growth as measured by weight gain. However, i.p. Lentinex® had an effect on the number and phenotype of the cells isolated from the peritoneum lavage. Orally exposed mice and those that received saline i.p were relatively similar; however, there was an increase in the total number of cells isolated from the peritoneum of i.p. Lentinex® treated mice. The increased number of cells was due mainly to an increase in neutrophils in the population. The relative contribution to the observed results by the PMN compared to macrophage cannot be estimated at this time, but might affect the difference observed differences in the i.p. groups. In contrast, the number and phenotype of the oral groups were very similar and their comparisons should be statistically valid. Intraperitoneal (i.p.) lentinan treatment caused a 4-fold increase in TNFα production at 12 hours by peritoneal exudate cells (PEC) and a 3-fold increase by splenocytes as well as increasing the amount of TNFα produced in response to LPS treatment in vitro by PEC (30% increase) and splenocytes (2-fold). Oral Lentinex® treatment had no significant effect on TNFα production by PEC or splenocytes. Interestingly, there was a modest but

significant decrease (20%) in TNFα production by PEC following in vitro LPS stimulation.

These results indicate that the effect on TNFα may be initiated at the local level and/or require direct exposure to Lentinex®. Both oral and i.p. Lentinex® treatment significantly enhance IL-1 production by PEC (18-fold and 6-fold, respectively). In addition, IL-1β in response to in vitro LPS was also increased more than 3-fold in i.p. treated mice Like TNF, IL-1β production by PEC from mice exposed to oral lentinan and stimulated in vitro LPS was decreased (2-fold). Splenocytes from treated and untreated mice produced small amounts of IL-1β in the first 12 hours of culture. When mice were exposed to Lentinex®, either orally or i.p., splenocytes from the mice were stimulated to produce IL-1β by treatment with either LPS or ConA (a T-cell mitogen), however the production was very low compared to PEC. In general, lentinan treatment had only small effects on IL-6 production by PEC, although this was only significant in the non-LPS treated cells. In contrast, IL-6 production was modestly elevated by unstimulated splenocytes but was modestly decreased by LPS or Con A stimulated splenocytes, both oral and i.p. exposure. IL-6 levels continued to rise over the next 12 hours of culture. Interleukin 12 production at 12 hours was at the limit of detection of the assay except in ConA stimulated splenocyte cultures. In the i.p. lentinan exposure, mice produce a modest but significant amount of IL-12 in response to LPS. Oral Lentinex® exposure generally decreased pro-inflammatory cytokine production by PEC and increased pro-inflammatory production by splenocytes suggesting the systemic response to Lentinex® (oral) may be different from the local responses measured in previous studies (nasal, i.p., i.v.).

Two additional studies were conducted by GlycaNova at the Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences. In the first study, 360 male broiler chickens were fed different experimental diets containing combinations of 0.01, 0.1 mg or 1.0 mg of Lentinex®/kg of feed (in a preparation called LentiGuard®) and/or a coccidiostatic agent (Monteban®). The objective was to study the effect of LentiGuard® in diets for broiler chickens. Weight gain did not differ significantly between any of the dietary treatments in any period. A numerical higher weight gain was observed for birds fed on diets with Monteban®, and birds on the treatment with the highest level of LentiGuard® showed the second highest weight gain. Because of the similar feed consumption among the treatments, the feed/gain ratio did not differ significantly between the treatments. No clinical problems related to dietary treatments were observed. No significant effects of Lentinex® on performance, mortality and litter dirtiness were observed.

The second study involved the feeding of three different levels (0.01, 0.10, or 1.0 mg/kg feed) of Lentinex® (delivered as LentiGuard®) or a control diet for four weeks, to 32 weaned piglets per group. On average, the pigs in the three Lentinex® groups consumed 7.5 mg, 77.8 mg and 797 mg Lentinex® respectively. The objective was to study the effect of LentiGuard® in diets for weaned piglets. No health problems related to the dietary treatments were observed. The piglets revealed good growth relative to what is found at commercial pig farms in Norway, on average for all of the pigs the daily gain (ADG) was 559 g. The piglets generally showed good viability, which was evaluated subjectively. Especially in Period 1, the piglets fed the highest level of LentiGuard® (2.0 mg/kg) were found to have a good viability compared to the other groups. There were no significant differences among the dietary treatment groups in either weight gain or daily feed intake of piglets during week 1-2, week 3-4, or the overall piglet period. During the two first weeks, the pigs fed the 2.0 mg LentiGuard®/kg diet numerically gained 12% faster relative to the control diet. During the two last weeks, and the overall piglet period, the differences among the treatment groups were small. The addition of LentiGuard® to diets had a significant effect on several blood haematological parameters including the index of anisocytis, neutrophilic granulocytes and lymphocytes. A significant positive linear effect of increasing dietary levels of LentiGuard® was also found for haemoglobin and haematocrit.

APPENDIX D – SAFETY STUDIES RELATED TO LENTINAN

The consensus of the scientific community is that Lentinan within defined dosage quantities is a safe compound for all species studied, is safe when taken chronically; and is safe regardless of the mode of administration. This consensus, especially with regard to oral administration, was achieved after Lentinan had been extensively studied during the 1980s (Wasser, 2005).

Safety studies, most of which have been published in peer-reviewed journals, included acute toxicity determination, subacute or subchronic toxicity determination, chronic toxicity determination, effect on fertility and reproduction and determination of teratogenesis. Lentinan was administered orally, by intravenous injection, by intraperitoneal injection, subcutaneous injection, intranasally and by inhalation. The effect of Lentinan administration was studied in several species, including mice, several strains of rats, guinea pigs, chickens, New Zealand white rabbits, beagle dogs and Rhesus monkeys. Most of these studies were rigorously controlled “pre-clinical” trials conducted by researchers from different countries in the Far East and in the West, in support of potential drug development programmes. (Full details of these safety studies can be included if considered relevant to the application).

This safety consensus eventually led to the commercialisation of Lentinan products throughout Asia and since the early 1990s in the United States where it is being sold as a dietary supplement under the auspices of DSHEA.

APPENDIX E – CoA FOR LENTINEX®

Glyca Nova

Certificate of Analysis

Identity of batch: 10-050530

Date of sampling: Nov. 20, 2006

Product: Lentinex (1mg/ml)

Country of origin: Norway

Parameter	Method	Spec.	Result
Appearance	Visual inspection	Light brown, slightly turbid	Approved
Microbiological data	Total viability count on PCA and MDSA	Sterile	Approved
Lentinan	GNM-003 (Ethanol precipitation)	1 mg/ml ± 0.2	1.1 mg/ml
Residual glucose	HPLC	<20 mg/ml	21 mg/ml(*)
Total protein	GNM-005 (Bradford)	< 100 µg/ml	50 µg/ml
pH	pH-meter	3.0 - 4.0	3.8

(*) Value out of spec. This is considered irrelevant because the low sensitivity method used at this time was later shown to over-estimate the amount of glucose.

Date: 6/11-07

Signature : TA
(QC) Med Dir.

Date: 6/11 - 07

Signatur :ATH Anne-Torill Hovland
Head of process developement

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Glyca Nova

Certificate of Analysis

Identity of batch: 10-070823

Date of sampling: Sep. 6., 2007

Product: Lentinex (1mg/ml)

Country of origin: Norway

Parameter	Method	Spec.	Result
Appearance	Visual inspection	Light brown, slightly turbid	Approved
Microbiological data	Total viability count on PCA and MDSA	Sterile	Approved
Lentinan	GNM-003 (Ethanol precipitation)	1 mg/ml ± 0.2	1.0 mg/ml
Residual glucose	Enzymatic	<20 mg/ml	15.1 mg/ml
Total protein	GNM-005 (Bradford)	< 100 µg/ml	31 µg/ml
pH	pH-meter	3.0 - 4.0	3.8

Date: 6/11-07

Signature : TA
(QC) Med Dir.

Date: 6/11-07

Signatur :ATH Anne-Torill Hovland
Head of process developement

Page 1 of 1

Glyca Nova

Certificate of Analysis

Identity of batch: 10-070511

Date of sampling: Mai 31., 2007

Product: Lentinex (1mg/ml)

Country of origin: Norway

Parameter	Method	Spec.	Result
Appearance	Visual inspection	Light brown, slightly turbid	Approved
Microbiological data	Total viability count on PCA and MDSA	Sterile	Approved
Lentinan	GNM-003 (Ethanol precipitation)	1 mg/ml ± 0.2	0.8 mg/ml
Residual glucose	Enzymatic	<20 mg/ml	16.5 mg/ml
Total protein	GNM-005 (Bradford)	< 100 µg/ml	22 µg/ml
pH	pH-meter	3.0 - 4.0	3.6

Date: 6/11-07

Signature : TA
(QC) Med Dir.

Date: 6/11-07

Signatur :ATH Anne-Torill Hoeland
Head of process developement

Page 1 of 1

Glyca Nova

Certificate of Analysis

Identity of batch: 10-071002

Date of sampling: Oct. 31., 2007

Product: Lentinex (1mg/ml)

Country of origin: Norway

Parameter	Method	Spec.	Result
Appearance	Visual inspection	Light brown, slightly turbid	Approved
Microbiological data	Total viability count on PCA and MDSA	Sterile	Approved
Lentinan	GNM-003 (Ethanol precipitation)	1 mg/ml ± 0.2	1.0 mg/ml
Residual glucose	Enzymatic	<20 mg/ml	15.6 mg/ml
Total protein	GNM-005 (Bradford)	< 100 µg/ml	39 µg/ml
pH	pH-meter	3.0 - 4.0	3.8

Date: 6/11-07

Signature : TA

(QC) Med Dir.

Date: 6/11-07

Signatur :ATH Anne-Torill Hovland

Head of process developement

Page 1 of 1

Glyca Nova

Certificate of Analysis

Identity of batch: 10-070921

Date of sampling: Oct. 15., 2007

Product: Lentinex (1mg/ml)

Country of origin: Norway

Parameter	Method	Spec.	Result
Appearance	Visual inspection	Light brown, slightly turbid	Approved
Microbiological data	Total viability count on PCA and MDSA	Sterile	Approved
Lentinan	GNM-003 (Ethanol precipitation)	1 mg/ml ± 0.2	1.1 mg/ml
Residual glucose	Enzymatic	<20 mg/ml	12.3 mg/ml
Total protein	GNM-005 (Bradford)	< 100 µg/ml	56 µg/ml
pH	pH-meter	3.0 - 4.0	3.8

Date: 6/11-07

Signature : TA
(QC) Med Dir.

Date: 6/11-07

Signatur :ATH
Head of process developement Anne-Torill Hoeland

Page 1 of 1

APPENDIX F – CoA FROM ANALYCEN

Certificate of Analysis

Lidköping

AnalyCen

+ GlycoNova Norge AS
Postboks 1045
Valaskjold
1705 Sarpsborg
NORGE

Report issued by
Accredited Laboratory

NORSK AKKREDITERING TEST043

Sample Number	NOE002421-07		Page 1 (1)
Customer Number	8184479-1166609		
Sample Type	Næringsmiddel		
		Sampled Date	2007-10-15
		Arrival Date	2007-10-16
		Report printed	2007-11-05
Sample Identity	Sample 2, 10-070823		

Analysis	Result	Units	Acc.	Method	Site
Mercury Hg	<0.020	mg/kg	± 30 %	NMKL 170 mod.; AFS (kallf.)	L
Cadmium Cd	<0.01	mg/kg	± 20 %	NMKL161 mod; ICP-MS	L
Cromium Cr	<0.05	mg/kg	± 30 %	NMKL161 mod; ICP-MS	L
Manganese Mn	0.28	mg/kg	± 15 %	NMKL161 mod; ICP-MS	L
Nickel Ni	<0.05	mg/kg	± 45 %	NMKL161 mod; ICP-MS	L
Lead Pb	<0.02	mg/kg	± 20 %	NMKL161 mod; ICP-MS	L
Copper Cu	0.27	mg/kg	± 15 %	NMKL161 mod; ICP-MS	L
Protein N*6,25	0.8	%	± 5 %	EU DIR 93/28 m	O

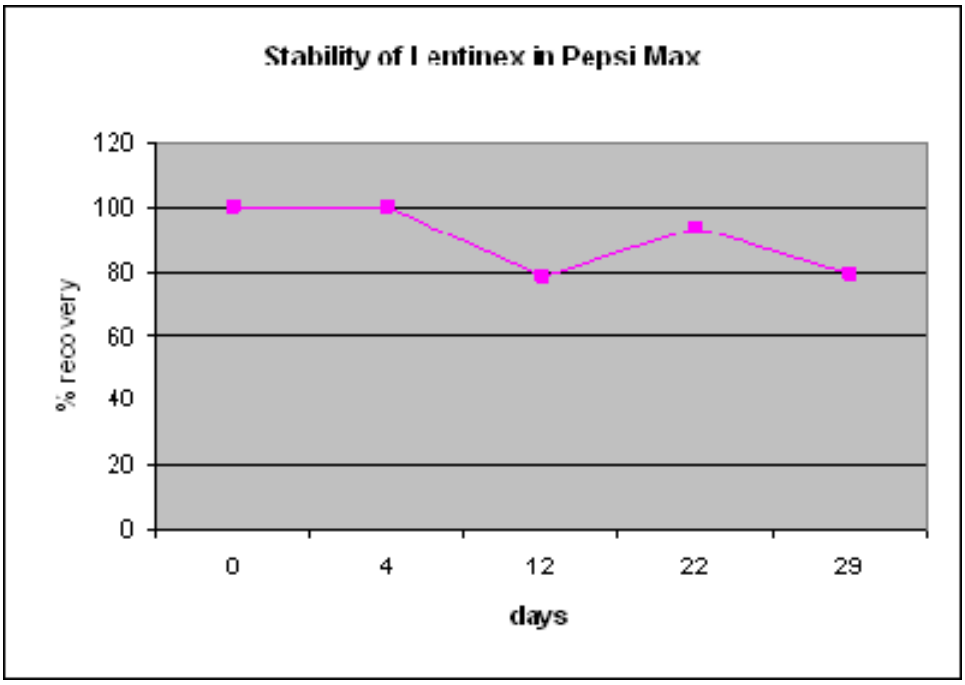
Karin Danielsen +47 69279817

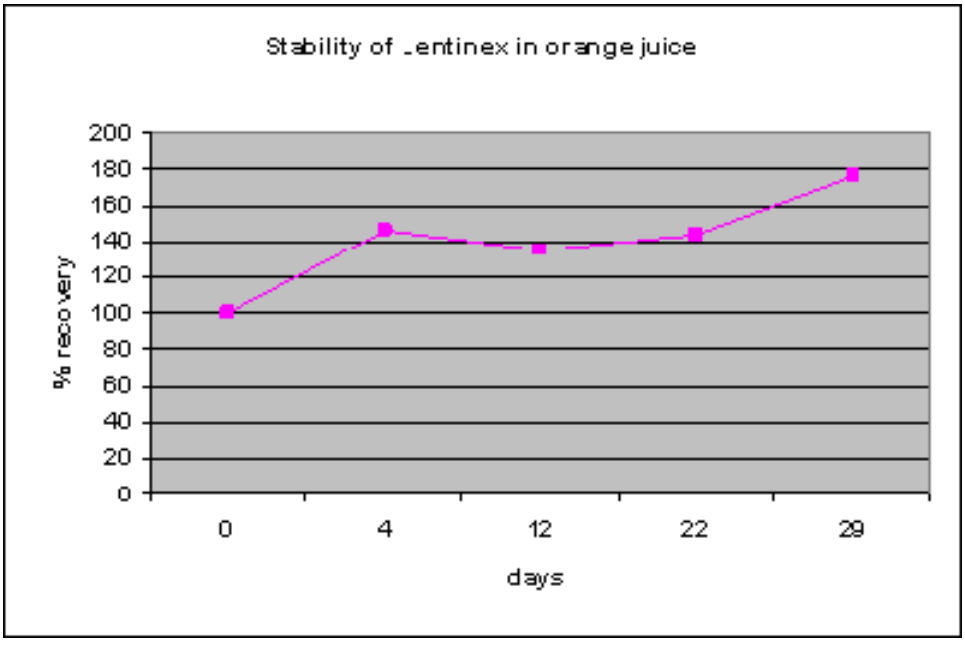
Lab. ingeniør

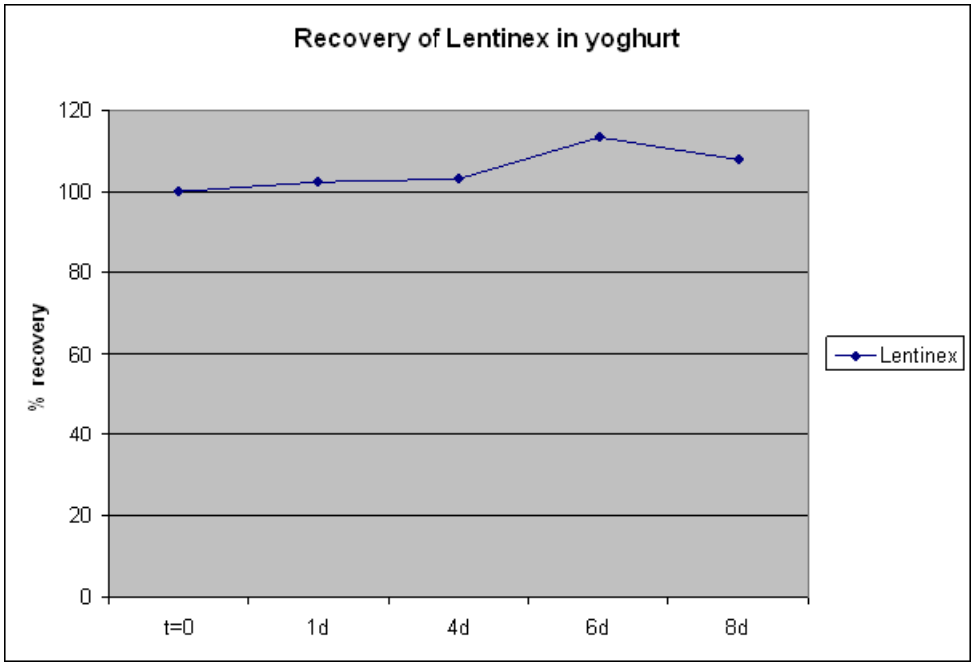
Explanation to abbreviations see reverse side.
Analysis marked * is a non accredited analysis.

APPENDIX G – ADDITION OF LENTINEX® TO SELECTED FOOD/DRINKS

Lentinex® was given to a soft drink, an orange juice and yoghurt. The products were tested for the duration of their perceived shelf life. The results show no adverse effect on Lentinex® during storage of the product.






Recovery of Lentinex in yoghurt
120
100
80
60
40
20
0
% recovery
t=0
1d
4d
6d
8d
Lentinex

APPENDIX H – ANALYTICAL PROCEDURES USED FOR LENTINEX®

Ethanol precipitation

Purpose:
The purpose is to estimate the content of polysaccharides, which is used to determine the strength of the product (ferment).

Procedure:
One volume of the test sample is mixed with two volumes of cold absolute ethanol in a beaker and shaken vigorously for 4 minutes. It is then left for four hours in a refrigerator at 4°C. The solution is centrifuged for 20 min. at 3750 rpm. After centrifugation the precipitate is washed with absolute ethanol and centrifuged for 20 min at 3750 rpm. The precipitate is dried for two hours at 105°C and thereafter weighed, using a watch glass.

Results
When the test is used to characterise a product for sale a minimum of 3 parallels should be analysed. The method is not valid for concentrations lower than 0.5 mg/ml. The result is given as weight/volume.

Analysis of sterility

Purpose:
The purpose of this sterility test is to check if the product batch contains living microorganisms on release from GlycaNova As.

Frequency and time of testing:
Testing of sterility is performed on the product in its final packing, i.e. on the product as it is distributed. The sampling frequency used depends on the size of product batch and the size of the packing, as given in the following table:

Batch size	Packing size	No. of packages (max)	No. of samples tested
0-50 litre	≤ 100 ml	500	5
0-50 litre	0.1 – 5 litre	10 - 500	5
50-500 litre	≤ 100 ml	5000	20

The samples should be selected at random, and should include the start, middle and finish of the production batch. The sampling frequencies should be reconsidered at regular intervals, and in particular if new packing equipment is implemented.

Procedure:
All samples are tested in parallel. Samples of 0.5ml are inoculated on PCA and MDSA **(**Medimush Double Strength Agar) Petri dishes using standard microbiological techniques. The PCA is incubated at 37 ºC for 48 hrs, and MDSA at 25 ºC for 48 hrs.

The plates are visually inspected after 24 and 48 hrs. This procedure is an adaptation of the accredited method NS 4789.

Results
Colony forming units are counted on all plates. If there is no growth (cfu = 0) on any plates the result is denoted as “Approved” on the CoA. If there is growth (cfu > 0) on any plate then the batch can not be approved for sale, and HPD must be informed immediately.

Analysis of protein (code GNM-005)

Purpose:
To determine the content of protein in the test substance.

Procedure:
Total protein content is measured in a standard Bradford assay as follows:

1. Apply 100ul sample per well in a microtitre plate (Nunc F)
2. Add 200ul Bradford reagent (Sigma Aldrich cat. no. B6916)
3. Mix by gentle shaking
4. Measure absorbance at 595 nm (Multiscan Ascent, Thermo Electron Corp.).

A standard curve is made by measuring different dilutions of BSA (Bovine serum albumin, Sigma Aldrich cat. no. 82516) according to the above steps, and the protein concentration in the sample is determined by referring to this standard curve.

Results:
The result is given as μg/ml

Analysis of free glucose

Purpose:
This SOP will define the procedure for the determination of free glucose in the fermentation broth, drug substance or the product.

Procedure:
5 ml of test-solution is sampled for this procedure. The procedure is as follows:

I. Dilute standard in distilled water as given in the table given below:

No	STD + H_2O	Vol (μL)	Glucose (mg/dL)
1	150μL + 0μL	150	300
2	100μL + 50μL	150	200
3	50μL + 100μL	150	100
4	25μL + 125μL	150	50
5	0μL + 150μL	150	0

Set up 1.5-mL centrifuge tubes. Transfer 5 μL diluted standards and samples to appropriately labelled tubes. Transfer 500 μL Reagent to each tube. Close the tubes tightly and mix. Store diluted standards at -20°C for future use.

2. Place the tubes in a tube holder and heat in a boiling water bath or for 8 mins. Cool down in cold water bath for 4 min.

3. Transfer 200 μL in duplicate into a clear bottom 96-well plate. Careful:
Avoid bubble formation. Read optical density at 620-650nm (peak absorbance at 630nm). Signal is stable for 60 min.

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