# CLINICAL TRIAL PROTOCOL

Protocol Title

A Randomized, Double-Blind, Placebo-Controlled Trial to Evaluate the Impact of a daily dose of AMAZ-02, a food derived ingredient, for 4 months on skeletal muscle energetics and function in Healthy Elderly

Short Title

BioENERGetics and Muscle function Improvement with AMAZ-02 in Elderly Skeletal MusclE (ENERGIZE Trial)

**Protocol N° 17.01.AMZ**

**Version 6.0 - Date: 05 June 2020**

**This is a Phase 2 randomized, double-blind, placebo-controlled study enrolling 66 healthy elderly subjects (33 placebo and 33 AMAZ-02 intervention) who are 65-90 years of age, who are otherwise healthy. AMAZ-02, a food derived ingredient, will be given as a daily oral dose for 4 months.**

# NAMES AND ADRESSES OF PARTICIPANTS

| | | |
|---|---|---|
| Sponsor | Amazentis<br>EPFL Innovation Park<br>Bâtiment C<br>1015 Lausanne<br>Switzerland | |
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|---|---|
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# CLINICAL STUDY PROTOCOL SIGNATURE PAGE

**Protocol N°: 17.01.AMZ Version 6.0 - Date: 05 June 2020**

**Title:** A Randomized, Double-Blind, Placebo-Controlled Trial to Evaluate the Impact of a daily dose of AMAZ-02, a food derived ingredient, for 4 months on skeletal muscle energetics and function in Healthy Elderly

The sponsor and the investigator agree to conduct the study in compliance with the clinical study protocol (and amendments), International Conference on Harmonization (ICH) guidelines for current Good Clinical Practice (cGCP) and applicable regulatory requirements.

**Sponsor:**

**AMAZENTIS SA**

EPFL Innovation Park, Batiment C

1015 Lausanne, Switzerland

Represented by:

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Date:

(dd-mm-yyyy) 08-06-2020

Signature:

# PROTOCOL SYNOPSIS

| | |
|---|---|
| **Name of Investigational Product:**<br>AMAZ-02, a food and dietary ingredient | |
| **Title of Study:** A Randomized, Double-Blind, Placebo-Controlled Trial to Evaluate the Impact of a daily dose of AMAZ-02, on Skeletal Muscle Energetics and Function in Elderly | |
| **Protocol No.: 17.01.AMZ** | |
| **Study center:** Single Center - University of Washington Medical Center, Seattle, WA, USA | |
| **Principle Investigator(s):** David Marcinek, PhD & Jose Garcia MD, PhD | |
| **Number of Planned Subjects:**66 | **Phase of Development:** Phase 2 |
| **Length of Study:** 4 months<br>Estimated first subject visit: February 5th, 2018<br>Estimated last subject visit: July 30th, 2020 | |
| **Study Design:** This is a randomized, double-blind, single-center, placebo-controlled trial enrolling 66 healthy elderly subjects (33 placebo and 33 AMAZ-02 administration) who are ≥65 and ≤ 90 years of age with evidence of low mitochondrial function. | |
| **Study Objectives:**<br>**Primary Objective:**<br>To evaluate the effect of an oral nutritional supplementation with AMAZ-02, a food derived ingredient, compared to placebo for 4 months in healthy elderly subjects on:<br>- 6 minute walking distance (6MWD)<br>- Maximum mitochondrial ATP production (ATPmax) measured via Magnetic Resonance Spectroscopy on a hand muscle (FDI- first dorsal interosseus)<br>**Secondary Objectives**<br>To evaluate the effect of 4 month oral administration of AMAZ-02 on:<br>- Muscle function measured via the single muscle fatigue test on a hand muscle (FDI- first dorsal interosseus)<br>- Maximum mitochondrial ATP production (ATPmax) measured via Magnetic Resonance Spectroscopy (on the *Tibialis Anterior* leg muscle)<br>- Muscle function measured via the single muscle fatigue test (on the *Tibialis Anterior* leg muscle)<br>- Exercise Performance (Leg power, time to fatigue, Borg perceived exertion scale and VO2) measured by cycle ergometry<br>- Short Physical performance Battery (SPPB)<br>- Hand grip strength | |

- Leg muscle strength (via Cybex one-repetition max and ten-repetition max testing)
- Muscle size (cross-sectional area of the muscles via MRI)
- Mitochondrial function on muscle biopsy (via high resolution respirometry)
- Mitochondrial gene and protein expression in skeletal muscle tissue (microarray and protein array)
- Acylcarnitine levels in plasma (via metabolomics)
- Quality of life via SF36 questionnaire
- Plasma lipid profile (Total Cholesterol, LDL, HDL and Triglycerides)
- Plasma circulating biomarkers (myostatin, follistatin, proteomics, metabolomics)
- Safety

**Investigational Product, Dosage, and Mode of Administration or Intervention:** AMAZ-02 provided as a softgel

Name of the compound: AMAZ-02

Form: Softgel containing 250 mg of AMAZ-02

Dose per intake: 4 softgels a day

Dosing's: 1000mg AMAZ-02

Timing for intake: Repeated oral dose administration from day 1 to day 120 according to the randomization. The administration will take place in the morning with around 200 ml of tap water in sitting position and in fasting conditions of 6-8 hours.

**Planned Duration of Intervention:** 4 months

Oral/placebo: 120 days

**Reference Product, Dose, and Mode of Administration or Comparative Intervention:** Placebo given as oral soft-gel

Name of the compound: Placebo

Form: Soft gel capsule containing lecithin, triglycerides, diglycerides.

Dose per intake: 4 softgels a day

Timing for intake: Repeated oral dose administration from day 1 to day 120 according to the randomization. The administration will take place in the morning with around 200 ml of tap water in sitting position and in fasting conditions of 6-8 hours.

**Primary endpoints**

- Change in 6 minute walking distance (6MWD) at the end of study intervention compared to baseline
- Percent change from baseline in ATP max in skeletal muscle (via MRS)

ATP max will be assessed on the hand muscle (FDI- first dorsal interosseus)

**Secondary endpoints**

- Percent change from baseline in contraction number during a single muscle fatigue test assessed on the hand muscle (FDI- first dorsal interosseus)
- Percent change from baseline in ATP max in skeletal muscle (via MRS) (on the *Tibialis Anterior* leg muscle)
- Percent change from baseline in contraction number during a single muscle fatigue test (on the *Tibialis Anterior* leg muscle)
- Change in SPPB Scores at the end of study interevention compared to baseline
- Change in leg power output, time to fatigue, Borg perceived exertion scale and VO2 at 85% of the estimated maximum heart rate (HRmax) will be determined at the end of study interevention compared to baseline (via cycle ergometry)
- Change in hand grip strength at the end of study interevention compared to baseline
- Change in leg muscle strength (via Cybex one-repetition max and ten-repetition max testing)
- Change in muscle size (cross-sectional area of the muscles) before and after intervention
- Change in mitochondrial function on muscle biopsy samples at the end of study interevention compared to baseline (respirometry)
- To assess the effect of AMAZ-02 on mitochondrial gene and protein expression in muscle tissue before and after study intervention
- To assess the effect of AMAZ-02 on plasma acylcarnitine levels
- To assess the effect of AMAZ-02 on quality of life questionnaire (SF36)
- Change from baseline in plasma lipid profile
- Change from baseline in plasma for circulating biomarkers (myostatin, follistatin, proteomics, metabolomics)

**Safety Assessments:**

- Number of adverse events
- Laboratory data
- ECG

- Physical examination
- Vital Signs and temperature

AEs will be coded according to the MedDRA.

**Statistical Methods:**

Primary efficacy endpoints

- Change in 6 minute walking distance (6MWD) at the end of study intervention compared to baseline
- Percent change from baseline in improvement in ATP max in hand skeletal muscle (via MRS)

The analysis of primary efficacy efficacy parameters is done using an analysis of co-variance (ANCOVA) using the factor treatment as independent factor, the baseline measurement as covariable and the change from baseline as dependent variable. 95% confidence intervals for treatment differences and corresponding non-adjusted p-values will be calculated.

A 5% significance level will be applied for the comparison of the AMAZ-02 dose versus Placebo.

In order to accont for the two primary efficacy endpoints, a priori-ordered hypothesis are stated, i.e. change in ATPmax will only be tested for confirmatory decisions if the change in 6MWD resulted in a statistical significant difference in AMAZ-02 dose compared to Placebo. If the AMAZ-02 dose resulted in a significant result for 6MWD, the testing procedure continues for testing ATPmax.

This trial is powered to detect a difference in change from baseline in 6-minute walk between treatment groups.

Previous studies in endurance training in subjects aged >65 to <90 years suggest a change in ATPmax can be achieved over a 6 month time period of 0.103 under treatment and -0.03 under Placebo. Based on a clinical relevant difference of 0.133 and a standard deviation of 0.149 under treatment and 0.154 under Placebo this results in a sample size of 27 per group using a 5% two-sided significance level and 88% power.

Assuming a 10% drop-out rate, 30 subjects per group will be enrolled. The sponsor (Amazentis) has observed however, in a parallel clinical study (NCT03464500) with a similar powering and utilizing similar study endpoints like peak Vo2 (a surrogate marker of mitochondrial function) and the 6 minute walk that a higher standard deviation was observed than initially anticipated on most endpoints. Sample size estimates performed by an independent biostatistician on these other study results showed that in order to hit statistical significance on the 6 minute-walk test, a n=35-40/ arm sample size would have been optimal, rather than a n=27-30. Based on the observed effects on the 6 minute-walk test that is also being utilized in the 17.01.AMZ study this will be the main study primary end-point and in light of the new information on the results from the other clinical trial and the impact of COVID-19 on future study recruitment, the sponsor has decided that this trial can stop at the current enrolment of 66 subjects (33/arm) into this study.

**Table of Contents**

## STUDY FLOWCHART

**Table 1: Schedule of Events**

| Parameters | Screening | Randomization | Visit 2 (Day 61) ±4days | Visit 3 (Day 119-121) ±4days |
|---|---|---|---|---|
| | Visit 1a and 1b (Day -30 – Day 0) | | | |
| Informed consent | X | | | |
| Randomization | | X | | |
| MRS (FDI & TA) | X | | | X |
| MRI (Muscle Cross-sectional area) | X | | | X |
| 6 minute walk test | X | | | X |
| Inclusion and exclusion criteria* | X | X | | |
| Medical history | X | | | |
| Physical examination(Body weight, height) | X | | | X |
| Blood Haematology | X | | | X |
| Visit Blood Chemistry | X | | | X |
| Temperature | X | | | X |
| ECG | X | | | X |
| Vital Signs (HR, BP, RR) | X | | | X |
| Study Product Dispensed | | X | X | |
| (Active / Placebo) administration | | Continuous | | |
| Plasma sample (AMAZ-02, myostatin, follistatin, Metabolomics) | X | | X | X |
| Lipid Prodile (Total cholesterol, LDL, HDL, triglycerides) | X | | | X |
| Muscle Fatigue Test | X | | X | X |
| Muscle Strength Test (Cybex) | X | | | X |
| Cycle Ergometer Test | X | | | X |
| SPPB | X | | | X |
| Quality of Life Questionnaires | X | | | X |
| Muscle biopsy– for microarray/ Oxygraph | X | | | X |
| (S)AE/Con-meds | X | Continuous | | |

*: Phone screening for majority of I/E criteria followed by ATPmax and walk test screening; FDI: first dorsal interosseus hand muscle; TA: tibialis anterior leg muscle; BP = Blood Pressure, HR = Heart Rate, RR = Respiratory Rate, SCR = Screening, 6MWT = 6 minute walk-test, ECG= Electrocardiography, , MRS = Magnetic Resonance Spectroscopy, SPPB: Short Physical Performance Test

## ABBREVIATIONS

| | | |
|---|---|---|
| **AE** | **:** | Adverse Event |
| **AESI** | **:** | Adverse Event of specific Interest |
| **AICAR** | **:** | 5-aminoimidazole-4-carboxamide riboside |
| **ALT** | **:** | Alanine Leucine Transferase |
| **AST** | **:** | Alanine serine transferase |
| **ATPmax** | **:** | Maximum phosphorylation capacity (of mitochondria) |
| **AUC** | **:** | Area Under the Curve |
| **BLQ** | **:** | Below the limit of quantification |
| **BMI** | **:** | Body Mass Index |
| **BP** | **:** | Blood pressure |
| **bpm** | **:** | beats per minute |
| **cGCP** | **:** | Current Good Clinical Practice |
| **cm** | **:** | centimeter |
| **Cmax** | **:** | Observed maximal plasma concentration |
| **CRO** | **:** | Contract Research Organisation |
| **CQA** | **:** | Clinical Quality Assurance |
| **DBP** | **:** | Diastolic blood pressure |
| **DNA** | **:** | Deoxyribonucleic acid |
| **ECG** | **:** | Electrocardiogram |
| **eCRF** | **:** | Electronic Case Report Form |
| **ET** | **:** | Ellagitannins |
| **ETC** | **:** | Electron Transport Chain |
| **FDA** | **:** | Food and Drug Administration |
| **FDI** | **:** | First dorsal interosseus |
| **GCP** | **:** | Good Clinical Practice |
| **GLP** | **:** | Good laboratory practice |
| **GRAS** | **:** | generally regarded as safe |
| **HBs** | **:** | Hepatitis B surface antigen |
| **HCV** | **:** | Hepatitis C virus |
| **HDL** | **:** | High density lipoprotein |
| **HIV** | **:** | Human Immunodeficiency Virus |
| **HIV1** | **:** | Human Immunodeficiency Virus type 1 |
| **HIV2** | **:** | Human Immunodeficiency Virus type 2 |
| **HR** | **:** | Heart Rate |
| **ICH** | **:** | International Conference on Harmonization |

| | | |
|---|---|---|
| ***i.e.*** | *:* | *id est(that is)* |
| **IP** | **:** | Investigational product |
| **IRB/IEC** | **:** | Institutional review board / ethical review board |
| **IWRS** | **:** | Interactive Web Response System |
| **Kg** | **:** | Kilogram |
| **L** | **:** | Liter |
| **LDL** | **:** | Low density lipoprotein |
| **LLQ** | **:** | Lower limit of quantification |
| **MRS** | **:** | Magnetic Resonance Spectroscopy |
| **MCH** | **:** | Mean Corpuscular Hemoglobin |
| **MCHC** | **:** | Mean Corpuscular Hemoglobin Concentration |
| **MCV** | **:** | Mean Corpuscular Volume |
| **MedDRA** | **:** | Medical Dictionary for Regulatory Activities |
| **mg** | **:** | Milligram |
| **min** | **:** | Minute |
| **ml** | **:** | Milliliter |
| **mmHg** | **:** | Millimeters of mercury |
| **ms** | **:** | Millisecond |
| **MRSD** | **:** | maximum recommended starting dose |
| **MTD** | **:** | Maximum Tolerated Dose |
| **NA** | **:** | Not applicable |
| **ng** | **:** | Nanograms |
| **NTEAE** | **:** | Non-Treatment Emergent Adverse Event |
| **PBS** | **:** | Phosphate buffered saline |
| **PCr** | **:** | Phosphocreatine recovery time |
| **PCR** | **:** | Polymerase chain reaction |
| **PI** | **:** | Principal Investigator |
| **PK** | **:** | Pharmacokinetics |
| **P**max | **:** | Leg power output at aerobic limit |
| **P/O** | **:** | Coupling coefficient |
| **RBC** | **:** | Red Blood Cells |
| **RNA** | **:** | Ribonucleic acid |
| **SAE** | **:** | Serious Adverse Event |
| **SAR** | **:** | Serious Adverse Reaction |
| **SBP** | **:** | Systolic Blood Pressure |
| **SD** | **:** | Standard Deviation |
| **SEM** | **:** | Standard Error of the Mean |

| | | |
|---|---|---|
| **SOC** | **:** | System organ class |
| **SPPB** | **:** | Short Physical Performance Battery |
| **SUSAR** | **:** | Suspected Unexpected Serious Adverse Reaction |
| **TA** | **:** | Tibialis Anterior muscle |
| **TEAE** | **:** | Treatment Emergent Adverse Event |
| **tmax** | **:** | First time to reach Cmax |
| **t1/2** | **:** | Plasma concentration half life |
| **VO2** | **:** | Volume of oxygen uptake |
| **WBC** | **:** | White Blood Cells |
| **WHO-DD** | **:** | World Health Organization - Drug Dictionary |
| **WHO-DRL** | **:** | World Health Organization - Drug Reference List |

# INTRODUCTION

## Background on AMAZ-02

Bioactives and ingredients derived from natural products have been demonstrated to exhibit a whole range of health promoting benefits ranging from inhibition of chronic inflammatory diseases to control of blood pressure, lowering of blood cholesterol, improving visual acuity, to a delay in cognitive decline associated with neurodegenerative disease. However, despite the promise the nutrition and health field has struggled to move these discoveries from the benchside to the clinic and to the consumer primarily because of poor characterization and understanding of biological pathways linked to the efficacy of these bioactive compounds and ingredients within dietary plants and fruits.

There is currently no effective treatment for age-related muscle decline in the elderly (Janssen et al. 2004). While it is natural to have a gradual decline in muscle mass and strength with aging (>60 years), a variety of environmental factors (diet, exercise, chronic diseases, polymedication) dictate whether with aging, elderly fall into healthy, pre-frail (i.e. decline in either muscle mass or function) or frailty (sarcopenia, i.e. > 2 standard deviations decline in both muscle mass and function) groups. Amongst the elderly, more than 50 percent males and 25 percent females fall into pre-frail category. Around 10-20 percent of the pre-frail elderly population subsequently transitions into frail category as this population advances into the next decades of their lifetime. The health economic costs of maintaining pre-frail and frail syndrome amount to over 20 billion USD in costs to the society and healthcare systems.

Nutritional interventions have been developed to support muscle mass in elderly. A few oral nutritional products worth noting in the nutrition sector include Abbott's Ensure and Juven products, Nestlé's Resource Senior Active, and Nutricia's Fortimel. These formulations are enriched with all essential minerals, vitamins and trace elements and are high in protein or essential amino acids content. Juven is a blend of beta-hydroxy-beta methylbutyrate, arginine, and glutamine, which are claimed to play an important role in building tissue by supporting repair after injury. However, they do not contain any ingredients that are intended to improve mitochondrial function according to product labeling and advertising. In the pharmaceutical sector, only sarcopenia (loss of muscle function and mass) has been targeted and there are no promising drugs in late-stage development.

Bioactives derived from natural products have been demonstrated to exhibit a whole range of health promoting benefits ranging from inhibition of chronic inflammatory diseases to control of blood pressure, lowering of blood cholesterol, improving visual acuity, to a delay in cognitive decline associated with neurodegenerative disease. However, despite the promise, the field has struggled to move these discoveries from the benchside to the clinic primarily because of poor characterization and understanding of biological targets linked to the efficacy of these bioactive compounds within dietary plants and fruits.

Amazentis is a Swiss life science company working in the field of health and nutrition and it focuses on developing bioactives derived from natural sources for food supplement and functional food applications. Amazentis, has discovered that AMAZ-02, a first in class dietary ingredient derived from food sources that is able to stimulate mitophagy, a process whereby faulty and dysfunctional mitochondria are cleared. Consequently, AMAZ-02 will be a completely new food product that will represent a notable breakthrough for the support of mitochondrial and muscle function during healthy aging. AMAZ-02 is derived from natural sources and is safe, bioavailable and addresses a longstanding unmet need using a unique and highly effective mechanism of action, i.e. the rejuvenation of mitochondria.

This breakthrough discovery positions AMAZ-02 as an innovative and pioneering product in the field of mitochondrial modulation. Age-related muscle decline will be the main clinical target for the development

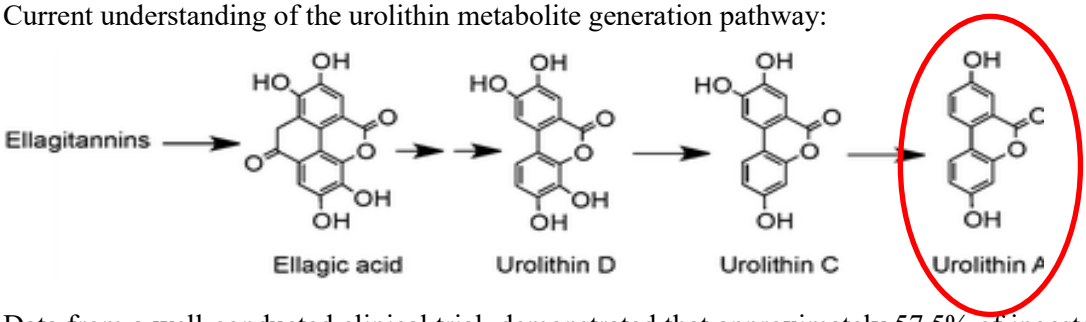
of a food supplement in the healthy aging category. As mentioned earlier, its closest competitor, resveratrol, suffers from safety issues and inferior efficacy, requiring much higher dosing as compared to AMAZ-02. AMAZ-02 is derived from the dietary components and is already naturally found in approximately 30-40% of the healthy population, this effect seems to be in part dependent on the gut microflora composition in humans.

Functional food and food supplements that have been precisely calibrated to deliver bioactive natural ingredients are a desirable way to unlock health benefits to a wider target population, i.e. those unable to derive the benefits of dietary components that impart various health promoting benefits. Amazentis has developed a food supplement that contains AMAZ-02 at specific levels after a controlled manufacturing process. In the case of AMAZ-02, which is a metabolite of a natural product present in food, one can only guarantee precise dosing levels by providing the bioactives in a specific formulation. Practically, it would not be possible to consume food products and receive the similar benefits of AMAZ-02 due to (1) seasonality (2) content level and (3) interpersonal variations of metabolism.

The synthetic compound AMAZ-02 produced by Amazentis SA (synthesized to a purity of over 97%) is identical in structure to Urolithin A (Uro A). Uro A is a metabolite, produced endogenously following consumption of ellagitannins (ET). ET are dietary polyphenols found mainly in fruits (pomegranate), nuts (walnuts, hazelnuts, pistachios, acorns, chestnuts, pecans), berries (strawberries, raspberries, blackberries, camucamu), muscadine grapes, oak aged wines and spirits, and medicinal plants and tisanes (geranium, oak leaves, etc.), as well as their derivatives such as juices, jams and jellies (González-Sarrias et al. 2010; Espin et al. 2013).

Dietary ellagitannins are converted into ellagic acid in the upper portion of the gastrointestinal tract and further metabolized in the large intestine to urolithins. There are large differences in urolithin production capacity among individuals likely due to differences in the colonic microflora responsible for ET degradation (Cerdá et al. 2004; Cerdá et al. 2005; González- Sarrías et al. 2010; García-Villalba et al. 2013). The urolithins are absorbed and may be conjugated in the liver, and may be subsequently excreted in the urine. Findings from several studies illustrate that Uro A and Uro A glucuronide are the predominant urolithin forms detected in urine (Cerdá et al. 2004; Cerdá et al. 2005b; Seeram et al. 2006; Seeram et al. 2008; Truchadoet al. 2011).

Current understanding of the urolithin metabolite generation pathway:




Data from a well-conducted clinical trial, demonstrated that approximately 57.5% of ingested ellagic acid (in this case from strawberries) was recovered as urolithins, and primarily in the form of Uro A. The table below demonstrates the potential levels of urolithin that a person might receive upon consumption of different foods containing ellagitannis.

| Food | Ellagitannin + ellagic acid content | Ellagic acid content after hydrolysis | Equivalent Urolithin Content [c] |
|---|---|---|---|
| 1 Liter Pomegranate Juice | 1600 - 2000 mg [a] | 200-1100 mg [b] | 115 – 633 mg |
| 50 g (8 nuts) Walnuts | 802 mg | 352 – 443 mg [d] | 202 - 255 mg |

| | | | |
|---|---|---|---|
| 1 g Pecans | 21 - 86 mg | 2 mg [d] | 1.2 mg |
| 100 g Raspberry | 263 - 330 mg | 124 mg [d] | 71.3 mg |
| 100 g Strawberry | 77-85 mg | 20-51 mg [d] | 18 mg [f] |
| 100 g Cloudberry | 315 mg | 57– 64 mg [e] | 33– 38 mg |

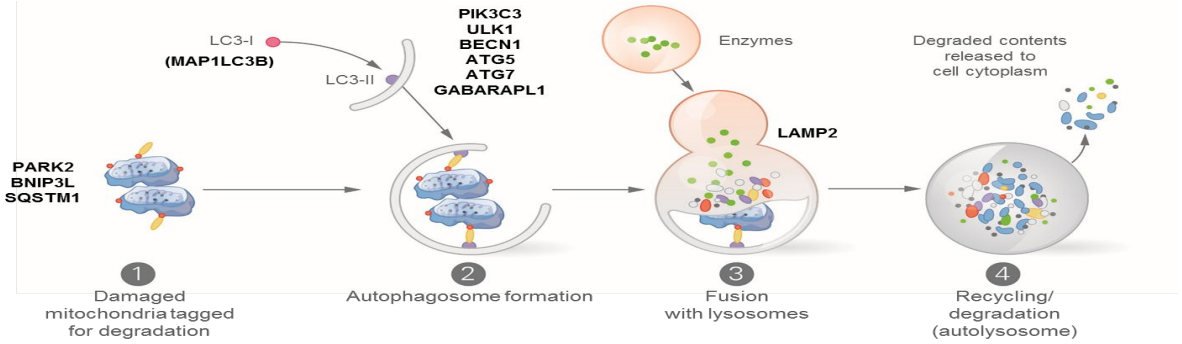
[a] Gil, et al. J. Agric. Food Chem 2000
[b] Uzuner, S. World Academy of Science, Engineering and Technology 2012
[c] The equivalent urolithin content was based on a 57.5% conversion of the available ellagic acid into urolithin, as described in Truchado et al., 2011
[d] Hollman et al. *"The Content of the Potentially anticarcinogenic Ellagic Acid in Plant Food", in Food and Cancer Prevention: Chemical and Biological Aspects, 1993*
[e] Häkkinen, S et al. Eur. Food Res. Technol., 2000
[f] Truchado et al. J. Agric. Food Chem 2011

Amazentis, the sponsor of the study, has conducted several preclinical studies showing that AMAZ-02 has a remarkable potential to improve muscle function in adult mice through the enhancement of mitochondrial function via mitophagy. Based on these pre-clinical findings of dramatic improvement of endurance (increase by 47%) and in hand grip strength (9% increase) in old animals, the sponsor has chosen age-related muscle decline for AMAZ-02 clinical development. Amazentis has conducted several preclinical studies showing that AMAZ-02 has a remarkable potential to improve muscle function in adult mice through the enhancement of mitochondrial function via mitophagy. Based on these pre-clinical findings of dramatic improvement of endurance (increase by 47%) and in hand grip strength (9% increase) in old animals, the sponsor has chosen age-related muscle decline as the primary clinical target population for AMAZ-02 clinical development.

Amazentis has also recently successfully performed a Phase 1 safety clinical study with AMAZ-02 in humans, where no product related serious or non-serious adverse events were observed in a single and multiple ascending dose study in elderly human subjects (NCT02655393). Consequently, Amazentis has decided to test AMAZ-02 in this Phase 2 human efficacy study (study code: 17.01.AMZ) designed to assess the effect of AMAZ-02 on improving mitochondrial function in a healthy elderly population.

**Mitochondrial and Muscle Function Decline with Aging**

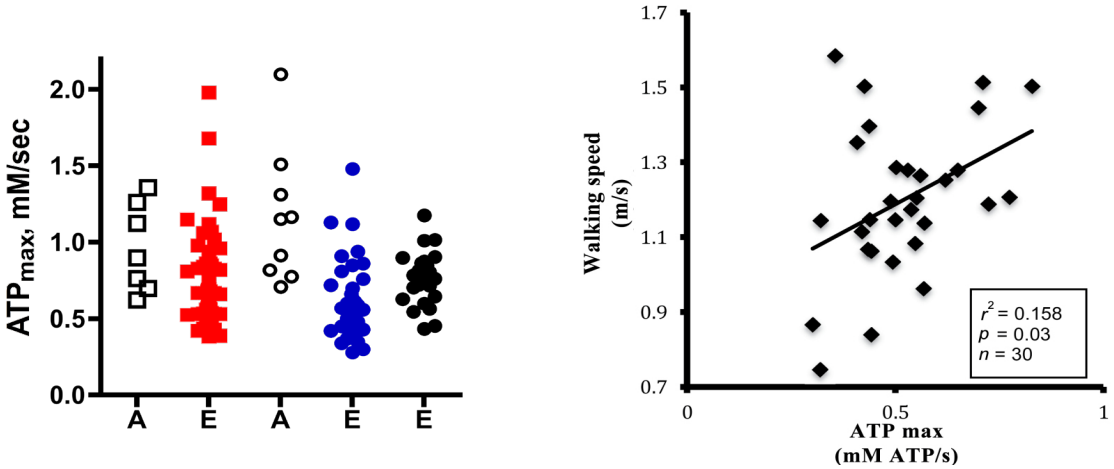
Elderly humans (≥60 years) currently represent around 20% of the total world population and the proportion is expected to increase to 29% by 2025. Age related diseases pose a burden for both the elderly and society as a whole. In recent years, evidence has shown that dysfunction of mitochondria plays an important role in age related diseases, such as Alzheimer's and Parkinson's diseases, diabetes mellitus type 2 and sarcopenia (Moon et al. 2015; Wang et al. 2014; Kamat et al. 2014). The mitochondrion is a central organelle that can drive both cellular life, i.e. by producing energy in the respiratory chain, and death, i.e. by initiating apoptosis. More recently, it was demonstrated that dysfunctional mitochondria can be specifically targeted for elimination by autophagy, a process that has been termed mitophagy. During aging, there is a progressive decline in the cell capacity to eliminate its dysfunctional elements by autophagy, as evidenced by the accumulation of oxidative damage and mutations in mitochondria and the decrease in autophagic flux. Therefore, restoring levels of autophagy and mitophagy in the elderly represents an interesting therapeutic approach to improve mitochondrial function. Most of the compounds that have been identified to improve mitochondrial function either stimulate mitochondrial biogenesis (e.g. 5-aminoimidazole-4-carboxamide riboside (AICAR), resveratrol, nicotinamide riboside) or the respiratory chain (e.g. coenzyme Q10), while no specific mitophagy inducer has been identified yet (Gruber et al. 2013; Ruetenik et al. 2015). AMAZ-02 (Uro A) specifically targets the mitophagy pathway shown in the figure below (Ryu et al. Nature Medicine, 2016), allowing clearing up of faulty mitochondria that potentially accumulate during the aging process. This leads to new and healthier mitochondria generation and optimal mitochondrial function.




Aging is associated with loss of muscle mass and strength that could predispose healthy elderly individuals to sarcopenia or frailty syndrome. This decline in muscle mass and function begins in the 5th decade of life and exaggerates after the eight decade of life. The loss of muscle mass and strength often reflects in elderly with reduced physical performance and endurance capacity (such as slow walking speed, faster time to fatigue, lower VO2 and lower muscle power).

Aging in parallel is also associated with a decline in both the capacity and efficiency of energy supply in the aged muscle. Mitochondria being the central and pivotal organelles that are often reffered to as the powerhouses of a cell, drive this energy supply demand in the skeletal muscle. Several studies using a variety of techniques have reported reduced capacity to generate ATP with age (Coen et al., 2013). The loss of skeletal muscle mitochondrial function has been widely hypothesized to contribute to the decline in endurance, phycial performance and muscle strength in elderly. The capacity for oxidative ATP synthesis, assessed with 31P Magentic Resonance Spectroscopy (ATPmax), has been shown to be lower in older adults with higher fatigue levels (Santanasto, 2015) and also shown conclusively to decline in elderly (E) compared to healthy adults (A- see figure below left panel), by research conducted over the years by Prof. Kevin Conley's group (original Priniciple Investigator of this Clinical trial).

31P-magnetic resonance spectroscopy has proven to be a potent tool for investigating energy metabolism in vivo. Recently, several studies reported skeletal muscle ATP production using the 31P-magnetic resonance spectroscopy (MRS) technique to study in vivo mitochondrial function in a non-invasive way. Lower ATP turnover (ATPmax) in the skeletal muscle was found in elderly with poor physical performance such as reduced walking speed via this MRS tool (see Figure below right panel- from J Gerontol A Biol Sci Med Sci. 2013 Apr; 68(4): 447–455).




ATPmax is measured by the PCr recovery (Figure above, right panel) rate after exercise and has been used extensively to investigate mitochondrial function in various study populations. The recovery rate constant (PCr recovery) is a function of the maximum rate of oxidative ATP synthesis in the skeletal muscle. Our

own non-interventional human studies (sponsored by Amazentis) have shown that PCr recovery (and thereby ATPmax) is delayed in elderly with lower muscle strength and sedentary status compared to active endurance trained elderly subjects. This reduced ATPmax correlates well with lower mitochondrial abundance in skeletal muscle biopsies of elderly subjects (*unpublished observations*).

Improving ATPmax (as measured by PCr recovery rates following exercise) in healthy elderly is the main study outcome of this Phase 2 trial initiated by Amazentis with Prof. Conley's team in University of Washington. Correlating these improvement ultimately to improvement in muscle function are key and as such we will be using well defined and characterized muscle function tests such as the exercise tolerance test muscle fatigue as the single muscle level and cycle ergometer test to measure leg muscle power.

**Duration of study intervention with AMAZ-02**

Decline in muscle and mitochondrial function with aging is a long term gradual process driven possibly by multiple factors such as physical activity status (active versus sedentary lifestyle), diet and chronic conditions in aging that lead to augmentation of sarcopenia and frailty. In general, clinical studies focussed on muscle and mitochondrial function have focussed on providing interventions for at least a 4- 6 month period before the expected health benefit can be observed.

By focusing on health elderly in the context of this efficacy trial and screening for healthy eldery who have low mitochondrial function at inclusion we believe we can stratify the elderly subjects on whom improvements are most likely to be observed with this dietary intervention. Still considering the pre-clinical evidence with AMAZ-02 and general evidence in the field that dietary interventions or even exercise regimens would need a longer intervention to manifest an improved effect, we have decided to provide oral supplementation with the higher 1000mg dose of AMAZ-02 compared to placebo for a duration of 4 months.

**Summary of available results with AMAZ-02**

**Pre-clinical efficacy studies**

Pre-clinical data generated by Amazentis demonstrate that AMAZ-02 is a novel, first-in-class natural compound that induces mitophagy both in vitro and in vivo following oral consumption and furthermore acts to improve muscle function in mice during aging. In the worm *C. elegans*, AMAZ-02 prevents the accumulation of dysfunctional mitochondria with age and extends lifespan. During aging AMAZ-02 prolongs normal activity, including mobility and pharyngeal pumping, while maintaining mitochondrial respiratory capacity. These effects translate to rodents, where AMAZ-02 is bioavailable in the muscle and stimulates mitophagy. AMAZ-02 was evaluated for the prevention of the onset of age-related muscle decline in 16 months-old male C57BL76J mice fed a high-fat diet. From the age of 16 months, the diet was supplemented with 50 mg/kg/d of AMAZ-02 for 8 months. Chronic AMAZ-02 administration led to a striking improvement in muscle function between 22 and 24 months of age, as manifested by a 9% increase in grip strength and a 57% increase in spontaneous exercise measured by using the running wheel. In a second mouse study, the impact of a shorter 6 weeks intervention with AMAZ-02 in already aged male C57BL/6J mice (22 months-old) that were fed regular chow diet was tested. Also, in mice fed chow diet, AMAZ-02 supplementation resulted in a robust increase in running endurance by 42%. The effect of AMAZ-02 on muscle function was also assessed in young rats. Male Wistar rats were fed chow diet containing AMAZ-02 at a concentration of 25 mg/kg/day starting at 5.5 weeks of age. Muscle function was

assessed by voluntary running in activity wheels to which they had access two nights per week from 7 weeks of age. On average, AMAZ-02 treatment significantly enhanced running capacity by 65%. Altogether, these studies highlight that AMAZ-02 improves muscle function throughout the different stages of aging with a relatively short-term intervention and across species border. Furthermore, it indicates that its impact on muscle function is independent of the diet composition. The table below sum up the key pre-clinical pharmacology experiments showing the effects of AMAZ-02 on mitophagy and muscle function in different experimental models:

**Table: Summary of preclinical pharmacological data showing the effect of AMAZ-02 on muscle function**

| Species/ Model | AMAZ-02 Intervention | Outcome at the phenotypic level related to muscle | Outcome at the macromolecular level | Other observations |
|---|---|---|---|---|
| *C. elegans* | 50uM | - Increase mobility<br>- Increase pharyngeal muscle pumping | - Induction of mitophagy in muscle as measured using GFP reporter strains, gene expression and quantification of mtDNA | - Extended lifespan<br>- No delay in development<br>- No impairment of fertility<br>- No change in food intake |
| C2C12 myoblasts | 50uM | | - Induction of mitophagy as measured by confocal microscopy and Western Blot | - no impairment of cellular viability |
| Mouse | 16-months old C57BL6/J treated for 6 months with AMAZ-02 admixed into HFD at 50mpk | - Increased grip strength<br>- Increased exercise capacity measured as voluntary running in running wheels | - Increased autophagy, as measured by gene expression and Western Blot | - no change in food intake<br>- no side effect |
| | 23-months old C57BL6/J treated for 6 weeks with AMAZ-02 admixed into CD at 50mpk | - Increased exercise capacity measured as endurance on treadmill | | - no change in food intake<br>- no side effect |
| Rat | 5 weeks-old Wistar-Han rats treated for 8 weeks with AMAZ-02 admixed into CD at 25mpk | - Increased exercise capacity measured as voluntary running in running wheels | | - no change in food intake<br>- no side effect |
| | 5 weeks-old Wistar-Han rats treated for 6 months with AMAZ-02 admixed into CD at 25mpk | | - Induction of mitophagy in muscle | - no change in food intake<br>- no side effect |

HFD: high-fat diet

CD: chow diet

### Pre-clinical Safety and Toxicology

Safety testing to date for AMAZ-02 has focused on the evaluation of both its genotoxic potential, as well as toxic effects after sub chronic exposure at high doses in line with the highest standards required for plant and food based ingredients to attain a generally regarded as safe (GRAS) food ingredient status. All assays were performed under GLP and were certified to be compliant with OECD and FDA Redbook guidelines, for the respective studies. Genotoxicity testing was carried out both in vitro and in vivo. To evaluate the potential of AMAZ-02 to induce DNA damage an AMES Reverse Mutation Assay was carried out, as per OECD 471. Even at the highest doses tested in this assay, no DNA damage was observed. To determine if administration of high doses of AMAZ-02 could cause cell damage during cell division leading to the formation of micronuclei (chromosomal fragments), both an in vitro and an in vivo micronucleus assay was carried out as per OECD 487 and 474, respectively. In the in vitro study, micronuclei formation was observed only in the absence of metabolic activation. However, the formation of a precipitate in tests at the higher doses showing positive micronuclei formation, the lack of dose dependence in the micronuclei formation, call into the question of the relevance of this observation to the in vivo situation. Indeed, AMAZ-02, when administered at the maximum recommended dose in the in vivo micronucleus assay, demonstrated no micronuclei formation, demonstrating that AMAZ-02 has no genotoxic impact in vivo even at very high doses. After the confirmation of the genotoxic safety of AMAZ-02, it was administered chronically to rats in the diet over a period of 28 days. This assay was performed as per OECD 407 guidelines for this assay. Briefly, we admixed the ingredient into the diet and made it available to rats at three dose levels for a period

of 28 days. The maximum dose tested, AMAZ-02 making up of 5% of the diet, was the maximum dose that could be achieved without causing dietary imbalances.

In this 28 days subchronic study, no adverse effects were observed at any of the doses tested. Diet consumption levels were normal, and no impact was observed on food consumption. Several endpoints were monitored over the 28 days treatment period for adverse effects of the treatment.

These included:

• Body weight

• Food consumption

• Functional Behavior

• Clinical Biochemistry

• Urinalysis

At the end of the treatment period, the animals were sacrificed and various tissues, as per the OECD and FDA Redbook guidelines, were examined by histology. None of the tissues examined demonstrated adverse effects at the highest doses tested. In addition to OECD Guideline 407 baseline recommendations, the study included ophthalmoscopic observations. AMAZ-02 was not toxic under the conditions of the study, and the No Observable Adverse Effect Level (NOAEL) were the highest doses tested. In males, the NOAEL was 4165 mg/kg bw/day and in females the NOAEL was 4705 mg/kg bw/day, based on mean test article intake.

To demonstrate the longer-term safety of AMAZ-02, the compound was administered chronically to rats in the diet over a period of 90 days. This assay was performed as per OECD 408 and FDA Redbook guidelines. Briefly, the ingredient was admixed into the diet and made it available to rats at three dose levels for a period of 90 days (respectively 1.25%; 2.5% and 5% of AMAZ-02 in diet). The dose levels were selected based on the safety data obtained from the 28 day repeated dose study which had demonstrated no toxicity at the highest dose tested. The maximum dose tested, AMAZ-02 making up of 5% of the diet, was the maximum dose that could be achieved without causing dietary imbalances. Several endpoints were monitored over the 90 day treatment period for adverse effects of the treatment. These included:

- Body weight
- Food consumption
- Functional Behavior
- Clinical Biochemistry
- Urinalysis
- Oestrous cycle
- Sperm motility
- Micronucleus formation
- Histology
- Macropathology

Diet consumption levels were normal, and no impact was observed on food consumption.

No adverse effects were observed at any of the doses tested.

At the end of the treatment period, the animals were sacrificed and various tissues, as per the OECD and FDA Redbook guidelines, were examined by histology. None of the tissues examined demonstrated adverse effects at the highest doses tested. No micronuclei formation was observed, and there was no impairement observed on the oestrous cycle or sperm motility.

AMAZ-02 was not toxic under the conditions of the study, and the No Observable Adverse Effect Level (NOAEL) were the highest doses tested. In males, the NOEL/NOAEL was 3,451 mg/kg bw/day and in females the NOEL/NOAEL was 3,826 mg/kg bw/day, based on mean test article intake.The results of this assay allowed an establishment of a No Observed Adverse Effect Level (NOAEL) of at least ~3,451mg/kg/day for AMAZ-02.

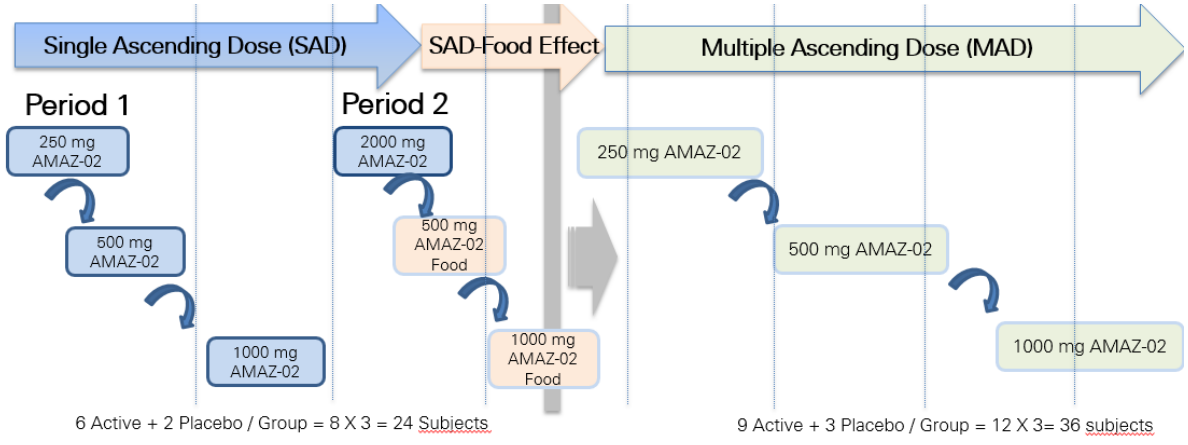
**Clinical Studies**

**Safety**

AMAZ-02 has recently been administered in Phase 1 human safety clinical trials in Phase 1A single ascending doses (SAD) (up to 2000mg) and multiple ascending doses (MAD) (up to 1000mg) in n=60 elderly subjects (>60+years old) for up to 4 weeks (NCT02655393). The safety profile of AMAZ-02 is very favorable as in both the SAD and MAD Phase 1 studies, **there were no reported product related serious or non-serious adverse events** in elderly human subjects. AMAZ-02 was bioavailable after single and multiple dosing's and after 4 week oral administration modulated muscle mitochondrial gene expression in a dose-dependent manner in health elderly.

**PHASE 1 HUMAN study with AMAZ-02**

Amazentis the sponsor has conducted a Phase 1 study to determine the safety and tolerability of AMAZ-02 in n=60, healthy elderly subjects (>60 years of age) following single ascending and multiple 28 days ascending dosing.




Phase 1 Study Design (NCT02655393)

**Part A: Single Ascending Dose**

This was a phase I, single-center, double-blind, randomized, single ascending dose (250mg, 500mg, 1000mg and 2000mg of Urolithin A delivered orally), study in 24 healthy elderly male and female volunteers. Each subject was randomised for two subsequent doses in three cohorts separated by a minimum washout period of 3 weeks between the two single dosing's.

**Part B: 28 day Multiple Ascending Dose**

This was a phase I, single-center, double-blind, randomized, multiple ascending doses (250mg, 500mg and 1000mg) study in 36 healthy elderly male and female volunteers. Each subject was randomised to receive study product or placebo for 28 days.

**Baseline characteristics of subjects included in Single Ascending Dose part A**

12 females and 12 males were included in the study. Age ranged from 61 to 82 years with a mean of 68.7±5.3 years and BMI ranged from 20.2 to 30.4 kg/m² with a mean of 24.60±2.72 kg/m². Recruited and randomized elderly subjects demographics are shown in the Table below by grouping- the groups were evenly matched for age, gender, BMI and the subjects were all of the same ethnicity.

| Demography | Statistics | Placebo (N=6) | 250mg (N=6) | 500mg (N=6) | 1000mg (N=6) | 2000mg (N=6) |
|---|---|---|---|---|---|---|
| Age (years) | N | 6 | 6 | 6 | 6 | 6 |
| | Mean±SD | 70.8±7.4 | 68.0±4.6 | 68.3±5.0 | 67.5±4.7 | 68.0±4.6 |
| | SEM | 3.0 | 1.9 | 2.0 | 1.9 | 1.9 |
| Gender: Male | N (%) | 3 ( 50.0) | 3 ( 50.0) | 3 ( 50.0) | 3 ( 50.0) | 3 ( 50.0) |
| Female | N (%) | 3 ( 50.0) | 3 ( 50.0) | 3 ( 50.0) | 3 ( 50.0) | 3 ( 50.0) |
| BMI (kg/m²) | N | 6 | 6 | 6 | 6 | 6 |
| | Mean±SD | 24.63±1.50 | 23.58±2.86 | 23.42±1.78 | 26.75±3.46 | 23.58±2.86 |
| | SEM | 0.61 | 1.17 | 0.73 | 1.41 | 1.17 |
| Ethnic origin: Caucasian | N (%) | 6 (100.0) | 6 (100.0) | 6 (100.0) | 6 (100.0) | 6 (100.0) |

Baseline of Phase 1 Part A Study Participants

**Baseline characteristics of subjects included in 28 day Multiple Ascending Dose part B**

24 females and 12 males were included in Part B of the study. Age ranged from 61 to 78 years with a mean of 66.4±4.9 years and BMI ranged from 18.8 to 30.6 kg/m² with a mean of 25.02±3.04 kg/m². Recruited and randomized elderly subjects demographics are shown in the Table below by grouping- the groups were evenly matched for age, gender, BMI and the subjects were all of the same ethnicity.

| Demography | Statistics | Placebo (N=9) | 250mg (N=9) | 500mg (N=9) | 1000mg (N=9) |
|---|---|---|---|---|---|
| Age (years) | N | 9 | 9 | 9 | 9 |
| | Mean±SD | 68.2±5.3 | 67.2±5.9 | 65.4±3.7 | 64.8±4.2 |
| | SEM | 1.8 | 2.0 | 1.2 | 1.4 |
| Gender: Male | N (%) | 3 (33.3) | 3 (33.3) | 3 (33.3) | 3 (33.3) |
| Female | N (%) | 6 (66.7) | 6 (66.7) | 6 (66.7) | 6 (66.7) |
| BMI (kg/m²) | N | 9 | 9 | 9 | 9 |
| | Mean±SD | 26.39±2.90 | 24.06±3.33 | 24.71±3.36 | 24.91±2.51 |
| | SEM | 0.97 | 1.11 | 1.12 | 0.84 |
| Ethnic origin: Caucasian | N (%) | 9 (100.0) | 9 (100.0) | 9 (100.0) | 9 (100.0) |

Baseline of Phase 1 Part A Study Participants

**Phase 1 Part A: Safety of AMAZ-02**

**Adverse Events (AEs)**

AEs were coded according to the Medical Dictionary for Regulatory Activities (MedDRA). They were classified into pre-defined standard categories according to chronological criteria:

**There were no serious adverse events (SAE) recorded in the study for any dosing.**

During the overall study period, 5/24 subjects reported the occurrence of 6 non-serious treatment emergent adverse events, of which none were related to product intake. 4 and 2 events were unrelated and unlikely related to study treatment respectively.

- 2 adverse events (myalgia and bronchitis) reported in the Placebo dose group, myalgia was linked to the study procedure: muscle biopsy and bronchitis was judged to be unlikely related
- 1 adverse event (myalgia) in the 250mg AMAZ-02, judged unrelated to product intake
- No adverse event in the 500mg AMAZ-02 capsule dose group
- 1 adverse event (orthostatic hypotension) in the 500mg AMAZ-02 powder + food dose group, judged unrelated to product intake
- 1 adverse event (myalgia) in the 1,000mg AMAZ-02 capsule dose group, judged unlikely related to product intake.
- 1 adverse event (headache) in the 1,000mg AMAZ-02 powder + yoghurt dose group, judged unlikely related to product intake.
- No adverse event in the 2,000mg AMAZ-02 dose group.

| Treatment group | Subject | PT MedDRA term | Relationship with study treatment |
|---|---|---|---|
| Placebo | 001-1001 | MYALGIA (after biopsy) | UNRELATED |
| | 001-1006 | BRONCHITIS | UNRELATED |
| 250mg | 001-1002 | MYALGIA | UNRELATED |
| 500mg powder | 001-1011 | ORTHOSTATIC HYPOTENSION | UNRELATED |
| 1000mg capsule | 001-1018 | MYALGIA (after biopsy) | UNLIKELY |
| 1000mg powder | 001-1018 | HEADACHE | UNLIKELY |

Adverse Events reported in Phase 1 Part A Single Dosing

All AEs were of mild to moderate intensity and were resolved before the end of the study

**Serum Biochemistry to assess Liver and Kidney Function**

The full laboratory tests evaluating liver and kidney function before and after single ascending dosing's comprised of:

- Creatinine,
- Uric acid,
- Alanine serine transferase (AST),
- Alanine leucine transferase (ALT),
- Gamma Glutamyl Transferase (GGT),
- Total and conjugated bilirubin;

24 hours after single dosing, no clinically significant abnormal laboratory test values from study baseline were observed for any of the biochemistry tests assessing liver and kidney function for any subjects at any of the doses orally administered during the course of the study.

**Hematology and Urinalysis**

The full laboratory tests comprised:

- Hematology: Hemoglobin, hematocrit, Red Blood Cells (RBC), White Blood Cells (WBC), differential count, platelet count, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC);

- Urinalysis: Semi-quantitative ("dipstick") analysis was performed for the following parameters: pH, ketone bodies, proteins, glucose, and blood.

No clinically significant abnormal laboratory test values from study baseline (D-1) were observed for any of the hematology and urinalysis tests for any subjects at any of the doses orally administered during the course of the study.

**Clinical Measures (Physical Examination, Vital Signs and ECG recordings)**

A physical examination included evaluation by a medical doctor of main body systems/regions, including: skin and mucous, ears/nose/throat, pulmonary, cardiac, gastro-intestinal and neurological systems before and after oral administration of AMAZ-02.

Vital signs consisted of measuring systolic (SBP) and diastolic (DBP) blood pressures and heart rate. The measurements were made after 10 minutes rest in the supine and after 2 minutes in the standing position.

Electrocardiogram (ECG): Each ECG consisted of a 10 second recording of the 12 leads simultaneously, leading to a12-lead ECG (25 mm/s, 10mm/mV) print-out with HR, PR, QRS, QT, QTc automatic correction evaluation, including date, time, initials and number of the subject, signature of the research physician, and at least 3 complexes for each lead. The Investigator medical opinion and automatic values were recorded in the eCRF.

• One abnormal physical examination was observed at D-1 in the placebo group (subject 001-1001: essential tremor (medical history).

• One clinically significant abnormal finding from study baseline was observed regarding vital signs for the subject 001-1011 (500mg dosing group at pre-dose): symptomatic orthostatic hypotension, with a normal control, in this subject exhibiting borderline high blood pressure at pre-dose. As this was prior to the dosing it was considered not related to the study product.

• One emergent value from period baseline (D-1 predose) to D2 was observed regarding QTc Fridericia in ECG findings for placebo group (subject 001-1001).

No other abnormal and clinically significant conclusions were observed for ECG findings for any subjects taking active intervention at any of the doses during the course of the study.

**It was concluded that single dosing of Urolithin A at the doses of 250mg, 500mg, 1000mg and 2000mg was safe and well tolerated.**

For the multiple dosing part B (250mg, 500mg, and 1,000mg) treatment for 4 weeks, safety in elderly after 4 weeks of AMAZ-02 oral administration was assessed.

**Adverse Events**

**No serious adverse events were reported during Part B of the study.**

During the overall study period, 31 non-serious AEs were collected for 15 subjects, of which only one was possibly related to study treatment (subject 001-2034 for 1000mg dose group: myalgia) and 26 were unrelated and 4 unlikely related to study treatment. There were no related non-serious adverse events.

**No product related adverse events were reported during the overall duration of the study.**

| Dose | Subject | PT MedDRA term | Relationship with study treatment |
|---|---|---|---|
| Placebo | 001-2014 | CONTUSION | UNRELATED |
| | | HEADACHE | UNRELATED |
| | | BACK PAIN | UNRELATED |
| | 001-2021 | HEADACHE | UNRELATED |
| | | ARTHRALGIA | UNRELATED |
| | 001-2027 | HEADACHE | UNLIKELY |
| | | LIMB DISCOMFORT | UNLIKELY |
| 250mg | 001-2001 | HEADACHE | UNRELATED |
| | 001-2003 | HEADACHE | UNRELATED |
| | | POST PROCEDURAL HAEMATOMA | UNRELATED |
| | | | UNRELATED |
| | | DIARRHOEA | UNLIKELY |
| | 001-2005 | PROCEDURAL PAIN | UNRELATED |
| | 001-2007 | CONTUSION | UNRELATED |
| | | POST PROCEDURAL HAEMATOMA | UNRELATED |
| | 001-2008 | PROCEDURAL PAIN | UNRELATED |
| 500mg | 001-2015 | POST PROCEDURAL HAEMATOMA | UNRELATED |
| | | PROCEDURAL PAIN | UNRELATED |
| | | | UNRELATED |
| | 001-2017 | POST PROCEDURAL HAEMATOMA | UNRELATED |
| | | SYNCOPE | UNRELATED |
| | 001-2019 | BACK PAIN | UNRELATED |

| Dose | Subject | PT MedDRA term | Relationship with study treatment |
|---|---|---|---|
| | | PAIN IN EXTREMITY | UNRELATED |
| | 001-2023 | BACK PAIN | UNLIKELY |
| | 001-2024 | HEADACHE | UNRELATED |
| | | | UNRELATED |
| | | | UNRELATED |
| | | THERMAL BURN | UNRELATED |
| 1000mg | 001-2034 | POST PROCEDURAL HAEMATOMA | UNRELATED |
| | | MYALGIA | POSSIBLE |
| | 001-2036 | POST PROCEDURAL HAEMATOMA | UNRELATED |

Adverse Events reported in Phase 1 Part B 28 day Multiple Dosing

Among the 31 AEs:

- 7 were reported in Placebo dose group, judged unrelated or unlikely related (1 headache and limb discomfort) to product intake.
- 9 AEs in 250mg UA, judged unrelated or unlikely related (diarrhea) to product intake.
- 12 AEs in 500mg UA group judged unrelated or unlikely related (back pain) to product intake.
- 3 AEs in 1000mg UA dose group, judged unrelated or possibly related (myalgia) to product intake.

All AEs were of mild to moderate intensity and were resolved before the end of the study. All AEs resolved.

**Serum Biochemistry to assess Liver and Kidney Function**

The full laboratory tests evaluating liver and kidney function before and after 28 day multiple ascending dosing's comprised of:

- Creatinine,
- Uric acid,
- Alanine serine transferase (AST),
- Alanine leucine transferase (ALT),
- Gamma Glutamyl Transferase (GGT),
- Total and conjugated bilirubin;

No clinically significant abnormal laboratory test values at Day 28 from study baseline were observed for any of the biochemistry tests assessing liver and kidney function for any subjects at any of the doses orally administered during the course of the study.

**Hematology and Urinalysis**

The full laboratory tests comprised:

- Hematology: Hemoglobin, hematocrit, Red Blood Cells (RBC), White Blood Cells (WBC), differential count, platelet count, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC);

- Urinalysis: Semi-quantitative ("dipstick") analysis was performed for the following parameters: pH, ketone bodies, proteins, glucose, and blood.

No clinically significant abnormal laboratory test values from study baseline to D28 were observed for hematology and urinalysis laboratory tests.

**Clinical Measures (Physical Examination, Vital Signs and ECG recordings)**

A physical examination included evaluation by a medical doctor of main body systems/regions, including: skin and mucous, ears/nose/throat, pulmonary, cardiac, gastro-intestinal and neurological systems.

Vital signs consisted of measuring systolic (SBP) and diastolic (DBP) blood pressures and heart rate. The measurements were made after 10 minutes rest in the supine and after 2 minutes in the standing position.

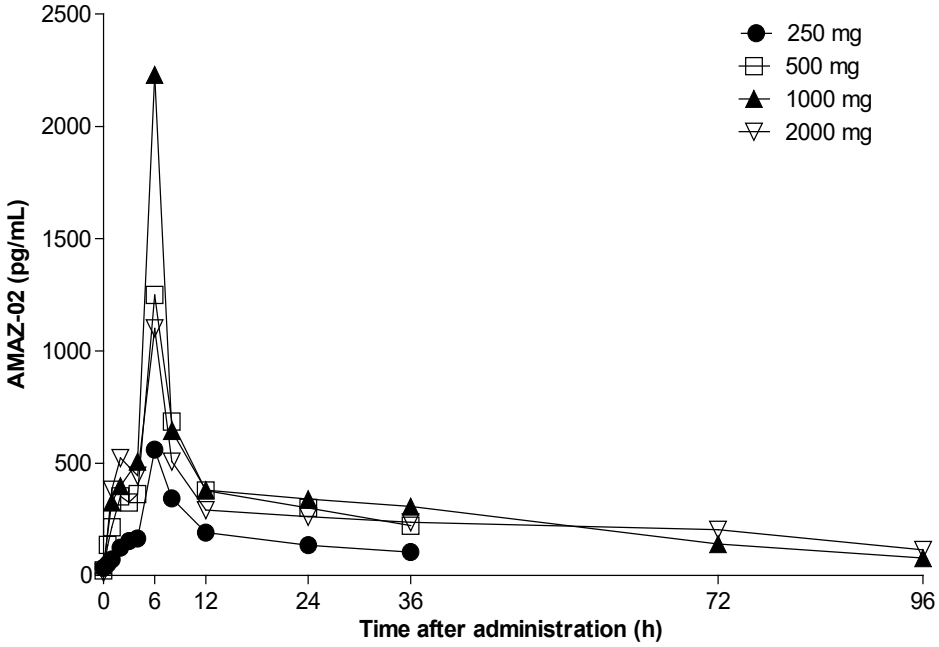
Electrocardiogram (ECG): Each ECG consisted of a 10 second recording of the 12 leads simultaneously, leading to a12-lead ECG (25 mm/s, 10mm/mV) print-out with HR, PR, QRS, QT, QTc automatic correction evaluation, including date, time, initials and number of the subject, signature of the research physician, and at least 3 complexes for each lead. The Investigator medical opinion and automatic values were recorded in the eCRF.

**It was concluded that 4-week, multiple dosing oral administration of AMAZ-02 (Urolithin A) at the doses of 250mg, 500mg, and 1000mg was safe and well tolerated in elderly subjects.**

**AMAZ-02 (Uro A) Pharmacokinetics in Phase 1 study with Healthy Elderly**
**Pharmacokinetics in plasma after single ascending dose administration**

Following single-dose administration of AMAZ-02 to subjects in the fasted state, plasma samples were collected at the following time-points: pre-dose, (after dosing) 1/2h, 1h, 2h, 3h, 4h, 6h, 8h, 12h, 24h, 36h. After 250 mg and 500 mg doses with soft-gels, the following time-points were added at 72h and 96h for the subsequent dosings. The plasma concentrations-time profiles of AMAZ-02 showed that this compound is absorbed with maximum plasma concentrations (Cmax) being reached 6 h (tmax) after administration. Thereafter, the AMAZ-02 concentrations decreased in a multi-phasic way. In this part of the study, the t1/2 could only be reliably estimated for the highest 2 doses, 1000 mg and 2000 mg. The reported values indicate that the t1/2 of AMAZ-02 is between 25.9h (1000 mg dose) and 41.7 h (2000 mg).

Plasma concentrations of the metabolites were higher when compared to that of parent compound. For example, at a dose of 250 mg, the Cmax of AMAZ-02 glucuronide and sulfate was 835- and 114-fold higher than that of AMAZ-02, respectively. The shape of the plasma concentration-time curves for the metabolites was similar than that of the parent compound. Values for the median tmax were either similar (4h-8h) or, for the 2000 mg dose group, shorter (2h-3h)when compared to parent compound. Exposure varied between the metabolites with that to AMAZ-02 glucuronide the highest. The t1/2 of the metabolites was in the same range as that of the parent compound (25.9h for parent vs. 23.8h for glucuronide at 1000 mg dose and 41.7 h for parent vs. 37.9h for glucuronide at 2000 mg dose. With increasing dose, the exposure to AMAZ-02 (based on both Cmax and AUC) increased and this increase appeared dose-proportional up to a dose of 500 mg (Cmax 601 pg/mL; AUC 6160 pg.h/mL at 250 mg dose vs. Cmax 1240 pg/mL; AUC 13300 pg.h/mL at 500 mg dose). At the next higher dose of 1000 mg, a less than dose proportional increase in exposure was observed (Cmax 1920 pg/mL; AUC 15800 pg.h/mL) whereas at the highest dose of 2000 mg, no dose proportionality was observed (Cmax 1040 pg/mL; AUC 12400 pg.h/mL).




Pharmacokinetic Profile of Single Ascending Doses of AMAZ-02

The Table below summarizes the clinical pharmacokinetic parameters for the different dosing's for AMAZ-02 and its main metabolite AMAZ-02 Glucuronide.

| Dose (mg) | Food status | $t_{max}$ (h) | $C_{max}$* (pg/mL) | $AUC_{0-\infty}$* (pg.h/mL) | $AUC_{0-36h}$* (pg.h/mL) | $t_{1/2}$ (h) |
|---|---|---|---|---|---|---|
| **AMAZ-02** | | | | | | |
| 250 | fasted | 6.01 (6.00-8.00) | 601 (38.9) | | 6160 (29.6) | |
| 500 | fasted | 6.00 (6.00-8.00) | 1240 (33.7) | | 13300 (36.0) | |
| 500 | fed | 6.00 (6.00-6.03) | 1720 (62.6) | 26700 (22.9) | 15300 (36.1) | 26.8 (35.4) |
| 1000 | fasted | 6.00 (4.00-6.00) | 1920 (76.3) | 34200 (46.6) | 15800 (53.3) | 25.9 (107.9) |
| 1000 | fed | 6.00 (4.00-6.55) | 2670 (36.1) | 38200 (44.7) | 20400 (29.3) | 31.3 (125.4) |
| 2000 | fasted | 6.00 (1.00-6.00) | 1040 (45.5) | 25100 (18.7) | 12400 (23.6) | 41.7 (13.6) |
| **AMAZ-02 glucuronide** | | | | | | |
| 250 | fasted | 7.00 (2.00-8.00) | 502 (25.8) | | 10600 (28.2) | |
| 500 | fasted | 6.00 (0.5-24.0) | 791 (33.5) | | 16300 (20.7) | |
| 500 | fed | 4.00 (1.00-6.00) | 914 (20.8) | 25200 (26.9) | 16300 (15.6) | 19.7 (44.6) |
| 1000 | fasted | 5.00 (1.00-6.00) | 1000 (35.9) | 36300 (38.1) | 19600 (32.1) | 23.8 (65.1) |
| 1000 | fed | 6.00 (1.00-8.00) | 1390 (10.3) | 43400 (45.3) | 23400 (31.4) | 22.6 (73.4) |
| 2000 | fasted | 2.00 (1.00-6.00) | 860 (46.5) | 41700 (52.0) | 17500 (28.5) | 37.9 (39.5) |

*Units are in ng for the AMAZ-02 glucuronide

Pharmacokinetic parameters of Single Ascending Doses of AMAZ-02

**Pharmacokinetics in plasma after 28 days (4 week) multiple-dose administration**

AMAZ-02 concentrations on Days 7, 8 and 28 were variable but, overall, indicated that steady-state had been reached by Day 7. Following multiple-dose administration of AMAZ-02 to elderly healthy subjects, AMAZ-02 is absorbed with Cmax being reached 6h after administration. Thereafter, the AMAZ-02 concentrations decreased in a multi-phasic way. The elimination phase was characterized by values of t1/2 of between 17.4h and 22.0h with no apparent effect of dose on this variable. Plasma concentrations of AMAZ-02 and it's main metabolites were similar to those observed with single dose oral administartion of the AMAZ-02 ingredient.

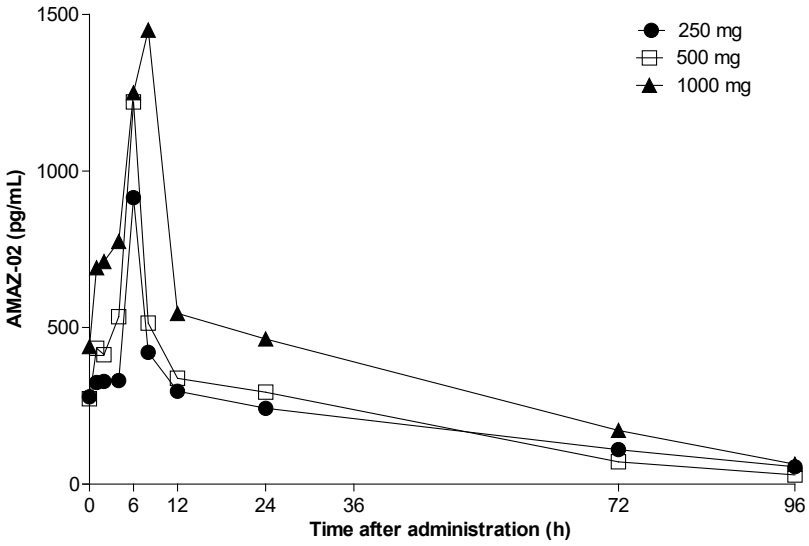



Figure: Pharmacokinetic Profile of Multiple Ascending Doses of AMAZ-02

| Dose (mg) | $t_{max}$ (h) | $C_{max}$* (pg/mL) | $AUC_{0-24h}$* (pg.h/mL) | $t_{1/2}$ (h) |
|---|---|---|---|---|
| | | **AMAZ-02** | | |
| 250 | 6.00 (0.00-6.00) | 830 (53.6) | 8370 (22.1) | 22.0 (52.3) |
| 500 | 6.00 (4.00-6.02) | 978 (76.7) | 9480 (49.5) | 20.0 (32.7) |
| 1000 | 6.00 (1.00-8.00) | 1710 (62.4) | 16600 (36.0) | 17.4 (90.8) |
| | | **AMAZ-02 glucuronide** | | |
| 250 | 6.00 (1.00-8.00) | 707 (28.4) | 10300 (22.0) | 21.1 (57.9) |
| 500 | 6.00 (1.00-8.00) | 943 (44.2) | 13900 (36.8) | 18.8 (30.0) |
| 1000 | 4.00 (1.00-8.00) | 1280 (23.5) | 18100 (18.1) | 18.3 (95.6) |

*Units are in ng for the AMAZ-02 glucuronide

***Table:*** Pharmacokinetic parameters of Multiple Ascending Doses of AMAZ-02

The pharmacokinetic profile in human biological fluids (plasma, urine) of dietary metabolites of ellagitannins has also been studied previously upon consumption of either pomegranate juice, after a dietary intake of walnuts or polyphenol enriched pomegranate extracts *(Seeram et al. 2006 ; Cerdá et al. 2005 ;*

*Tomás-Barberán et al. 2014 ; Espín et al. 2013 ).* In these studies when pomegranates juice or an ellagitannin enriched extract were orally administered, it was observed that the ellagitannins were absorbed in the proximal part of the gastrointestinal tract and subsequently detected in plasma with concentrations of approximately 100 nM, 1 hour following oral intake *(Mertens-Talcott 2006 ; Seeram et al. 2004)*. The peak plasma levels of urolithin A were 14–25 μM depending on the volunteers. These metabolites started to appear in plasma 6–8 hours after the intake, which confirms Uro A production in the large intestine, and persisted in urine and plasma up to 48-72 hours after the oral intake *(Tomás-Barberán et al. 2009; Cerdá et al. 2004; Cerdá et al. 2005 ; Seeram et al. 2006)*.

The bioavailable presence of Uro A after consumption of ellagitannins, however, was highly dependent on the host gut microflora profile and certain subjects in these studies were "non-responders", i.e. they did not make Uro A. Amazentis has also confirmed by observation the presence of responders and non-responders, prior to dosing in our Phase 1 study.

Seeram et al. (2007) described the pharmacokinetics and tissue distribution of urolithins in mice after oral or intra-peritoneal administration of chemically synthesized Uro A. Urolithin A peaked in plasma after 2h and reached the highest concentrations in the prostate followed by the small intestine and colon, with a peak at 4h. Uro A, Uro A sulfate, and Uro A glucuronide were mainly detected in the prostate gland whereas urolithin A glucuronide was primarily detected in liver and kidney tissues. This rapid absorption of AMAZ-02 was confirmed by us in our ADME study, where we observed a $T_{max}$ of between 10 minutes and 2h in males. Other studies with animal models, including rats and pigs, confirmed the production of urolithin in the colon, and the sequential loss of hydroxyls, as the metabolites advance in the intestinal tract *(Tomás-Barberán et al. 2009; Cerdá et al. 2005)*.

Taken together the information on the bioavailabilty of Uro A suggests that the highly variable production linked to the gut microflora composition of the individual subjects and the different dietary exposure to ellagitannin containing foods (such as pomegranates, nuts and berries) play a vital role in derving the health benefits associated with Uro A. By delivering carefully calibrated dietary supplementation with Uro A doses, one can negate this variabilty and Uro A bioavailability can be achieved as has been done by Amazentis via dietary supplementation with AMAZ-02 (Uro A) in Phase 1 studies in elderly. Uro A delivered via oral supplementation in doses from 250-1000mg is bioavailable in a dose response manner both with single and multiple dosing, gets cleared and the kinetics are very similar between single and repeat dosing's in elderly subjects.

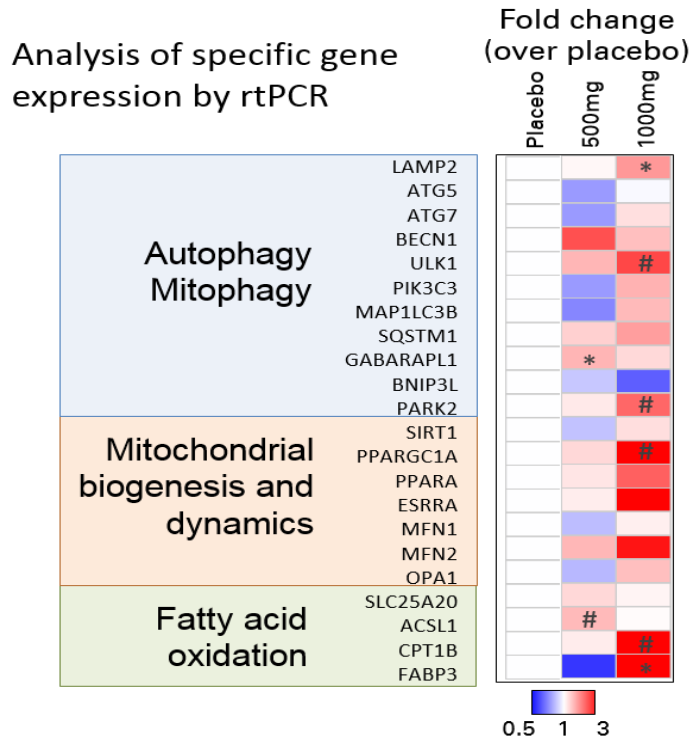
**AMAZ-02 impacts Mitochondrial biomarkers in Skeletal Muscle of Elderly Subjects in Phase 1B**

During the Phase 1 part B, 28 day study, muscle biopsies from the right leg *vastus lateralis* muscle were collected from healthy elderly subjects before the study intervention started (pre-dose) and after the last intake of the study product (at Day 28) under fasted state using the Bergström biopsy needle technique. RNA was extracted from the muscle tissue and a RNA microarray and follow-up with qPCR for target genes linked to mitochondrial pathways was performed along with housekeeping genes. The Table below summarizes the global impact on mitochondrial genesets in elderly skeletal muscle observed in a dose dependent manner with 500mg and 1000mg doses (the 1000mg dose is selected for this Phase 2 clinical study).

**TABLE: Upregulated mitochondria-related pathways in the 500 and 1000mg AMAZ-02 dosing group comparisons normalized to placebo**

| Genesets | 500 mg change over time normalized over placebo | | 1000mg change over time normalized over placebo | |
|---|---|---|---|---|
| | NES | FDR | NES | FDR |
| GO_MITOCHONDRIAL_ENVELOPE | 1.438442 | 0.2308831 | 1.762903 | 0.0387385 |
| GO_MITOCHONDRIAL_MATRIX | 2.525320 | 0.0020414 | 1.987229 | 0.0069189 |
| GO_MITOCHONDRIAL_PART | 2.078387 | 0.0751073 | 2.090689 | 0.0021444 |
| GO_MITOCHONDRION | 2.192505 | 0.0432333 | 1.939153 | 0.0102209 |
| GO_MITOCHONDRION_ORGANIZATION | 1.252384 | 0.3308285 | 1.757141 | 0.0398731 |
| GO_INNER_MITOCHONDRIAL_MEMBRANE_PROTEIN_COMPLEX | NA | NA | 1.639517 | 0.0855748 |
| GO_MITOCHONDRIAL_MEMBRANE_PART | NA | NA | 1.697246 | 0.0621488 |
| GO_MITOCHONDRIAL_PROTEIN_COMPLEX | NA | NA | 1.668623 | 0.0728421 |

The Figure below also shows the impact in a dose dependent fashion of AMAZ-02 at 500mg and 1000mg doses on up-regulating targeted mitochondrial gene expression in health elderly skeletal muscle compared to placebo (n=9 subjects /group).




#0.05<p<0.15

*p<0.05

**p<0.01

***p<0.001

Figure: Impact of AMAZ-02 on skeletal muscle mitochondria after 4 week oral administration

**Summary of the known and potential risk and benefits to humans**

The subjects enrolled in the present study being healthy are not expected to derive any therapeutic benefit from participating in the present study. This is a food ingredient that evolutionary has been derived from dietary sources that are present in the human diet and has demonstrated a extremely safe profile in pre-clinical toxiciology and Phase 1 human studies in healthy elderly subjects. The only expected risks are those arising from the puncture of venous blood through a catheter and from the muscle biopsy procedure.

**Description of and justification for the dosage regimen**

Briefly, the NOAEL from rats was taken as 4165 mg/kg bw/day. The dose was then converted into an equivalent human equivalent dose (HED) based on body surface area using Table 1 in the above mentioned guidance.

$$HED = \frac{NOAEL\ in\ rodents}{Species\ Conversion\ Factor\ from\ Table\ 1}$$

$$HED = \frac{4165\frac{mg}{kg}/d}{6.2} = 672\frac{mg}{kg}/d$$

The equivalent human dose was determined to be 672 mg/kg/d. This dose was then divided by a 10X safety factor. The 10X safety factor was chosen to take into account inter-individual variability. This resulted in a final maximum recommended starting dose (MRSD) for humans as 67.2 mg/kg/d

$$MRSD = \frac{HED}{10\ fold\ safety\ factor}$$

$$MRSD = \frac{672\frac{mg}{kg}/d}{10} = 67.2\frac{mg}{kg}/d$$

A MRSD of 67.2 mg/kg/d is equivalent to a daily consumption of 4,704 mg/day in a 70 kg individual.

In our Phase 1 human safety study single ascending doses in the range between 250 mg and 2,000 mg were administered without any adverse events as well as upto 1000 mg for upto 4 weeks with no serious or product related adverse events, well below the calculated MRSD. **The 1000 mg dose was bioavailable and gave apparent effects on mitochondrial biomarkers after the 4 week intervention Phase 1 study. Hence, the chosen dose for this Phase 2 efficacy study will be 1000 mg of AMAZ-02.**

**Ethical considerations**

The study will be carried-out in accordance with the Declaration of Helsinki as modified in Fortaleza (2013), the recommendations on Good Clinical Practice (GCP) (ICH E6) and any applicable local regulatory requirement(s). The clinical study will start only upon receipt of the final approval from a central Institutional Review Board (WIRB) committee.

**Description of the population to be studied**

<u>80 healthy elderly</u> male and female volunteers, aged from 65 to 90 years will be included in the study. They will be recruited from volunteers' database of the clinical unit. Newspaper and online

advertisements, radio spots, posters, mailing, specific press inserts, broadcast message or clinical unit recruitment website may be used. Only study-specific recruitment tools approved by IRB will be used.

## STUDY OBJECTIVES

**Primary Objective(s)**

To evaluate the effect of an oral nutritional supplementation with AMAZ-02, a food derived ingredient, compared to placebo for 4 months in health elderly subjects on:

- 6 minute walking distance (6MWD)
- Maximum mitochondrial ATP production (ATPmax) measured via Magnetic Resonance Spectroscopy on a hand muscle (FDI- first dorsal interosseus)

**Secondary Objectives**

To evaluate the effect of 4 month oral administration of AMAZ-02 on:

- Percent change from baseline in contraction number during a single muscle fatigue test assessed on the hand muscle (FDI- first dorsal interosseus)
- Maximum mitochondrial ATP production (ATPmax) measured via Magnetic Resonance Spectroscopy (on the *Tibialis Anterior* leg muscle)
- Muscle function measured via the single muscle fatigue test ((on the *Tibialis Anterior* leg muscle)
- Exercise Performance (Leg power, time to fatigue, Borg's perceived exertion scale and VO2) measured by cycle ergometry
- Short Physical performance Battery (SPPB)
- Hand grip strength
- Leg muscle strength (via Cybex one repetion maximum and ten repetition maximum testing)
- Muscle size (cross-sectional area of the muscles via MRI)
- Mitochondrial function on muscle biopsy (via high resolution respirometry)- this will be an optional and exploratory endpoint
- Mitochondrial gene and protein expression in skeletal muscle tissue (microarray and protein array) - this will be an optional and exploratory endpoint
- Acylcarnitine levels in plasma (via metabolomics)
- Quality of life via SF36 questionnaire
- Plasma lipid profile (Total Cholesterol, LDL, HDL and Triglycerides)
- Plasma circulating biomarkers (myostatin, follistatin, metabolomics)
- Safety

# STUDY DESIGN AND INVESTIGATIONAL PLAN

## Summary of Study Design

The goal of the trial is to assess the impact of oral ingestion of AMAZ-02 treatment on mitochondrial, skeletal muscle and physical function in elderly subjects. This will be a randomized, double-blind trial enrolling 66 subjects (33 placebo and 33 AMAZ-02 administration) aged ≥65 and ≤ 90 years of age, both male and female elderly with evidence of skeletal muscle mitochondrial dysfunction. The goal of the trial is to assess the impact of the AMAZ-02 treatment on skeletal muscle and physical function in healthy elderly.

Skeletal muscle function will be determined using 3 independent measurements: 1) in vivo 31P nuclear magnetic resonance measures (MRS) to assess mitochondrial phosphorylation (ATPmax) and muscle size, 2) a muscle strength and fatigue test and 3) a SPPB test. In addition, physical function measures such as endurance (cycle ergometer exercise test) and performance (6 minute walk) will be assessed. Mitochondrial function will be determined by measuring the capacity for ATP generation using magnetic resonance spectroscopy and by high resolution respirometry. A muscle strength and fatigue test will be performed at the intermediate visit for all subjects (Day 61). Comprehensive hematology and physiological testing will be obtained at baseline and 4 month time-points along with safety adverse event recording to document the long- term safety of AMAZ-02 administration.

**Discussion of Design and Control**

This will be a randomized, double-blind trial enrolling 66 subjects (33 placebo and 33 AMAZ-02 administration) aged ≥65 and ≤ 90 years of age with evidence of skeletal muscle mitochondrial dysfunction. The goal of the trial is to assess the impact of the AMAZ-02 treatment on skeletal muscle and physical function in elderly. Muscle function will be determined using 3 independent measurements: 1) in vivo 31P nuclear magnetic resonance measures (MRS) to assess mitochondrial phosphorylation (ATPmax) and muscle size, 2) a muscle strength and fatigue test and 3) a SPPB test. In addition, physical function measures such as endurance (cycle ergometer exercise test) and performance (6 minute walk) will be assessed. Mitochondrial function will be determined by measuring the capacity for ATP generation using magnetic resonance spectroscopy and by high resolution respirometry.

**Clinical trial visits and study procedures**

Study participants will provide informed consent, and be asked to visit the University of Washington and Fred Hutchinson Cancer Research Center about 3-4 times. Each visit will take about 4 hours. The tests and the typical activities for a session are described below.

**Screening and Randomization Visit (Visit 1a and 1b, Day -30 to Day 0)**

See also Table 1 (pg. 12) for a detailed study flowchart and schedule of events at each visit

**University of Washington Medical Center**

Subjects will be asked to visit the Translational Center for Metabolic Imaging (TCMI) and Translational Research Unit (TRU) on the campus of the University of Washington Medical Center (UWMC). In the TRU, subjects will be asked to provide informed consent, have vital signs taken, complete an ECG and 6MWT. In the TCMI, subjects will fill out a questionnaire, will undergo MR scans that involve single muscle exercise tests and will perform fatigue tests of single muscles outside the MRI. The muscle fatigue tests of single muscles will be undertaken twice for each muscle before and after a break. Tests will happen at the beginning and at the end of the study. The procedures will take about 4 hours from the time subjects arrive to the time they leave the facility.

**MRI/MRS Tests**

Potential study participants will go over a screening form to allow them to participate in the MRI testing. An MRI and a hand and leg exercise tolerance test will be performed. There will be a test on two separate muscles involving magnetic resonance spectroscopy (MRS) to determine muscle energetics. For this test, a small device will be taped onto subjects hand or leg. This device is the antenna coil. During the testing session, subjects will sit or lie on a padded table with their right hand or leg inside the magnet tube. Subjects will be asked to exercise at 3 levels. The first exercise, the subject will be asked to repeatedly push their finger or pull their leg against a bar as fast as possible for 20-30 seconds followed by ~6 min of recovery. In two additional exercises the subject will also push or pull the muscle to a pace set by a beeping sound for 3 min each followed by recovery (5 min each). During some parts of the MR tests, subjects will wear earplugs to muffle banging sounds. It is important that subjects remain as still as possible when they are in the magnet.

**Exercise Tolerance Test**

During these sessions, subjects will be asked to exercise their hand or leg. First, they will be asked to push their finger or pull their leg against a bar as hard as possible and hold for 5 seconds. Subjects repeat this 3 times This will tell the investigators about the maximum strength of the muscle. For a second type of exercise, subjects will be asked to push their finger or pull their leg against the bar in a rapid motion for up to 5 minutes and to increase the rate every minute. A beeping sound will tell them when to push or pull. Subjects will try to push or pull each time at about 70% of their maximum strength. Subjects will watch a light display that tells them how hard they are pushing or pulling.

**Fred Hutchinson Cancer Research Center: physical exam and exercise testing** Subjects will be asked to visit the Fred Hutchinson Cancer Research Center Prevention Center (FHCRC) located in the Public Health Sciences building on the FHCRC campus at Southeast Lake Union. At that time, they will be screened for eligibility, given a physical exam, and asked to perform a series of tests: a hand grip strength test and a set of tests of leg strength and walking. The screening visit will take about 3 hours.

**1. Physical exam**

A physical examination will be done which will include general appearance, skin, head, eyes, ears, nose, throat, neck (including thyroid), lymph nodes, chest, heart, abdomen, extremities, nervous

system, weight, and height. Subjects vital signs (breathing rate, heart rate, temperature, and blood pressure) will be measured. An electrocardiogram ([ECG], an electrical tracing of the heartbeat) will be done while subjects exercise, and a 6MWT. A blood sample will be taken for clinical laboratory tests.

**2. Questionnaires and Physical Function**

Subjects will be asked to fill out the SF36 quality of life survey. We will also ask them to walk a second time for 6 min. at the highest comfortable pace, which typically covers 300-500 yards. Subjects will also perform a short physical performance battery test (SPPB test). Once subject's are found eligible, they will be randomized to the nutritional supplement group or the placebo group.

**3. Exercise test and Cybex leg muscle strength:**

Subjects will be asked to take of test of your heart and fitness level with a resting electrocardiogram and a cycle ergometer test. The test will involve pedaling a cycle ergometer for 8 to 12 minutes while breathing into a measurement tool. Each session will last about 3 hrs from the time your arrive to the time you leave the facility.

Leg muscle strength testing will be perfomed with one-repetition max testing and ten-repetition max testing using a Cybex®leg extension machine (Cybex International, Medway, MA). Subjects are seated with the resistance bar positioned in line with the medial malleolus. Subjects lifted the weight to near full extension of the knee.

**4. Blood Sampling**

Each subject will receive (3) blood draws through the course of the study: at the start of the study (i.e. before start of dosing), after 60 days, and at the end of the study at 4 months. About 70 mL of blood (about 5 tablespoons) will be drawn during the study. Additional blood samples may be required if any laboratory values are abnormal. It is possible that more than one attempt to obtain a blood sample may be necessary. The timing of the blood sampling will be recorded in the eCRF.

**5. Muscle Biopsy (Optional)**

The subjects will be asked to fast overnight and arrive at the FHCRC in the morning. Once in the facility, the subjects will be asked to lie down on a padded table and given a local anesthetic (2% lidocaine plus epinephrine solution) just under the skin. A small piece of fine muscle, approximately the size of a pencil eraser (50-100mg), will be removed through a 3/16 inch slit in the skin and the underlying tissue, using a hollow biopsy needle to remove the most sample. This procedure will be performed by a physician at the Fred Hutchinson Cancer research Center prevention Center, and will require approximately one hour.

**Visit 2 (Day 61) +/- 4 days**

Subjects will come to the UWMC to get a new 2-month supply of AMAZ-02 on one occasion. They will be asked to return unused pills and given a new two month supply. The subjects will also be asked to repeat the exercise tolerance tests for the hand and leg.

Subjects will come to the FHCRC for a blood draw. The timing of the blood sampling will be recorded in the eCRF, but has no relationship to the last study product intake.

**End of Study Visit 3 (Day 119- Day 121) +/- 4 days**

Subjects will be asked to return to the Fred Hutchinson Cancer research Center prevention Center. A final physical exam will be given. The handgrip, SPPB and 6 min walk will be repeated. Subjects will undergo the cycle ergometry whole body exercise test at the end of the study. A blood sample and optionally a muscle biopsy will be taken. Subjects will fill out the SF36 quality of life survey. Subjects will be asked to return to the UWMC for a final MRI/MRS and hand and leg exercise tolerance tests.

**Study risks and discomforts**

Certain side effects and discomforts associated with the study may occur. There may also be side effects and discomforts that are not yet known.

**Blood Sampling**

The risks from blood sampling are rare but possible. These risks may include pain, bleeding, hematoma (blood collecting under the skin) or infection at the needle insertion site, damage to surrounding tissue or nerves, fainting, lightheadedness, nausea and vomiting. The timing of the blood sampling will be recorded in the eCRF, but has no relationship to the last study product intake.

**ECG**

An ECG could cause skin irritation. This is rare but could occur during an ECG from the electrodes or gel that is used to perform the test.

**MRS**

It is important to keep any magnetic objects away from subjects and the magnet in the MRS. Subjects will be asked if they have any metal objects or implants before they undergo the MRS study procedure. Some subjects may feel uncomfortable while remaining still and holding the same position during the session. This will disappear soon usually after subjects get up and walk around the MRS procedure is done.

**Cycle Ergometer Test for Evaluating Exercise Performance**

Subjects may feel tired after exercising during the ergometer test.

Recovery generally occurs within 30 minutes. Subjects will be continuously monitored and the test will be stopped at any time they ask (an emergency stop mechanism is available for subjects to use). An exercise physiologist with Advanced Cardiac Life Support (ACLS) certification will be present at all times. In persons with an underlying heart condition, there is a slight (less than 1 chance in 1,000) risk of having a sudden heart attack and an even slighter (less than 2 chances in 10,000) risk of sudden death. Persons with a serious heart condition will not be eligible for this study.

**Leg Muscle Strength Testing (Cybex)**

Leg muscle strength testing will be perfomed with one-repetition max testing and ten-repetition max testing using a Cybex®leg extension machine (Cybex International, Medway, MA). Subjects are seated with the resistance bar positioned in line with the medial malleolus. Subjects lifted the weight to near full extension of the knee.

**Single Muscle Exercise Tolerance Test**

Subjects may feel tired after exercising their hand and leg. Recovery generally occurs within 30 minutes but they may have some soreness up to a day.

**Muscle Biopsy (Optional)**

Subjects may find the initial injection of the anesthetic to be uncomfortable. The biopsy needle may exert pressure during the procedure. There may be some discomfort after the procedure such as a dull pain similar to a bruise or mild "charlie horse". This discomfort should goes away in a day or two after the biopsy. If discomfort lasts more than 2 days or bleeding the subject will be advised to contact Dr. Jose Garcia, MD at (206) 764-2984.

**Duration of follow-up**

During the last visit, subjects will undergo a complete clinical and biological examination, identical to the examination at the start of the study. (AEs) will be recorded, and if they are on-going a further follow-up will be arranged; follow-up will continue until the event is resolved or the condition is unlikely to change or the subject is lost to follow-up.

**Measures taken to minimize and avoid bias**

**Randomization**

The randomization list will be created by SynteractHCR. Particularities of the randomization – such as variable or fixed block length will be stated in the randomization list itself.

The investigators will be able to randomize subjects with a web-based interactive-web response system (IWRS) which is fully intergrated within the e-CRF.

Patients who meet all inclusion and exclusion criteria and have given written informed consent will be randomized in a 1:1 ratio to either AMAZ-02 Dose or Placebo via an Interactive Web Response System (IWRS).

The product will be allocated at Day 0 of the study.

**Blinding**

The following measures are taken to avoid bias:

- Double Blind Study,
- The softgels containing active product and placebo will be indistinguishable in appearance.

The analytical centre as well as the Investigator and the Investigator's team and the subject will be in blind conditions. Emergency unblinding can be done within this IWRS system.

The CRO staff and Sponsor's clinical trial team members will not have permission to unblind the treatment code in the IWRS system.

The code for any study participant should only be broken by the Investigator or authorized person if it is absolutely necessary.

In case of emergency, for a subject in particular, the code may be broken to ascertain the type of treatment/product given to study participant concerned. If so, the Investigator (or a person designated from his team) must document the unblinding in the IWRS with his e-signature and the reason for code breaking

and the Sponsor must be notified within 24 hours and a full written explanation must be provided. The Investigator has to inform the Clinical Project Manager as soon as possible.

## STUDY POPULATION

### Inclusion Criteria

Subjects are eligible to be included in the trial only if they meet all of the following criteria:

1. Adults ≥65 and ≤90 years of age
2. Able to travel to and from the UW and FHCRC Prevention Center
3. Informed consent obtained
4. 6 minute walk distance of <550 meters
5. ATP max ≤1.0 mM/sec (in the hand muscle FDI)

### Exclusion Criteria

Subjects will be excluded from the trial if they meet any of the following criteria:

1. Subjects who have significant disease(s) or condition(s) which, in the opinion of the investigator, may put the subject at risk because of their participation in the trial or may influence either the results of the trial or the subject's ability to participate in the trial
2. Hospitalization within 3 months for major atherosclerotic events (defined as combined incidence of myocardial infarction, urgent target-vessel revascularization, coronary bypass surgery and stroke) and for any hospitalization within 2 months.
3. Have any metal implants in the right limbs, including non-MRI compatible metal stents, titanium pins/markers, etc.
4. Have an implanted cardiac pacemaker or other implanted non-MRI compatible cardiac device
5. Chronic, uncontrolled hypertension as judged by the Investigator (i.e., Baseline SBP >150 mm Hg, DBP >90 mm Hg) or a SBP > 150 mm Hg or DBP > 95 mm Hg at the time of screening or baseline. If the initial BP reading is above these values, the reading may be repeated one time within 20 minutes of the initial reading.
6. Body mass index <18 or >32 kg/m2
7. Severe chronic kidney disease requiring treatment with hemodialysis or peritoneal dialysis.
8. Additional laboratory abnormalities determined as clinically significant by the Investigator.
9. Clinically significant abnormalities on physical examination (as judged by the Investigator)
10. Clinically significant and chronic uncontrolled renal, hepatic, pulmonary, endocrine, neurologic disorders, bone, or gastrointestinal system dysfunction
11. History of seizures or epilepsy
12. History of serious mental illness as judged by the Investigator

13. Oral temperature >37.5°C at the time of the physical examination
14. Suspicion, or recent history, of alcohol or substance abuse or tobacco use
15. Subjects who in the opinion of the Investigator have a clinically significant abnormal 12-lead ECG during the screening period. Presence of atrial fibrillation, varying degrees of AV block, existence of a left bundle branch block, or evidence of previous myocardial infarction.
16. Subjects who are either unwilling to agree to refrain from using or are found to be using supplementary antioxidant vitamins (e.g., Coenzyme Q10, resveratrol, L-carnitine) from 7 days prior to dosing and throughout the treatment period
17. Subjects who are either unwilling to agree to refrain from using or are found to be using the following dietary restrictions (pomegranate juice, walnuts, pecans, strawberry, raspberry blackberry) from 7 days prior to dosing and throughout the treatment period
18. Are currently enrolled in a clinical trial involving an investigational product or non-approved use of a drug or device or concurrently enrolled in any other type of medical research judged not to be scientifically or medically compatible with this study
19. Have participated, within the last 30 days from a clinical trial involving an investigational product. If the previous investigational product has a long half life, 3 months or 5 half-lives (whichever is longer) should have passed

**Discontinuation of Subjects**

The criteria for enrollment must be followed explicitly. If the Investigator site identifies a subject who did not meet enrollment criteria and who was inadvertently enrolled, the Sponsor must be notified. If the Sponsor identifies a subject who did not meet enrollment criteria and who was inadvertently enrolled, the Investigator site will be notified. A discussion must occur between the Sponsor clinical research physician and the Investigator to determine whether the subject may continue in the study.

**Investigator Decision**

The Investigator decides that the subject should be discontinued from the trial for any reason if the subject, for any reason, requires treatment with another therapeutic agent that may effect the efficacy outcomes of this trial or is prohibited by this study protocol.

**Subject Decision**

The subject or the subject's designee requests to be withdrawn from the study. No further assessments will be performed, unless the subject provides written consent for follow-up data assessments to be collected.

**Adverse Event**

If the Investigator decides that the subject should be withdrawn because of an SAE or a clinically significant abnormal laboratory value, the investigational product is to be discontinued and appropriate measures are to be taken. The Sponsor or its designee is to be alerted immediately. Any subject withdrawn from the trial will be followed until the SAE resolves.

**Subject Identification**

Each subjects screened and signed an informed consent will be allocated to a 3-digit screening number comprising the prefix 'S' and a 3-digit number representing the sequential order in which they are screened, e.g. S001, S002 etc. The number will be assigned by the e-CRF/IWRS upon entry in the system.

**Randomization**

At randomization, the e-CRF/ IWRS will randomly assign eligible patients to one of the treatments (AMAZ-02 dose or placebo)

## INVESTIGATIONAL PRODUCT

**Description of the Investigational product**

Name of the product: **AMAZ-02**

Form: 1100 mg softgel containing 250 mg of AMAZ-02

Dose per intake: 1000 mg (4 softgels)

Timing for intake: Single oral dose administration according to the randomization for 120 days. The administration should take place in the morning, with around 200 ml of tap water, in sitting position and in fasting conditions of 6-8 hours.

Product formulation:

| FILL | | |
|---|---|---|
| INGREDIENTS | AMOUNT (MG) /CAP | % TOTAL |
| **AMAZ-02** | **250** | **22.73%** |
| **Lecithin NF (35% Total PC) (Epikuron 135 F IP) - E322** | **284.25** | **25.84%** |
| **Medium Chain Triglycerides (MCT)** | **284.25** | **25.84%** |
| **Glycerol Monostearate (40-55) EP, Mono- and Diglycerides NF** | **11.5** | **1.06%** |
| **Fill weight** | **830 mg** | **75.47%** |
| SHELL | | |
| INGREDIENTS | AMOUNT (MG) /CAP | % TOTAL |
| **Gelatin EP, NF** | **165.97** | **15.09%** |
| **Glycerol – E422** | **80.01** | **7.27%** |
| **Water** | **21.62** | **1.96%** |
| **Titanium Dioxide EP – E171** | **1.96** | **0.18%** |
| **DualDustmaster FD&C Blue #1 (Brilliant Blue FCF - E133)** | **0.234** | **0.021%** |

| | | |
|---|---|---|
| **Sodium Copper Chlorophyllin Powder (min 95%) - E141** | **0.196** | **0.018%** |
| **Shell weight** | **270 mg** | **24.539%** |
| **Total Capsule weight** | **1100 mg** | **100%** |

Name of the compound: **Placebo**

Form: Softgel containing lecithin, triglycerides and diglycerides.

Dose per intake: 4 softgel pills for blinding

Timing for intake: Single oral dose administration according to the randomization for 120 days. The administration should take place in the morning with around 200 ml of tap water, in sitting position and in fasting conditions of 6-8 hours.

Product formulation:

| FILL | | |
|---|---|---|
| INGREDIENTS | AMOUNT (MG) /CAP | % TOTAL |
| **Lecithin NF (35% Total PC) (Epikuron 135 F IP) – E322** | **371.49** | **36.14%** |
| **Medium Chain Triglycerides (MCT)** | **371.49** | **36.14%** |
| **Glycerol Monostearate (40-55) EP, Mono- and Diglycerides NF** | **15.03** | **1.46%** |
| **Fill weight** | **758 mg** | **73.74%** |
| SHELL | | |
| INGREDIENTS | AMOUNT (MG) /CAP | % TOTAL |
| **Gelatin EP, NF** | **165.97** | **16.15%** |
| **Glycerol – E422** | **80.01** | **7.78%** |
| **Water** | **21.62** | **2.10%** |
| **Titanium Dioxide EP – E171** | **1.96** | **0.19%** |
| **DualDustmaster FD&C Blue #1 (Brilliant Blue FCF - E133)** | **0.234** | **0.023%** |
| **Sodium Copper Chlorophyllin Powder (min 95%) E141** | **0.196** | **0.019%** |
| **Shell weight** | **270 mg** | **26.26%** |
| **Total Capsule weight** | **1028 mg** | **100%** |

**Unit form, packaging and labelling**

This trial involves a comparison of an oral dose of AMAZ-02 supplement versus a placebo alone for 4 months. The AMAZ-02 will be provided as a maximum of 4 softgels, with each softgel containing 250mg AMAZ-02 or placebo.

Primary packaging of the softgel are blister packs. They are made of Polyvinyl chloride (PVC) coated with a layer of Polyvinylidene Chloride (PVDC). The PVC provides structural rigidity and physical protection of the product. The lidding material is aluminum.

AMAZ-02 or Placebo softgels will be packaged into blister packs.

Primary packaging of the softgels is managed by:

**PCI Clinical Services**

**4545 Assembly Drive,**

**Rockford, IL 61109**

**USA**

For the purpose of the study, the daily dose of AMAZ-02 or placebo will be delivered by employing secondary packaging in wallets containing an 8 day supply . Each day supply will have 4 softgels. Subjects will take 4 softgels a day:

- For Placebo daily dose: 4 Placebo softgels

- For 1000mg AMAZ-02 daily dose: 4 AMAZ-02 softgels

4 wallets (32 day supply) will be tertiary packaged in a box to deliver a month's clinical supply to the study participants. The study labels will be inserted on top of the secondary and tertiary packaging with the necessary instructions on intake along with the blinded study codes.

These will be sent to the investigational site in University of Washington. Each subject will receive 2 boxes for 2 months supply at Day 0 and Day 61 of the study.

Labelling will be in accordance with local regulatory specifications and requirements.

The following information will be reported on boxes, cases and vials, in English language:

- Name, address and telephone number of sponsor,
- Name of the investigator,
- Trial reference code (Sponsor),
- Packaging batch number ,
- Supplement dosage form, route of administration, quantity of dosage units, and name/identifier and strength/potency,
- Directions for use ,
- The storage conditions,
- The mention " Product for clinical use only" and "Do not leave within the reach of the children".
- Kit number
- Verifier (a 4-digit alphanumeric code that needs to be re-entered into the IWRS to validate the correct assignment to study products)

The Investigator or his/her designee is responsible for the following:

- Explaining the correct use of the investigational agent(s) to the site personnel
- Verifying that instructions are followed properly
- Maintaining accurate records of investigational product dispensing and collection

Subjects will be instructed to contact the Investigator as soon as possible if he or she has a complaint or problem with the investigational product so that the situation can be assessed.

**Method of Assignment to Treatment**

Subjects who meet all criteria for enrollment will be randomized 1:1to double-blind treatment by the IWRS.

**Rationale for Selection of Doses in the Study**

The 1000 mg/day amount of AMAZ-02 proposed for this study has been taken in a Phase 1 human clinical trial without notable side-effects. The other ingredients in the softgels containing AMAZ-02 are excipients, i.e., non-primary ingredient materials, such as dietary oil for diluting or gelatin for softgels. Therefore, the AMAZ-02 exposure is consistent with what has been shown to be safe in previous human subject studies. As a result, the dosing regimen being employed should be well-tolerated.

**Selection and Timing of Doses**

A dose-response relationship for the AMAZ-02 on clinical and physiological outcomes has not been established previously, but favorable biologic effects on mitcohondrial gene expression in elderly skeletal muscle and on plasma levels of acylcarnitines (indicator of mitochondrial function) following a 4 week oral supplementation in Phase 1 human studies have been found with AMAZ-02 dose of 1000 mg/day.

**Continued Access to Investigational Product**

The AMAZ-02 will not be made available to subjects after conclusion of the trial.

**Concomitant Therapy**

The excluded medications and procedures are provided under exclusion criteria. For randomized subjects these will be recorded 30 days prior to start of study upto the end of study visit.

**Study Product Compliance**

The AMAZ-02 will be administered as a daily oral dose.

The softgels and empty wallets remaining from each month's supply will be returned and counted. Follow-up phone calls will be done to subjects at Day 30 (± 4) and Day 90 (± 4) to check subjects compliance. Urolithin A levels will be measured before and after the end of the study in the collected plasma samples as an additional sign of product compliance.

## ASSESSMENT OF EFFICACY AND SAFETY

### Efficacy Measures

#### Skeletal Muscle Energetics

The skeletal hand muscle energetics will be evaluated by changes from baseline in muscle and mitochondrial energetics determined by the 31P MRS: ATPmax (phosphorylation capacity per unit muscle volume).

#### Skeletal Muscle Function

The skeletal muscle performance will be evaluated by changes from baseline in muscle strength (maximum voluntary contraction) and endurance (number of muscle contractions and force time integral) determined from the results of exercise testing. A hand grip ergometer will be used to measure arm strength.

#### Exercise Tolerance Test (Single Muscle Fatigue Test)

During these sessions, subjects will be asked to exercise their hand or leg. First, they will be asked to push their finger or pull their leg against a bar as hard as possible for 5 seconds and to repeat this 3 times.. This will tell the investigators about the maximum strength in that muscle. For a second type of exercise, subjects will be asked to push their finger or pull their leg against the bar in a rapid motion for up to 5 minutes and to increase the rate every minute. A beeping sound will tell subjects when to push or pull. Subjects will try to push or pull each time at about 70% of their maximum strength. Subjects will watch a light display that tells them how hard they are pushing or pulling. Muscle strength (maximum voluntary contraction) and endurance (number of muscle contractions to fatigue and force time integral) will be determined on each muscle.

#### Cycle Ergometer Test for Exercise Performance

Subjects may feel tired after exercising during the exercise ergometer test.

Subjects will be asked to take of test of their heart and fitness level with a resting electrocardiogram and a cycle ergometer test. The test will involve pedaling a cycle ergometer for 8 to 12 minutes while breathing into a measurement tool. Each session will last about 3 hrs from the time they arrive to the time subjects leave the facility.

Recovery generally occurs within 30 minutes. Subjects will be continuously monitored and the test will be stopped at any time they ask (an emergency stop mechanism is available for subjects to use). An exercise

physiologist with Advanced Cardiac Life Support (ACLS) certification will be present at all times. In persons with an underlying heart condition, there is a slight (less than 1 chance in 1,000) risk of having a sudden heart attack and an even slighter (less than 2 chances in 10,000) risk of sudden death. Persons with a serious heart condition will not be eligible for this study. The leg power output, time to fatigue and Borg's perceived exertion scale and VO2 at 85% of the estimated maximum heart rate (HRmax) will be determined.

**Cybex Leg Muscle Strength Testing**

Leg muscle strength testing will be perfomed with one-repetition max testing and ten-repetition max testing using a Cybex®leg extension machine (Cybex International, Medway, MA). Subjects are seated with the resistance bar positioned in line with the medial malleolus. Subjects lifted the weight to near full extension of the knee.

**Physical function**

A short physical performance battery assessing lower extremity function will be made at baseline and 4 months. Measurements include balance, gait, ability to stand with the feet together in the side-by-side, semi-tandem, and tandem positions, time to walk 8 feet, and time to rise from a chair and return to the seated position 5 times, hand grip and distance covered in 6 minutes in a walk (Guralnik et al, 1994).

**Safety and Biomarker Parameters**

Blood samples will be taken about three (3) times throughout the course of the study: at the start of the study, after 60 days, and at the end of the study. About 70 mL of blood (about 5 tablespoons) will be drawn throughout the study. Additional blood samples may be required if any laboratory values are abnormal. It is possible that more than one attempt to obtain a blood sample may be necessary. The following blood analysis will be conducted:

| **Hematology**: | **Clinical Chemistry**: |
|---|---|
| Hemoglobin | Total bilirubin |
| Hematocrit | Direct bilirubin |
| Erythrocyte count (RBC) | Alkaline phosphatase |
| Mean cell volume | Alanine aminotransferase (ALT) |
| Mean cell hemoglobin concentration | Aspartate aminotransferase (AST) |
| Leukocytes (WBC) | Blood urea nitrogen (BUN) |
| Neutrophils, segmented | Creatinine |
| Lymphocytes | Uric acid |
| Monocytes | Calcium |

| | |
|---|---|
| Eosinophils | Lipid panel (LDL, VLDL, Triglycerides, Cholesterol) |
| Basophils | Albumin |
| Platelets | Creatine kinase (CK) |
| | Acylcarnitine (Metabolomics) |
| | AMAZ-02 levels |

**Skeletal Muscle Biopsy (Optional)**

Subjects will be asked to lie down on a padded table and given a local anesthetic (2% lidocaine plus epinephrine solution) just under the skin. A small piece of fine muscle, approximately the size of a pencil eraser (approx. 100mg of muscle tissue), will be removed through a 3/16 inch slit in the skin and the underlying tissue, using a hollow biopsy needle to remove the most sample. This procedure will be performed by physician at the Fred Hutchinson Cancer research Center prevention Center, and will require approximately one hour. After the samples are obtained, the fat will be carefully dissected from the muscle tissue before performing further analysis and storage.

**High-resolution Respirometry (Optional)**

**Permeabilizing human muscle fibers:**

Biopsies are obtained from vastus lateralis muscle of human donors and 2 to 4 4-8mg pieces are dissected from the muscle longitudinally along fiber length. Small muscle pieces are immediately placed into cold Isolation buffer (CaK2EGTA (20mM), K2EGTA (20mM), Na2ATP (5.8mM), MgCl2-6H2O (6.6mM), Taurine (20mM), Na2Phosphocreatine (15mM), Imidazole (20mM), DTT (0.5mM), MES (50mM), pH 7.1) and the remaining muscle frozen in liquid nitrogen and stored in a -80˚C freezer. Under a dissection microscope, the fibers from the small muscle pieces are teased apart with tweezers to facilitate better diffusion. Two pieces of muscle are then placed into 2 mL of cold isolation buffer containing 50ug/ml saponin and allowed to permeabilize for 30 minutes on a rocker. The permeabilized fibers are moved to 2mL of Isolation buffer to rinse for 5 minutes, and then rinsed in Buffer Z (EGTA (1mM), MgCl2 (5mM), K-MES (105mM), KCl (30mM), KH2PO4 (10mM), pH 7.1) twice for 5 minutes each. The permeabilized fibers are then placed into the two chambers of the Oroboros O2K oxygraph for respirometric analysis.

**Repirometric analysis of permeabilized fibers:**

After oxygen and hydrogen peroxide calibration, permeabilized fibers are placed into the two respiration chambers of the Oroboros O2K. The chambers are oxygenated with pure oxygen until saturation reaches approximately 500-600 nmol/ml. Once stabilized and background readings taken, mitochondrial respiration and H2O2 production are simultaneously measured under Forward Electron Transfer (FET) and Reverse Electron Transfer (RET) conditions. FET is defined as the sequential delivery of substrates that promotes the flow of electrons from complex I and II to complex IV (state 3 respiration), resulting in oxygen consumption and minimal hydrogen peroxide production. RET is state 4 respiration where the principle substrate is succinate and facilitates reverse electron flow from complex II through complex I and maximum hydrogen peroxide production.

FET is obtained by the addition of malate (final concentration of 2mM), pyruvate (5mM), and glutamate (10mM), sub-saturating ADP (0.045mM), saturating ADP (2.5mM), and Succinate (10mM), allowing at least 5 minutes to elapse between injections to observe stable respiration rates. State 3U is also measured by adding sequential 0.2uM concentrations of the uncoupler FCCP until maximal respiration is reached. Non-mitochondrial respiration is measured for background subtraction purposes by injecting 1.5mM potassium cyanide. The sequential addition of 0.05, 0.1, 0.5, 1, and 2 mM succinate in the absence of ADP was used for RET analysis.

Media and tissue are collected from the chamber following the assay, and fragments of the permeabilized muscle are dried and weighed for assay normalization.

Summary of measurements:

Respiration and H2O2 production under the following conditions:

- State 4
- State 3
- Uncoupled (FCCP)
- Complex II
- Complex IV
- H2O2 production with reverse electron transfer using multiple succinate concentrations

**EXERCISE TESTING DESCRIPTION (University of Washington & Fred Hutchinson Clinical Research Center)**

Overview: Each subject will be tested at baseline and at month 4 in the MRI. An exercise fatigue test will also be performed at month 2, during the visit to replenish investigational product for the remaining 2 months. Listed below is a detailed description of each test followed by the execution sequences on the experimental days. One session will be dedicated to each muscle.

**Magnetic Resonance Spectroscopy (MRS) Method**

*Inside the Magnet separately on 2 single muscles: FDI (hand muscle) and TA (leg muscle)*

I. Screening (~0.5 hr.)

1) Screening: Screen for suitability for MR measures, explain protocols, take blood pressure.

II. Positioning/Scanning (0.25 hr)

1) Limb positioning: Fit limb into MR cradle to position leg for force measurement, secure MR coil to limb with tape and secure limb to cradle with Velcro.

2) Subject positioning: Move subject into magnet room, position subject in supine position on gurney, place MR holder in magnet and adjust subject's position on gurney to ensure comfort.

3) Cradle positioning in magnet: Adjust position of MR cradle in magnet bore to center of magnet and attach all electronic connections.

4) Scanning: Provide subject with earplugs, inform about MR generated noises, tune MR probe, close door, optimize MR measures, take fully relaxed spectrum and muscle images .

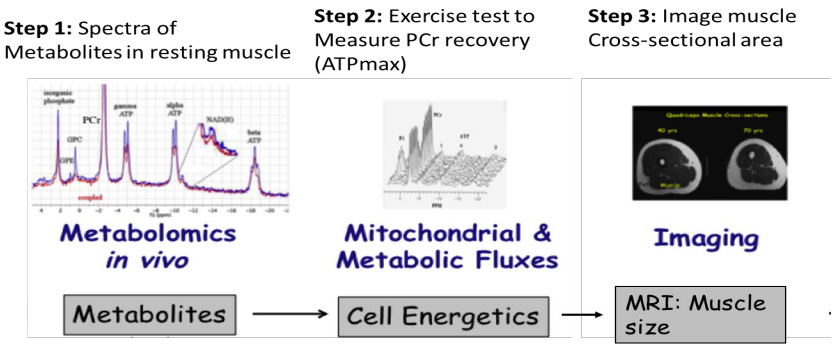
2) Execution of protocol.

III. Muscle burst exercise protocol (0.25 hr)

1) Exercise instruction: Review exercise protocol to re-familiarize subject. ATPmax test: instruct subject to exercise as fast as possible when instructed and to stop immediately when instructed (~20 sec protocol).

2) ATPmax Test (~15 min): Start scan of resting muscle (min 0); warn subject about onset of exercise (min 7); instruct subject to start exercise (min 8); contract muscle as fast as possible until instructed to stop (~20 sec; min 8.5), allow muscle to recover until min 15.5 (total recovery=6 min).

*MRS measurements flow for single muscle assessments*




**Single Muscle Exercise Tolerance Test**

IV. Fatigue test (0.5 hr): *Outside the Magnet on the FDI (hand muscle) and TA (leg muscle)*

1) Practice runs: Review exercise protocol which involves instructing subject to exercise at ~70% MVC (as shown by the light box) at rate set by a metronome for 1 min and to increase rate with a metronome every 1 min until the subject can no longer exercise.

2) Muscle force measure: The subject is requested to pull against a force transducer to generate a muscle maximum voluntary contraction (MVC). Follow force by the number of lights activated on a LED panel. The subject is instructed to activate ¾ the number of lights (70% MVC) during the exercise period.

3) Fatigue test (0.25 hr). The subject is warned about onset of exercise; the subjects is instructed to start exercise at ~70% MVC at the rate set by a metronome and to increase exercise rate with the faster beat of the metronome, which occurs in 1 min increments until the subject can no longer exercise.

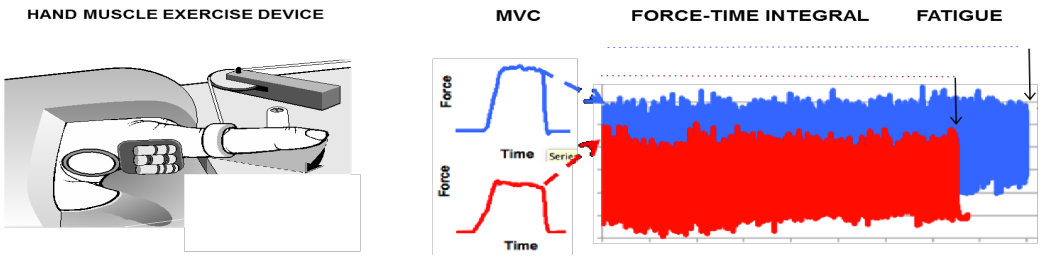


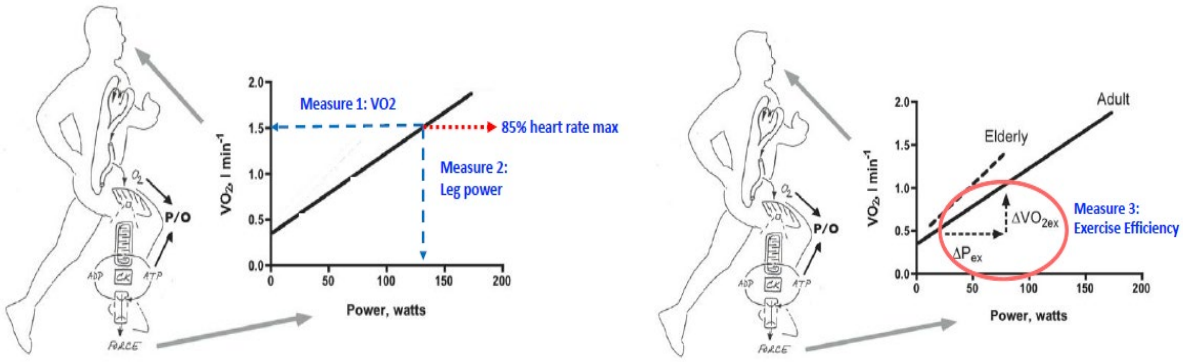

**Figure**: Exercise device (left panel) and force measurements (right panel) used to determine the maximum voluntary contraction (MVC) and force-time integral (FTI) to fatigue. The left panel shows the exercise device with the magnetic resonance coil positioned over the first dorsal interosseous muscle of the hand that was used to determine ATP flux. The right panel shows the force records generated by the hand muscle to determine MVC. The dashed arrows connect the single contraction of the MVC to the repeated

contractions that comprise the FTI. These repeated contractions continue until fatigue (indicated by the solid arrows pointing down). The integral of force of each contraction is summed over the exercise test (the force time integral (FTI)). A example is shown for an exercise test at baseline (red) and the greater FTI and sum of contractions to fatigue after treatment (blue).

**Cycle Ergometer Exercise Performance Test (Fred Hutchinson)**

Subjects will be asked to take of test of their heart and fitness level with a resting electrocardiogram and a cycle ergometer test. The test will involve pedaling a cycle ergometer for 8 to 12 minutes while breathing into a measurement tool. Each session will last about 3 hrs from the time they arrive to the time they leave the facility.

Recovery generally occurs within 30 minutes. Subjects will be continuously monitored and the test will be stopped at any time they ask (an emergency stop mechanism is available for subjects to use). An exercise physiologist with Advanced Cardiac Life Support (ACLS) certification will be present at all times. The leg power output, and VO2 at 85% of the estimated maximum heart rate (HRmax) will be determined (see Figure below depiciting how the measures will be derived).




**SF-36 Quality of Life Questionnaire**

Subjects will be asked to respond to a single SF-36 questionnaires that surveys quality of life. This is a standardized survey used in a variety of studies to provide an objective measure of their physical and mental health status. We will ask them to provide answers to these questionnaires before and after the study.

**Safety Evaluation**

Investigators are responsible for monitoring the safety of subjects who have entered this trial and for alerting Sponsor or its designee to any event that seems unusual, even if this event may be considered an unanticipated benefit to the subject.

The Investigator is responsible for the appropriate medical care of subjects during the study.

The Investigator remains responsible for following, through an appropriate health care option, AEs that are serious, considered related to the study treatment or the study, or that caused the subject to discontinue before completing the study. The subject should be followed until the event is resolved or explained. Frequency of follow-up evaluation is left to the discretion of the Investigator.

**Adverse Events (AE)**

The Sponsor has standards for reporting AEs that are to be followed regardless of applicable regulatory requirements that may be less stringent.

Lack of investigational product effect is not an AE in clinical studies, because the purpose of the clinical trial is to establish investigational product efficacy.

Trial site personnel will record the occurrence and nature of each subject's preexisting conditions, including clinically significant signs and symptoms of the disease under treatment in the study.

After the informed consent form (ICF) is signed, site personnel will record any change in the condition(s) and the occurrence and nature of any AEs. All AEs related to protocol procedures are reported to the Sponsor or designee.

In addition, all AEs occurring after the subject receives the first dose of investigational product must be reported to the Sponsor or its designee via designated data transmission methods.

Any clinically significant findings from ECGs, labs, vital sign measurements, or other procedures that result in a diagnosis should be reported to the Sponsor or its designee.

Investigators will be instructed to report to the Sponsor or its designee their assessment of the potential relatedness of each AE to investigational product via designated data transmission methods.

The Investigator decides whether he or she interprets the observed AEs as either related to disease, to the study medication, trial procedure, or other concomitant treatment or pathologies. To assess the relationship of the AE to the study investigational product, the following terminologies are defined:

- Related: a direct cause and effect relationship between the study treatment and the AE is likely
- Possibly related: a cause and effect relationship between the study treatment and the AE has not been demonstrated at this time and is not probable, but is also not impossible
- Unrelated: without question, the AE is definitely not associated with the study treatment

For reporting purposes, all "related" and "possibly related" AEs and SAEs will be defined as related to study investigational product.

If a subject's dosage is reduced or treatment is discontinued as a result of an AE, trial site personnel must clearly report to the Sponsor or its designee via designated data transmission methods the circumstances and data leading to any such dosage reduction or discontinuation of treatment.

**Serious Adverse Events (SAE)**

Serious adverse event collection begins after the subject has signed informed consent and has received investigational product. If a subject experiences an SAE after signing informed consent, but prior to receiving investigational product, the event will NOT be reported as serious unless the Investigator feels the event may have been caused by a protocol procedure.

Planned surgeries should not be reported as SAEs unless the underlying medical condition has worsened during the course of the study.

Trial site personnel must alert the Sponsor or its designee of any SAE within 24 hours of Investigator awareness of the event via a Sponsor-approved method. If alerts are issued via telephone, they are to be

immediately followed with official notification on study-specific SAE forms. This 24-hour notification requirement refers to the initial SAE information and all follow-up SAE information.

An SAE is any AE from this trial that results in one of the following outcomes:

Death

Initial or prolonged inpatient hospitalization

A life-threatening experience (that is, immediate risk of dying)

Persistent or significant disability/incapacity

Congenital anomaly/birth defect

Considered significant by the Investigator for any other reason

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse events when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Serious adverse events occurring up to and including the subject's last trial visit will be collected, regardless of the Investigator's opinion of causation, in the clinical data collection database and the pharmacovigilance system at the Sponsor.

If an Investigator becomes aware of SAEs occurring to a subject after the subject's participation in the trial has ended, the Investigator should report the SAEs to the Sponsor, regardless of the Investigator's opinion of causation, and the SAEs will be entered in the pharmacovigilance system at the Sponsor.

Information on SAEs expected in the trial population independent of investigational product exposure and that will be assessed by the Sponsor in aggregate periodically during the course of the trial may be found in the IB.

**Suspected Unexpected Serious Adverse Reactions**

Suspected unexpected serious adverse reactions (SUSARs) are serious events that the Investigator identifies as related to investigational product or procedure. United States 21 CFR 312.32 and European Union Clinical Trial Directive 2001/20/EC and the associated detailed guidances or national regulatory requirements in participating countries require the reporting of SUSARs. The Sponsor has procedures that will be followed for the recording and expedited reporting of SUSARs that are consistent with global regulations and the associated detailed guidances.

Complaint Handling

The Sponsor collects product complaints on investigational products and delivery systems used in clinical studies in order to ensure the safety of trial participants, monitor quality, and to facilitate process and product improvements.

For blinded studies, all product complaints associated with material packaged, labeled, and released by the Sponsor or delegate will be reported.

The Investigator or his/her designee is responsible for handling the following aspects of the product complaint process in accordance with the instructions provided for this study:

Recording a complete description of the product complaint reported and any associated AEs

Faxing the completed product complaint information within 24 hours to the Sponsor or its designee.

Sample Collection and Testing

Blood samples will be collected to determine whether subjects meet inclusion and exclusion criteria and to monitor subject health.

Blood will be obtained for assay of AMAZ-02 levels at baseline, two months and four month time-points.

Muscle biopsy will be optionally offered to study subjects. This will be performed under local anaesthesia on the *vastus lateralis* muscle on the opposite leg (i.e the one not subjected to MRS). The aim will be to have approximately 15 subjects per intervention subjected to muscle biopsy to look for mitochondrial gene and protein biomarkers and perform in vivo mitochondrial function assessment on muscle biopsy samples via the Oroboros technique (perform gene microarray on muscle tissue)

## INFORMED CONSENT AND IRB REVIEW

### Informed Consent

The Investigator is responsible for ensuring that the subject understands the potential risks and benefits of participating in the study, including answering any questions the subject may have throughout the trial and sharing in a timely manner any new information that may be relevant to the subject's willingness to continue his or her participation in the trial.

The ICF will be used to explain the potential risks and benefits of trial participation to the subject in simple terms before the subject is entered into the study, and to document that the subject is satisfied with his or her understanding of the risks and benefits of participating in the trial and desires to participate in the study.

The Investigator is responsible for ensuring that informed consent is given by each subject or legal representative. This includes obtaining the appropriate signatures and dates on the ICF prior to the performance of any protocol procedures and prior to the administration of investigational product.

### IRB Review

The Sponsor or its representatives must approve all ICFs before they are used at investigative sites(s).

Documentation of IRB approval of the protocol and the ICF must be provided to the Sponsor before the trial may begin at the investigative site(s). The IRB(s) will review the protocol as required.

The trial site's IRB(s) should be provided with the following:

ICF

Relevant curricula vitae

The majority of evaluations, reported in the electronic Case Report Form (eCRF), must be supported by appropriately signed identified source documentation.

**Data Collection**

All clinical data will be reported electronically by the Investigator or authorised designee on a web-based electronic Case Report Form (eCRF). This eCRF is specifically designed for the study and developed by the Data Management Department of SynteractHCR using the software Marvin from the company ©XClinical GmbH (www.xclinical.com/). Marvin is compliant with all FDA legislation relevant to electronic data capture (FDA 21 CFR Part 11, GCP). Should a correction be made, the corrected information will be entered in the eCRF and the initial information will be tracked in the audit trail.

**Responsibilities**

The Investigator or authorised designee is responsible for the timeliness, completeness, and accuracy of all observations and other data pertinent to the clinical investigation in the eCRFs.

The Investigator will ensure that all data are entered promptly (within 4 days) after the evaluation has occurred, in accordance with source documents and specific instructions accompanying the eCRFs, designed specifically for the study.

The Data Management Department of SynteractHCR will provide all tools, instructions, and training necessary to complete the eCRF, and each user will be issued a unique username and password.

The data management of SynteractHCR will be responsible for data processing, in accordance with the CRO data management procedures.

**Data Management**

During the study, through regular data collection and monitoring, clinical data reported in the eCRFs will be integrated into the clinical database. Computerised logic and/or consistency checks will be systematically applied in order to detect errors or omissions. Queries will be generated and submitted through the electronic data capture (EDC) system to the investigator sites for resolution (queries should be answered within 7 days).

Correction will be made either automatically from the immediate completion or following the review of the data during the SynteractHCR monitoring. An audit trail, which will be initiated at the time of the first data entry, allows tracking all modifications.

The Data Management Department of SynteractHCR may generate additional requests to which the Investigator must respond electronically by confirming or modifying the data questioned. The requests with their responses will be implemented to the eCRFs.

Each step of this process will be monitored through the implementation of individual user passwords and regular backups to maintain appropriate database access and to ensure database integrity.

When eCRFs are complete and all queries have been answered, the Investigator has to sign the eCRFs . Then eCRFs are locked and no modification is possible anymore.

After integration of all corrections in the complete set of data, the database will be locked and saved before being released for statistical analysis.

After database lock, archival PDFs containing the e-CRF data and complete audit trail will be created. A CD-ROM of the archival PDFs will be kept at site and another one will be sent to the sponsor for archiving.

**Study Monitoring**

SynteractHCR will perform the study in accordance with this protocol, GCP and the applicable regulatory requirements and the contract with the sponsor.

The Investigator is required to ensure compliance with the Investigational Product schedule, visit schedule and procedures required by the protocol. The Investigator agrees to provide all information requested in the Case Report Form in an accurate and legible manner according to the instructions provided and to ensure direct access to source documents to Sponsor representatives.

The Sponsor of this study is responsible to Health Authorities for taking all reasonable steps to ensure the proper conduct of the study as regards ethics, protocol compliance, integrity and validity of the data recorded on the Case Report Forms. Thus, the main duty of the Monitoring Team is to help the Investigator and the Sponsor maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the study.

**Access to data by Subjects**

In conformity with the law, any subjects who so wish may access any data concerning them, at the end of the research. They should address their request in writing to the Investigator, and will obtain a response within 8 working days.

## STATISTICS

### Determination of Sample Size

Revised Primary Endpoint: This trial is powered to detect a difference in change from baseline in the 6-minute walk test between treatment groups. Following completion of a double-blind, randomized, placebo controlled in middle aged, overweight subjects (https://clinicaltrials.gov/ct2/show/NCT03464500) that was also a 4-month interventional trial comparing 6 minute walk distance (in meters) before (baseline Day 0) and after Day 120 between the same active and placebo treatment (2 arms)used in tis trial, the sponsor observed an improvement of 33.4meters between the two treatment groups i.e. Active 33.43 $\pm$ 51.07 m (n=25) and Placebo -0.52 $\pm$59.08 m (n=27). Amazentis expects improvements to be in a higher range (>40meters improvement) in the ongoing trial with similar SD, than the above completed trial as the ongoing trial is in a more homogenous elderly (>70 yrs. old) population that have a bigger compromise in mobility vs. this trial in healthy middle-aged subjects (40-60 yrs.). As such to target an improvement of 45m with the same SD in the two groups, then 29 patients per group will be required.

Previous studies in endurance training in subjects aged 65 to 80 years suggest a change in ATPmax can be achieved over a 6 month time period of 0.103 under treatment and -0.03 under Placebo. Based on a clinical

relevant difference of 0.133 and a standard deviation of 0.149 under treatment and 0.154 under Placebo this results in a sample size of 27 per group using a 5% two-sided significance level and 88% power.

Assuming an approximately 10% drop-out rate, 33 subjects per group will be enrolled (66 subjects enrolled in total so far in the study)

In order to accont for the two primary efficacy endpoints, a priori-ordereed hypothesis are stated, i.e. ATPmax will only be tested for confirmatory decisions if 6-minute walk distance resulted in a statistical significant difference in AMAZ-02 dose compared to Placebo.

**Statistical Analysis Plan**

**General considerations**

All data obtained in this study and documented in the Case Report Forms will be listed and summarized with descriptive statistics or frequency tables as appropriate. A Statistical Analysis Plan (SAP) that details the planned statistical analysis and may include necessary adaptations to the planned statistical analysis will be prepared before unblinding of the data.

Raw data listings and summary tables will be generated using the software SAS© Version 9.4.

**Classification of patients to subsets**

The data sets defined in Table below will be used for the statistical analysis.

**Data Analysis Sets**

| | |
|---|---|
| Safety analysis (SA) set | All patients who were randomized and received at least one dose of study medication. |
| Full analysis set (FAS) | All patients of the SA set with at least one measurement of the primary efficacy variable(s) following at least one dose of study medication. |
| Per Protocol (PP) set | All patients of the FAS who completed the study and for whom no relevant protocol deviations were documented. |

The decision whether a protocol deviation is relevant or not for the exclusion of patients from the Per Protocol (PP) set will be made case-by-case in a blind data review meeting.

The primary analysis of the primary efficacy endpoint(s) will be based on the FAS data set. A secondary analysis of the primary efficacy parameter will be based on the PP set. All other secondary efficacy analyses will be based on the FAS.

Safety analyses will be based on the safety analysis (SA) set.

**Analysis of Efficacy Data**

**Primary efficacy endpoints**

- Change in 6 minute walking distance (6MWD) at the end of study intervention compared to baseline
- Percent change from baseline in ATP max in skeletal muscle (via MRS)

ATP max will be assessed on the hand muscle (FDI- first dorsal interosseus)

The analysis of primary efficacy efficacy parameters is done using an analysis of co-variance (ANCOVA) using the factor treatment as independent factor, the baseline meassurement as covariable and the change from baseline as dependent variable. 95% confidence intervals for treatment differences and corresponding non-adjusted p-values will be calculated.

A 5% significance level will be applied for the 2 comparisons of doses versus Placebo.

In order to account for the two primary efficacy endpoints, a priori-ordereed hypothesis are stated, i.e. per cent ATPmax will only be tested for confirmatory decisions if 6 minute walking distance resulted in a statistical significant difference for the active dose compared to Placebo.

**Secondary efficacy endpoints**

- Percent change from baseline in contraction number during a single muscle fatigue test assessed on the hand muscle (FDI- first dorsal interosseus)
- Percent change from baseline in ATP max in skeletal muscle (via MRS) (on the Tibialis Anterior leg muscle)
- Percent change from baseline in improvement in the number of contractions in the single muscle fatigue test (on the Tibialis Anterior leg muscle)
- Change from baseline in ATP max in skeletal muscle (via MRS) (on the Tibialis Anterior leg muscle)
- Change from baseline in contraction number during a single muscle fatigue test (on the Tibialis Anterior leg muscle)
- Change in SPPB Scores at the end of study interevention compared to baseline
- Change in leg power output, time to fatigue, Borg's perceived exertion scale and VO2 at 85% of the estimated maximum heart rate (HRmax) will be determined at the end of study interevention compared to baseline
- Change in hand grip strength at the end of study interevention compared to baseline
- Change in muscle size (cross-sectional area of the muscles) before and after intervention
- Change in mitochondrial function on muscle biopsy samples at the end of study interevention compared to baseline (respirometry)
- To assess the effect of AMAZ-02 on mitochondrial gene and protein expression in muscle tissue before and after study intervention
- To assess the effect of AMAZ-02 on plasma acylcarnitine levels

- To assess the effect of AMAZ-02 on quality of life questionnaire (SF36)
- Change from baseline in plasma lipid profile
- Change from baseline in plasma for circulating biomarkers (myostatin, follistatin, proteomics, metabolomics)

Methods for statistical analysis of secondary efficacy parameters will be specified in the SAP.

**Safety Data**

Results of physical examination, vital signs and temperature and ECG will be summarized with descriptive statistics by treatment group.

Clinical laboratory test results will be marked whether the result is below, within or above the respective reference range. Descriptive statistics will be prepared. For categorigical results, the number and percentage of each outcome will be summarised by treament group. The number of values outside of the reference range will be counted per treatment group.

AEs will be coded according to the MedDRA.

The analysis of adverse events will include summary tables displaying counts and percentages of subjects experiencing adverse events by system organ class and preferred term.

**Further Data**

All other data obtained in this study and documented in the Case Report Forms will be tabulated with descriptive statistics or counts/ percentages depending on the variable and where appropriate.

**Withdrawals, Drop-outs, Missing Data**

Analysis of efficacy and safety variables will be done on a valid case basis, i.e. for missing observations no imputation technique like LOCF (last observation carried forward) will be applied.

**Interim Analysis**

No interim analysis is planned.

## SAMPLE MANAGEMENT AND ANALYSIS

**Plasma Samples**

Blood sampling will be performed for analysis on plasma at the exact time-points described in the Table 1. A 10 mL blood sample should be drawn into K2-EDTA coated tube. The blood samples will be gently inverted a few times for complete mixing with the anticoagulant. The exact time of sample collection will be recorded on the eCRF, which has no relationship with the timing of study product intake. Within 30 minutes following blood collection, centrifuge each blood sample at 1500 g for 10 minutes at 4°C.

Within 30 minutes after the centrifugation, the top layer of human plasma will be transferred into two pre-labelled polypropylene tubes, containing approximately 1500µL of plasma each (2 aliquots per time point).

Blood cells should not be transferred. All sample tubes must be clearly and appropriately labelled. Tubes will be capped immediately from each time point and the plasma will be frozen in an upright position at approximately -80°C for storage. The samples will be shipped on dry ice by a specialized carrier. Temperatures will be monitored using data logger during all transport. Shipment will only be carried out using a Sponsor approved carrier.

The total amount of blood collected during the study will be approximately:

| | |
|---|---|
| Safety:..... | 20 mL |
| Lipid Profile ..... | 20 mL |
| AMAZ-02, Biomarkers ..... | 30 mL |
| **Total volume:** | **70 mL** |

Measurement of markers of muscle and mitochondrial function: Markers of muscle function, including, myostatin and follistatin will be measured using an ELISA based method along with targeted metabolomics for muscle and mitochondrial biomarker metabolites such as acylcarnitines.

**Muscle Biopsy (Optional)**

Muscle biopsy will be collected on Day 0 pre-dose and at Day 121 before the meal. The amount of muscle tissue collected will be approximately 100 mg.

After carefully dissecting the fat out from the muscle biopsy samples, the collected muscle tissue will be divided into 3 portions: one third for high-resolution respirometry (see Section 6.1.6), one third for protein analysis and one third for gene expression.

The one third for gene expression (≈30mg) will be further divided into two equal portions: one portion for RNA analysis (≈20mg) and one portion for DNA analysis (≈10mg), both in a Safe-lock Tubes 2.0 ml, Eppendorf. Muscle tissue will be snap frozen using liquid nitrogen immediately after collection and further, long term storage will be in a -80ºC freezer. A set of genes involved in autophagy, mitophagy, mitochondrial biogenesis and fatty acid oxidation will be measured by qPCR and RNA microarray. Quantification of mtDNA over nuclear DNA will provide another measure of mitochondrial abundance.

The last third (≈30mg) will be frozen for analysis of proteins by protein array. The shipment will be done in a dry ice by a specialized carrier. Temperatures will be monitored using data logger during all transport. Shipment will only be carried out using a Sponsor approved carrier.

## QUALITY CONTROL AND ASSURANCE

**Quality Assurance**

The study will be carried out in conformity with legal conditions and with respect to GCP (ICH E6).

**Quality Control**

The main study stages (coherence between source and CRF for: eligibility criteria, main evaluation criteria, AEs) will undergo to a quality control process.

The study may be subject to on-site audit visit by the Sponsor Representative and inspection in order to verify the study is conducted in compliance with the principles of GCP and with the study protocol. The auditor/inspectors will have direct access to medical records, source documents, and all documents and facilities relevant to the clinical trial.

The Investigator agrees to allow the auditors/inspectors to have direct access to study records for review, being understood that this personnel is bound by professional secrecy, and as such will not disclose any personal identity or personal medical information.

The confidentiality of the data verified and the anonymity of the subjects should be respected during these inspections.

## ETHICS

### Informed consent form

The persons participating in the study will be selected during a screening visit. During this meeting, the study objectives and methodology will be explained.

The volunteer subjects will receive a synthesis document explaining the requirements of research, the title and the objectives of the study, the detailed research protocol and the risks and constraints of the research.

Before being included in the study, each participant must give his/her written consent. The text of the consent is to be signed and dated by the subject and dated and signed by the Investigator.

### Ethics Committee and Competent Authority

The study will be carried out in conformity with the principles of the Declaration of Helsinki as modified in Fortaleza (2013).

This study will be undertaken after approval by the WIRB Committee.

### Protocol amendments

Neither the Investigator nor the Sponsor can modify the protocol without agreement from the other party.

Any protocol modifications must be the topic/matter of an amendment which will be dated and signed by the two parties and must be included as an addendum to the protocol.

Depending on the importance of the changes to the study conditions, the amendment will be sent to the WIRB for approval or for information.

### Protocol deviations

No deviations are systematically tolerated. Any protocol deviations will be notified to the Monitor/Sponsor on an ongoing basis, and no later than the date of the blind review, and will give rise to a discussion to define their status (minor – major).

## DATA HANDLING AND RECORD KEEPING

### Archival

All documents relating to the study must be kept by the Investigator in appropriate files. The archives of the subjects, original informed consent forms, source documents, case report forms, inventory of study products, correspondence with the Sponsor and Ethics committee relating to the study, must be filed.

The Investigator authorizes direct access to the source documents for monitoring, audits and inspections.

These documents must be kept on the Investigator site until at least fifteen years. Even at the end of this period, no destruction can be achieved unless authorized in writing by a duly mandated Sponsor's representative.

If the Investigator/Institution is no longer able to be responsible for essential documents the Sponsor must be notified in writing of this change and informed as to whom the responsibility has been transferred.

### Ownership of results

The Sponsor is the sole owner of the data and research results. He reserves the right to use them in any form whatsoever, to submit them to the Health Authorities of any country.

Should the study generate results likely to be patented, then only the Sponsor will be authorized to depose such a patent, in his name and at his costs.

## FINANCING AND INSURANCE

The Sponsor certifies that it has taken out a liability insurance policy which covers the current research in accordance with local laws and requirements.

An insurance certificate will be provided to the Investigator in countries requiring this document.

## PUBLICATION

All information issuing from the study will be considered to be confidential, and must not be divulged without the Sponsor's prior agreement.

The study results may be published or presented by the Investigator or analysis experts, in collaboration with the Sponsor, with the sponsor's written permission. The Sponsor may use the study results for any publication or communication, with the written agreement of the Investigator or the analysis experts if they are cited.

## LIST OF APPENDICES

APPENDIX I DECLARATION OF HELSINKI

APPENDIX II SF-36 Questionnaire

**APPENDIX1**

Declaration OF Helsinki

# WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

## Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

## General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words,

"The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by

individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

## Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

## Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

## Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

## Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and

standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

## Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

## Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain

for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

## Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

## Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

## Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made

publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

## Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

**APPENDIXII**

SF36 Questionnaire

## SF-36 QUESTIONNAIRE

Name:_____ Ref. Dr:_____ Date: _____

ID#: _____ Age: _____ Gender: M / F

Please answer the 36 questions of the **Health Survey** completely, honestly, and without interruptions.

**GENERAL HEALTH:**
**In general, would you say your health is:**

◯ Excellent ◯ Very Good ◯ Good ◯ Fair ◯ Poor

**Compared to one year ago, how would you rate your health in general now?**

◯ Much better now than one year ago
◯ Somewhat better now than one year ago
◯ About the same
◯ Somewhat worse now than one year ago
◯ Much worse than one year ago

**LIMITATIONS OF ACTIVITIES:**
The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

**Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.**
◯ Yes, Limited a lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

**Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf**
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

**Lifting or carrying groceries**
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

**Climbing several flights of stairs**
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

**Climbing one flight of stairs**
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

**Bending, kneeling, or stooping**
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

**Walking more than a mile**
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

**Walking several blocks**
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

**Walking one block**
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

**Bathing or dressing yourself**

◯Yes, Limited a Lot ◯Yes, Limited a Little ◯No, Not Limited at all

**PHYSICAL HEALTH PROBLEMS:**

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

**Cut down the amount of time you spent on work or other activities**

◯Yes ◯No

**Accomplished less than you would like**

◯Yes ◯No

**Were limited in the kind of work or other activities**

◯Yes ◯No

**Had difficulty performing the work or other activities (for example, it took extra effort)**

◯Yes ◯No

**EMOTIONAL HEALTH PROBLEMS:**

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

**Cut down the amount of time you spent on work or other activities**

◯Yes ◯No

**Accomplished less than you would like**

◯Yes ◯No

**Didn't do work or other activities as carefully as usual**

◯Yes ◯No

**SOCIAL ACTIVITIES:**

**Emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?**

◯Not at all ◯Slightly ◯Moderately ◯Severe ◯Very Severe

**PAIN:**

**How much bodily pain have you had during the past 4 weeks?**

◯None ◯Very Mild ◯Mild ◯Moderate ◯Severe ◯Very Severe

**During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?**

◯Not at all ◯A little bit ◯Moderately ◯Quite a bit ◯Extremely

**ENERGY AND EMOTIONS:**
These questions are about how you feel and how things have been with you during the last 4 weeks. For each question, please give the answer that comes closest to the way you have been feeling.

**Did you feel full of pep?**
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

**Have you been a very nervous person?**
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

**Have you felt so down in the dumps that nothing could cheer you up?**
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

**Have you felt calm and peaceful?**
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

**Did you have a lot of energy?**
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

**Have you felt downhearted and blue?**

- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

**Did you feel worn out?**

- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

**Have you been a happy person?**

- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

**Did you feel tired?**

- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

**SOCIAL ACTIVITIES:**
**During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?**

- ◯ All of the time
- ◯ Most of the time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

**GENERAL HEALTH:**
**How true or false is each of the following statements for you?**

**I seem to get sick a little easier than other people**
○ Definitely true ○ Mostly true ○ Don't know ○ Mostly false ○ Definitely false

**I am as healthy as anybody I know**
○ Definitely true ○ Mostly true ○ Don't know ○ Mostly false ○ Definitely false

**I expect my health to get worse**
○ Definitely true ○ Mostly true ○ Don't know ○ Mostly false ○ Definitely false

**My health is excellent**
○ Definitely true ○ Mostly true ○ Don't know ○ Mostly false ○ Definitely false