1.1 Clinical Study Identifier

Efficacy of New Antiviral Therapy (ENAT) (Fit for Health 2.0, Project Nr. 81)

1.2 Study design and endpoints

Stage 1 Study is envisioned as a pilot study on a small number of patients with various viral pathologies, with a total study duration of 4-6 months. It aims to enroll *up to 300 patients in 3 sites:* in Romania (Bucharest) Greece (Athens) and Spain (Terrassa).

Given the fact that the natural progression (evolution of the viral infections without treatment) which are being treated during this study is well known, the fact that administration of a placebo in such instances will not be an ethical approach, and spontaneous remissions in such cases are extremely rare as reported by literature, we will perform an open-label prospective study – a study in which all patients are given the treatment in study, no patient is given a placebo, and administering the treatment in study is known to both patients and the doctors administering it.

Primary endpoints are:

- For patients with viral diseases in which a viral load can be measured (Hepatitis C, B, D, E, infections with HIV, parvovirus B19, in transplant patients CMV, BKV):
    - decrease in viral load of more than 100 fold (2 log) following 1 month of treatment
    - sustained virologic response (SVR) at 12 weeks –defined as undetectable viral load
    - sustained remission (defined as viral load not increasing more than 10% from the previous testing) after 6 months post-treatment
- For patients in whom a viral load is not usually performed (influenza, herpes simplex, West Nile, etc.):
    - Change in overall clinical status of the patient by quantifiable assessment tools (temperature, rhinorea, coughing, improvement in lung auscultation, overall tonus and strength, intestinal motility, skin modifications – vesicles, swelling and color, for which pictures are taken pre- and post- treatment -, etc.)

Secondary endpoints are represented by the improvement in the clinical status of the patient on various aspects:

- Subjective improvement reported by patient – relief of pain and discomfort, energy level, coughing, night sweats, diarrhea, skin modifications, etc.
- Additional laboratory results indicating improving health status (AST/ALT, markers for inflammation such as C-reactive protein, TNF-alpha, cytokines, lymphocyte number and ratio, etc.)

1.3 Scientific advice / protocol assistance / communication with regulatory / competent authorities / ethics committees

The proposed clinical study was not registered yet with a national regulatory authority or an ethics committee. This was due in part because the treatment consists of a specific combination of *over-the-counter (OTC) products*, in quantities that are not considered as posing danger to humans. So far it was used with very good results and no adverse effects on more than 20 patients in outpatient setting. The following activities are planned, contingent with the approval for funding of this project:

- An informed consent form will be given, explained and signed for all participants in the study
- The study will be registered as a clinical trial in the respective countries
- The ethics committees overseeing each sites' activities will be properly informed and permission for the clinical study will be obtained before the study is begun

1.4 Subjects/population(s)

Outpatients or inpatients from the participating countries mentioned (3 in Stage 1, 4 in Stage 2) will be asked to participate in the study.

General *inclusion criteria* are :

- Adults aged 18 years or more;
- Not currently receiving any other antiviral medication;
- Have been diagnosed with a viral infection and have a viral load performed before the study
- Have clinical manifestations of a viral infection and known risk factors of contagion (group 2, 3 and 4 patients)

Exclusion criteria are:

- Patients enrolled in other clinical study or a planned treatment which involves administration of antiviral therapy for at least 90-day
- Patients currently undergoing treatment which impacts upon the immune system (cortisol-type treatments, methotrexate, cyclosporine, other immune suppressants, chemo- and radio-therapy, etc.)
- Patients who are currently taking and unwilling to discontinue during the 30-day treatment, administration of supplements with known activity on the immune system (Echinacea, agaricus, ganoderma, reishi, etc.)

Group 1. Patients with *known Hepatitis C infection*, with documented viral load (number of virus copies in blood) who previously received treatment with interferon and ribavirin and had recurrence of disease. Preference is given to patients who are not planned to receive antiviral therapy with known medications (including sofosbuvir and ledipasvir).

Group 2. Patients with *other viral diseases than Hep C*, for whom a quantitative evaluation can be performed. Examples are Hepatitis B/D/E with viral load, or combination of any of these, HIV with viral load, Cytomegalovirus (CMV), etc.

Group 3. Patients with viral infections for which a viral load is not usually performed and recovery is evaluated clinically: *influenza* of any serological type; Patients with acute manifestation of *herpes simplex* reactivation as either Zona Zoster or herpes labialis (cold sores)

Influenza virus sensitivity – chemillumiscence (50 Eur)

All patients, Urine summary with vit C determination

1.5 Sample size

For the first primary endpoint (a reduction of the viral load of more than 50% in a patient population with a mean viremia of 1.000.000 +/- 500.0000) using the 1-way ANOVA pairwise 1 sided test, type I error rate of 0.05, power of 0.80 and a sampling ratio of 1, the number of patients needed to show statistical significance is just 9.

*For the second primary endpoint (*decrease in viral load of more than 90% following 1 month of treatment) *using the same patient groups as above and the ANOVA test, an even smaller number of patients will suffice.*

*However for the third primary endpoint(*percentage of patients in remission - defined as viral load not increasing from the previous testing - after 6 months post-treatment), using same 1 way ANOVA pairwise 1 sided test, *type I error rate of 0.05, power of 0.80 and a sampling ratio of 1,we will need a much bigger number of patients to show statistical significance. Estimating a mean viral load post-treatment of about 100.000 +/- 45.000, and the need for the final testing to be within 10% of the post-treatment result (110.000 +/- 45.000), then the patients needed are 251.*

All these 251 patients belong to Group Study 1 (as defined above); for the other three groupsan additional 12-15 each are added, which brings the final total of patient number to approximately 300 (and a final total of about 310-320 patients).

1.6 Statistical methods

The statistical method used is the 1-way ANOVA pairwise 1 sided test, type I error rate of 0.05, power of 0.80 and a sampling ratio of 1. The calculations were performed using the following formula, taken from

http://powerandsamplesize.com/Calculators/Compare-k-Means/1-Way-ANOVA-Pairwise-1-Sided

we may have k groups, meaning there are a total of K≡(k2)=k(k−1)/2 possible pairwise comparisons. When we test τ≤K of these pairwise comparisons, we have τ hypotheses of the form

$$H0:\mu A=\mu B$$
$$H1:\mu A<\mu B$$

or

$$H0:\mu A=\mu B$$
$$H1:\mu A<\mu B$$

where μA and μB represent the means of two of the k groups, groups 'A' and 'B'. We'll compute the required sample size for each of the τcomparisons, and total sample size needed is the largest of these.

Formulas

This calculator uses the following formulas to compute sample size and power, respectively:

$$nA=(\sigma 2A+\sigma 2B/\kappa)(z1-\alpha/\tau+z1-\beta\mu A-\mu B)2$$

$$nB=\kappa nA$$

$$1-\beta=\Phi\left(\left|\mu A-\mu B\right|nA---\sqrt{}\sigma 2A+\sigma 2B/\kappa----------\sqrt{}-z1-\alpha/\tau\right)$$

where

- κ=nA/nB is the matching ratio
- σ is standard deviation
- σA is standard deviation in Group "A"
- σB is standard deviation in Group "B"
- Φ is the standard Normal distribution function
- Φ−1 is the standard Normal quantile function
- α is Type I error
- τ is the number of comparisons to be made
- β is Type II error, meaning 1−β is power

The statistical analysis will be conducted with specialized software, namely NCSS, each site is entering the raw data in MS Excel and the PI will import results into NCSS. Responsible for the accuracy of data entered will be both the site coordinator, who will log in the results from the laboratory testing, and final responsibility will rest with the overall monitor and Principal Investigator of the ENAT study, Dr. Felician Stancioiu.

1.7 Conduct

this section needs input from you

Description of planned strategy for study management,

Participating clinical centers are located in Romania (Bucharest) Greece (Athens) and Spain (Terrassa).

	Patient visit 1	Patient visit 2 (4 weeks)	Patient visit 3 (12 weeks)	Patient visit 4 (6 months)	Cost (Euro)
Doctor examination	Comprehensive 100 Eur	Follow-up 60	Follow-up 60	Follow-up 60	280
Laboratory test	Viral load Quantitative IgM/IgG, C3, C4, (complement), AST/ALT, TNF-alpha, CRP, fibrinogen, IL-1, IL-6, IL-2, Lymphocyte T4 & T8 ratio, B lymphocyte type, NK type	Viral load Quantitative IgM/IgG, C3, C4, (complement), AST/ALT, TNF-alpha, CRP, fibrinogen, IL-1, IL-6, IL-2, Lymphocyte T4 & T8 ratio, B lymphocyte type, NK type	Viral load Quantitative IgM/IgG, C3, C4, (complement), AST/ALT, TNF-alpha, CRP, fibrinogen, IL-1, IL-6, IL-2, Lymphocyte T4 & T8 ratio, B lymphocyte type, NK type	Viral load Quantitative IgM/IgG, C3, C4, (complement), AST/ALT, TNF-alpha, CRP, fibrinogen, IL-1, IL-6, IL-2, Lymphocyte T4 & T8 ratio, B lymphocyte type, NK type	400 400
Treatment given	yes	Yes/no	Yes/no	No	100

Monitoring of the sites will be done on a weekly basis via electronic means and monthly visits for the Spanish, Greek and Cyprus sites, by the principal investigator (PI) of the study – Dr. Felician Stancioiu.

- data management – recording the clinical report forms is done by each site, copies are sent electronically to the PI and any issues will be reported to the site manager and the PI
- planned schedule for study conduct
  date for 'First Patient, First Visit' – first patient visit can take place approximately 6-8 weeks after the study is granted approval, and at about the same time the contractual agreement is signed. The site in Bucharest can begin enrolling within 6 weeks after approval of the study grant. If the other two sites (Spain and Greece) are experiencing delays in approvals and enrolling, more patients will be enrolled in Bucharest for Stage 1 by using multiple medical providers for patient enrollment but same laboratory for testing.

- *timelines for ethics, further administrative approvals :*

Each site is responsible for obtaining the approvals from the respective ethics committees and regulatory authorities. It is estimated that necessary approvals for conducting the clinical study at all sites will be obtained within 6-8 weeks from a positive answer for the study's funding

All the medication is available as over-the –counter (OTC) medication in each of the countries participating; it is an open-label study.

1.8 Orphan designation

An orphan designation has not been requested for the proposed antiviral treatment. Should the results of the testing suggest such use, it will be applied for.

1.9 'Unit costs per patient' for clinical trials / studies / investigations

We have pondered the use of unit costs and have encountered the following issues:

- *Costs of doctor's visit* in the three countries included (Romania, Spain and Greece) are different; it can vary three-fold (from about 50 to 150 Euro per patient visit, total 150 – 450 Euro/patient) also depending on the doctor's specialty (general practice, family medicine, internal medicine, emergency medicine, infectious disease), the complexity of medical consult and the time spent.
- *Cost of laboratory testing* can also vary about two-fold (from 750 to 1500 Euro/patient) depending on whether in-house laboratory is used, contractual agreements for discounts are used, etc.
- *Cost of treatment* can also vary two-fold (70 – 140 Euro/patient) depends on the local cost of medication or if transportation and storage will be needed, if one site wants to use same medication from another site.

Therefore we consider that we need to make use of the actual costs method in this situation

A reasonable cost per study patient – the actual cost of medical care for each patient for 6 months – will be about 1.500 Euro/ patient (of this, about 1.000 Euro will be laboratory testing costs).