Theoretical background and the main results of the application EBOO RU for the treatment of chronic hepatitis C.

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First, a bit about the pathogen of the disease and the available treatment options.

The virus consists of a nucleocapsid and surrounded by a lipid membrane. Viral genome encodes the synthesis (core protein), envelope glycoproteins - E1 and E2, and nonstructural proteins (NS2, NS3 NS4, NS5). Portion of the virus genome which coding of the envelope proteins E1 and E2 rapidly mutating. The immune system is more inertial, which leads to chronic disease in 85% of cases. In 10-15% of sufferers of hepatitis develop cirrhosis or liver cancer within 10-30 years. According to various estimates, the number of infected on Earth is from 200 to 500 million people.

Currently, the gold standard treatment for hepatitis C is a combined treatment with interferon-alpha and ribaverin. This is really a gold standard - 12 month course is now about 18,000 usd. How is result of the treatment according with this standard?
There are three types of treatment response (Fig.1) on interferon and ribavirin: 1) a minor response, 2) a significant but insufficient fall of viral load - is a partial response, and 3) reduction below the threshold of detection of virus in the blood after 12 weeks of treatment, which is called a complete response. If viruses are not detected at 24 weeks after completion of treatment, the answer is said to be sustained (SVR). The probability of sustained response is 65-76%, if full of viral response was achieved. What is the probability that the patient will receive a sustained virologic response as a result of treatment?
It depends on the status of gene of the IL28B interleukin localized to human chromosome 19. Probability SVR for variations C / C of IL28B of about 80%, C / T - 20-40%, while for T / T of 20 to 25%. An average of about 50%. (Fig.2)

**Note:** In the spring of 2011, the arrival of telaprevir (Incivek) on the market seemed to be a big win for the Hepatitis C community. Approved by the FDA to treat Hepatitis C in unison with interferon and ribavirin, telaprevir appeared to improve treatment success rates in a shorter period of time without adding any substantial safety concerns. However, it was just a matter of time before the safety profile of telaprevir associated with some disturbing side effects, including: Nausea/vomiting, Change in ability to taste, Rash/blisters, Skin or mouth sores, Itching, Red, swollen, itchy or teary eyes, Hemorrhoids, Discomfort, burning, or itching around the anus, Dizziness/shortness of breath, Tiredness/weakness [http://www.hepatitis-central.com/mt/archives/2013/04/the-dark-side-of-telaprevir.html](http://www.hepatitis-central.com/mt/archives/2013/04/the-dark-side-of-telaprevir.html). “Just under half (48.6%) of patients receiving telaprevir experienced a serious adverse event as did 38% of those treated with boceprevir. This high prevalence of serious side-effects contrasted to the rates of between 9 and 14% observed in the phase III studies which led to the licensing of the drugs. 14.5% of the telaprevir-treated patients and 7% of the boceprevir-treated patients discontinued treatment as a result of serious adverse events.” [http://www.aidsmap.com/Telaprevir-and-boceprevir-show-high-rate-of-serious-side-effects-in-hepatitis-C-patients-with-urgent-need-of-treatment/page/2323755/](http://www.aidsmap.com/Telaprevir-and-boceprevir-show-high-rate-of-serious-side-effects-in-hepatitis-C-patients-with-urgent-need-of-treatment/page/2323755/)

**Note:** SVR does not guarantee the absence of relapse. For treatment at 24 weeks the probability of relapse is 40%. Course in 48 weeks reduces the risk of relapse, but does not eliminate it. **It is shown that in 10 of 12 patients with long-persistent viral response was detected viral RNA in liver biopsies.** Hepatitis viruses are deposited in the capillaries of the kidneys, in the bone marrow, adrenal cortex, and mononuclear cells. These depots are a time bomb actions, which can explode at any time. Thus, the gold standard of modern pharmacotherapy HCV is far from ideal.

**Ozone therapy - an effective non drug treatment for chronic hepatitis C**

There are many literature reports of the use of ozone for the treatment of viral hepatitis. Ozonation found to be effective way to disinfect blood virus (18th Annual Meeting of the International Society of Blood Purification ".)
The basic premise of the antiviral effect of ozone therapy is that the extracellular form of the virus is covered with a lipid-fatty shell. (Fig.3) This shell protects the virus, from immune control of organism and plays a crucial role in the mechanism of penetration of the virus into the cell. Lipids and fats are the main targets of ozone. Obviously, the destruction of transport vesicles has antiviral effect.

The second possible mechanism of action of ozone - is the destruction peplomery of virus. Peplomers, the viral glycoproteins protuberances which connect to host cell receptors are likely sites of ozone action. Alteration in peplomer integrity impairs attachment to host cellular membranes.
foiling viral attachment and penetration. Introduction of ozone into the serum portion of whole blood induces the formation of lipid and protein peroxides. While these peroxides are not toxic to the host in quantities produced by ozone therapy, they nevertheless possess oxidizing properties of their own which persist in the bloodstream for several hours. Peroxides created by ozone administration show long-term antiviral effects which serve to further reduce viral load. (Fig.4).

Fig.5

The third possible mechanism - the destruction of the lipid envelop of the virus itself. (Fig.5) The presence of numerous double bonds in these unsaturated molecules makes them vulnerable to the oxidizing effects of ozone which readily donates its oxygen atom and accepts electrons in these redox reactions. Double bonds are thus reconfigured, molecular architecture is disrupted and widespread breakage of the envelope ensues. Deprived of an envelope, virions cannot sustain nor replicate themselves. Ozone proper, and the peroxide compounds it creates, may directly alter structures on the viral envelope which are necessary for attachment to host cells.

All of these mechanisms of action of ozone, inevitably reduces the virulence of viruses. Obviously, that ozone inhibits the virulence of all quasispecies of virus in blood. This gives the possibility immune system to produce antibodies to all quasispecies and thus eliminate the virus. In essence, it is a kind of self-inoculation of the organism.

In addition to the direct effects on viral particles, ozone has immunostimulatory effects: normalizes impaired cellular immunity, accelerates chemotaxis, adhesion and reduces the virus, activates digestion of viruses by phagocytes. Ozone therapy stimulates the production of cytokines (interferons, interleukins), lymphocytes and monocytes.
It looks incredible, but ozone is even able to reduce the already existing level of fibrosis, which is the main cause of worsening the prognosis of hepatitis. As can be seen from the table ozone decreases by almost half had already developed liver fibrosis in laboratory animals.

Hepatitis C (1b). Dynamics of indexes in a current of 2.5 years. A course - six AHT-O3, 4 mg (50 mg/kg/kg)
Ozone really normalizes biochemical indicators of hepatitis. The graph (Fig.7) shows the level of transaminases and bilirubin. the patient was receiving six courses of procedures AHT every 6-7 months. Based on the level of bilirubin, periodic courses of ozone therapy in general, improved the condition of the patient. However, the dynamics of AST and ALT shows periodic deterioration typical for chronic hepatitis. Clearly, one of the main disadvantages of courses AHT-O3 is the small volume of processed blood.

\[
C = C_0 \times \frac{e^{-t \cdot \frac{q}{M}}}{M}
\]

where \(C\) is the initial level of virus copies per ml, \(q\) - flow rate through the filter mL / min, \(t\) - min

\[
V = M \times (1 - \frac{m}{M})^N
\]

where \(M\) – total volume of blood, \(m\) – part of blood for one AHT-O3, \(N\)-number of procedures of AHT-O3

The diagram in the slide (Fig.8) shows that method of the sequential dilution of reduces clearance of blood. Suppose we want to clear 85% of the blood (5 L). In according to the formula for this, we should provide 94 procedures of AHT-O3 in 100 milliliters of blood or 46 procedures in 200 ml. The duration of such a course of 6-12 months. It is clear that continued viral replication will prevent disinfection. EBOO use in blood flow rate of 50 ml / min to solve the same problem for 190 minutes, which is quite acceptable.
The graph shows the relative effectiveness of procedures AHT-O3 and EBOO for the treatment of hepatitis D. Before the course EBOO, the patient received 60 treatments AHT-O3 without visible results. After four extracorporeal treatments (blue arrows) viral load fell sharply. To date, we have only a one-year experience in the treatment of chronic hepatitis C by EBOO RU. The treatment of 11 patients with hepatitis C (genotype 1b), stage of reactivation, hepatitis moderate activity, the degree of fibrosis, F1-F2. In all cases, we have noticed a dramatic and long-term improvement of health of patients. In 5 cases EBOO course led to sustained virologic response. In other cases, 5 months after the end of treatment we received a stationary regime of viral load in range 20000-80000 ME / ml at a moderate (no more than 1.5 fold above normal) level of ALT.
All patients received four treatments EBOO once a week on the background course of rectal insufflation of ozone. In the slide, we show a timeline of protocol of a treatment of patient by name Kirov.

Before the course viral load was 210,000 ME, ALT is normal, AST - one and a half times the norm. All patients were tested using O3Navigator. Program defines the estimated age of the patient according to the psychosomatic testing (Psychotest) and leucocyte count (Leykotest). Typically, the value of calculated age by program Psychotest and Leykotest, for relatively healthy patients, have similar values and estimated are equal to the actual age of the patients. Differences between calculated and real age show pathological processes in the body of the patient.
The patient Kirov (Fig.11) complained of low efficiency, constant fatigue, apathy and subdepressive state of mind. As can be seen from the graph of the initial state of psychosomatic patient was 52 years, with of the real age of 35. Estimate age of the blood of patient <16 years. Such "scissors", mean that the adaptive system the patient is in a state areactivity. This is the indications for the application of ozone therapy. Indeed, after the first procedures EBOO, process begins sharp activation of adaptive system, reducing the tension of adaptive reactions, followed by the return of the estimated age of the patient to the age norm. Subjectively meaningful to patients is a sharp improvement of health - health, appetite, sleep and emotional state.
Fibrosis - a major risk factor for chronic hepatitis. The program has O3Navigator liver panel, which holds the calculation known indicators FORNS, FIB-4, APRI, HALT-C, MDA level of AST, ALT, GGTP, alkaline phosphatase, albumin, platelets, and others. The graph shows, that the course of procedures EBOO RU shifted all of the indicators below the green line, which corresponds to the current entry-level fibrosis equal to F1 and low probability of progression of fibrosis. Figure 12 shows the changes in the above design characteristics during the course EBOO for patient. Dynamics of clinical and biochemical parameters of a patient Kirov is typical of the whole group.
Treatment by EBOO has one unusual feature. After a week or two weeks after the first EBOO treatment occurs, a sharp increase in values PCR test with equally sharp decrease in value by the second or third week. For example, for a patient Kirov after the patient's first treatment EBOO viral load increased from 210,000 to 98 million ME / ml (Fig.13). A week later this level drops to 350,000. After completion of the procedures for 3 months, the virus load spontaneously reduced 800 ME / ml. The gigantic increase in viral load is not accompanied by significant changes in the activity of AST, ALT, GGT, or the balance of oxidative and antioxidant system of the patient.
The dynamics of the PCR test patients treated by EBOO RU

### Fig.14

Dynamics of PCR tests of other primary patients was similar (Fig.14). The exception is the patient N who previously received EBOO procedures for which registered only insignificantly (twice) jump of PCR test. What is the nature of the primary abrupt increase values PCR test?

### Fig.15

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**Western blot analysis**

- **aHCV**
  - Positive: 1.820, 0.124
  - Weakly Positive: 0.336, 0.307

**Spectrum**

- aHCV core
- aHCV S3
- aHCV S4
- aHCV S5

(abSENT)
As the analysis of the spectrum of antibodies to HCV in patients treated with a course EBOO, antibodies to aHCV NS5 was absent. This is evident from the example of the spectrum of patient by name Kirov (Fig.15).

![Image](image1.png)

Maximum immunogenicity in acute and remission has nucleocapsid (core) and nonstructural protein NS3 protein. Minimal immunogenicity have NS4 and NS5 nonstructural proteins.

Anti-HCV IgG antibodies of different specificity are part of the CIC in patients with HCV. Qualitative method of immunoblotting allowed to detect 100% of their participation in the formation of the CIC with NS3 antigens. Frequently (87-90%) was involved in the formation of the CIC IgG specific antibodies to antigens core. The part in the formation CIC of IgG specific NS4 antibody was less pronounced and virtually no immune complexes were constructed on the basis of antibodies to nonstructural proteins of NS5.

Fig.16

Absence of antibodies aHCV NS5 is the visit card of the spectrum of antibodies which was extracted from of circulating immune complexes. We believe that the release of copies of the virus after EBOO is due to desorption and decomposition of circulating immune complexes of vascular and renal depot, just as it was shown in patients suffering from systemic lupus erythematosus Fig.16.
This conclusion is supported by a joint study of the dynamics of the PCR test and the level of circulating immune complexes in the blood (Fig. 17). The graphs show that the increase level of copies of the virus in the blood occurs only if there is a jump in the level of immune complexes.

**Scheme of therapeutic action of ozone therapy of chronic HCV in the version EBOO**

Fig. 18
The mechanism of ozone therapy in the form EBOO presented on the slide. In the period of the replicative form of the disease, circulating immune complexes are formed and deposited in the capillaries and cause toxic effects. After the first procedure, these complexes are desorbed. After the second procedure, most of the damaged viruses destroy by ozone and are eliminated from the blood. The remaining viruses activate the immune cells that produce a corresponding vaccine to hepatitis. At the end of the course in the blood exist only are not functional viral particles, that are removed from the body for a long time. Elimination of immune complexes from the body, probably explains fact of the sharp improvement of health of patients after procedures EBOO.

In general, the treatment method has EBOO efficiency comparable to the effectiveness of treatment with interferon and ribavirin. EBOO treatment method dramatically improves the patient feels, has no side effects and empties depot virus. The latter is the most important. It is promising application EBOO in combination with antiviral agents.