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NOVEL ANTI HIV/AIDS PREPARATION LIPOHIVIR (FTL/AZT/PEBA)

(Short dossier)

Summary.

Lipohivir (FTL/AZT/PEBA) is a liposome preparation for treatment of HIV/AIDS infected people. It is based on the combination of the HIV RT blocker Azydothymidine (AZT) and PEBA (Potent enzyme blocking agent) a transmembrane cell signalling blocker both encapsulated in liposomes. The preparation was evaluated *in vitro*, *in vivo* and a pilot clinical trial was performed on 8 AIDS patients. The obtained up to now results and the advantages over the existing drugs may be summarized as follows:

- Lack of toxicity evaluated on cell cultures, experimental animals and AIDS patients.
- Sixty seven times higher anti-HIV efficacy in comparison with AZT-free, evaluated on HIV infected cells.
- Depot effect over 2 weeks evaluated on HIV infected cell cultures.
- Very good increasing of CD4+ T-cells in patients (up to the normal values).
- Administrative intervals from once weekly to two times every week.
- Convenient rectal administration.
- No need of using low fat and protein food during the treatment as some existing anti HIV/AIDS drugs require.
- Blocking of the polyphosphoinositide transmembrane cell signalling system which is vitally important for HIV replication cycle is a new approach for attacking the virus. See E.E. Gabev et al. Blocking of the polyphosphoinositide transmembrane signalling system is a novel and promising approach for AIDS therapy. European Journal of Parenteral Sciences (UK), volume 7, No 1, p.3-11, 2002. The full text is given at http://www.geocities.com/hivaidsbg/.
- Expressed anti-HIV activity in both the peripheral blood as well as in the reservoir organs gives us grounds to expect eradication of the virus and full cure of the patients.
- Under treatment of AIDS patients with Lipohivir we have obtained for the first time a zero trend linear decay of the viral load (Figure 1). This fitting analysis also serves as an evident that a whole body eradication of the virus is achievable in reasonable time. In contrast to our data the obtained under conventional treatment (HAART) viral load exponential decay with non zero tend (Figure 2) clearly indicates that such an eradication is not possible.

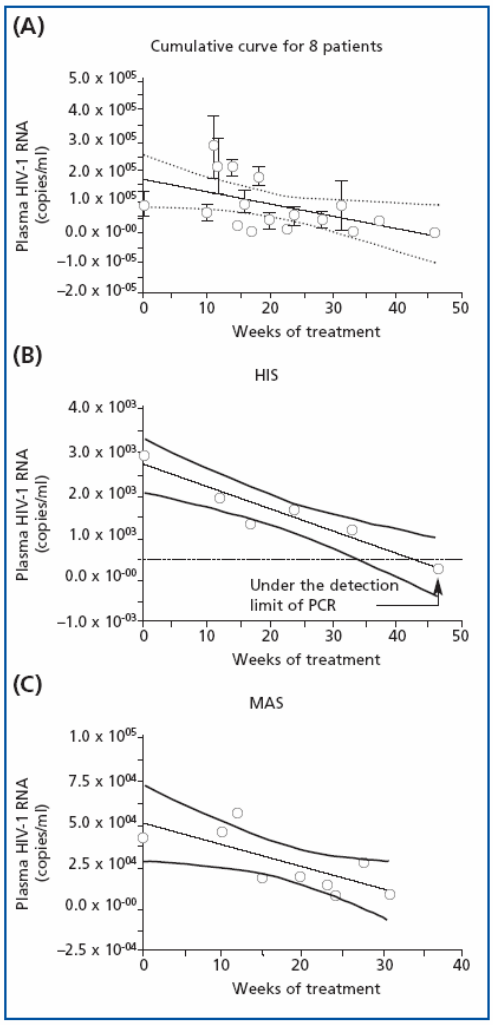

Figure 1. The estimated best fit line (linear regression) of the viral load (VL) decay obtained under treatment with Lipohivir (FTL/AZT/PEBA). (A) Cumulative curve for 8 AIDS patients P =0.041, r^2 = 0.25. The calculated time for obtaining zero VL is 51 weeks and 53 and 83 weeks for four and five orders of magnitude bellow zero respectively. (B) Representative example for patient (HIS) whose VL reached the detection limit of our PCR (500 copies/ml HIV-1 RNA, indicated by horizontal dashed line) within 46 weeks of treatment (P = 0.0041, r^2 = 0.8973). We calculated that 52 weeks are necessary for the patient's VL to reach the mathematical zero and respectively 71 and 244 weeksfor 3 and 4 orders of magnitude bellow zero. (C) Representative example for patient (MAS) whose VL sharply decreased but did not reach the detection limit of our assay within 31 weeks of treatment. We calculated that 38 weeks are necessary to obtain zero VL and respectively 46 and 116 weeks for VL of four and five orders below the mathematical zero. We used Curve Expert version 1.36 (Hyams Development, USA) for preliminary automatic fit with 36 models and interpolation of the viral load with time. We used Prism version 2.01 (20) for further detailed analysis of the best fit linear regression line and estimation of the best fit parameters and calculation of the theoretical end point time necessary to obtain different viral loads. We also used Prism to construct the graphs. Amplicor HIV-1 Monitor test (Roche Diagnostic Systems, USA) was used to measure of HIV-1 RNA copies. From E. E. Gabev et al. Fitting analysis provides further evidence for eradication of HIV/AIDS infection under combined liposomes drug delivery treatment. From: Drug Delivery Systems and Sciences (UK), volume 3, No 2, p.49-51, 2003. (See Selected publications in http://www.geocities.com/hivaidsbg/.

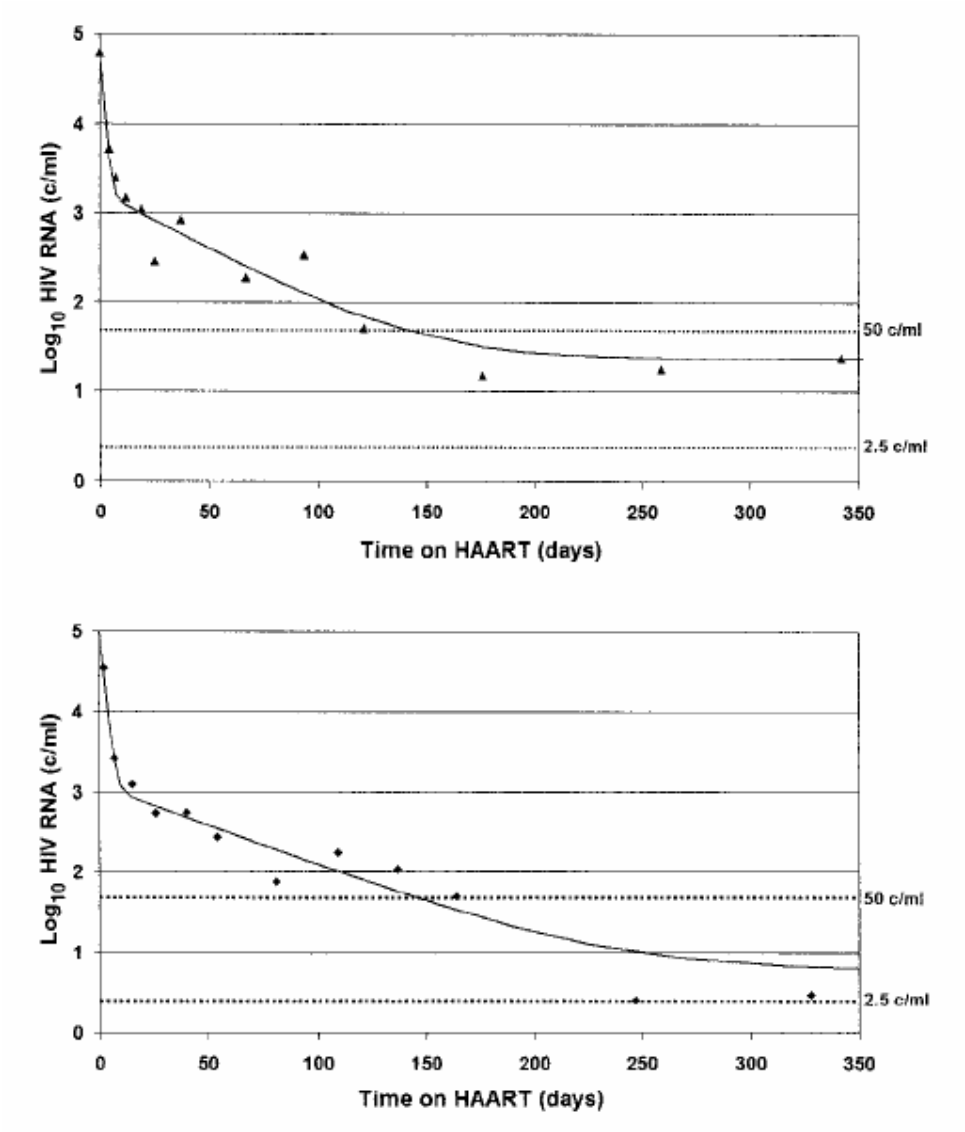

Figure 2. Typical exponential to non zero constant viral load decay obtained under treatment with the conventional therapy (HAART). From Havlir D. et al. Productive Infection Maintains a Dynamic Steady State of Residual Viremia in Human immunodeficiency Virus Type 1-Infected Persons Treated with Suppressive Antiretroviral Therapy for Five Years. Journal of Virology, volume 77, No 20, p.11212-11219, 2003.

Present state of hiv/aids infection and its treatment.

For over 20 years since 1981 the HIV/AIDS pandemic is a prior health problem with demographic, social, economic and even political menace for the World with fatal consequences (Figure 3) and lack of a radical treatment nor effective prevention.

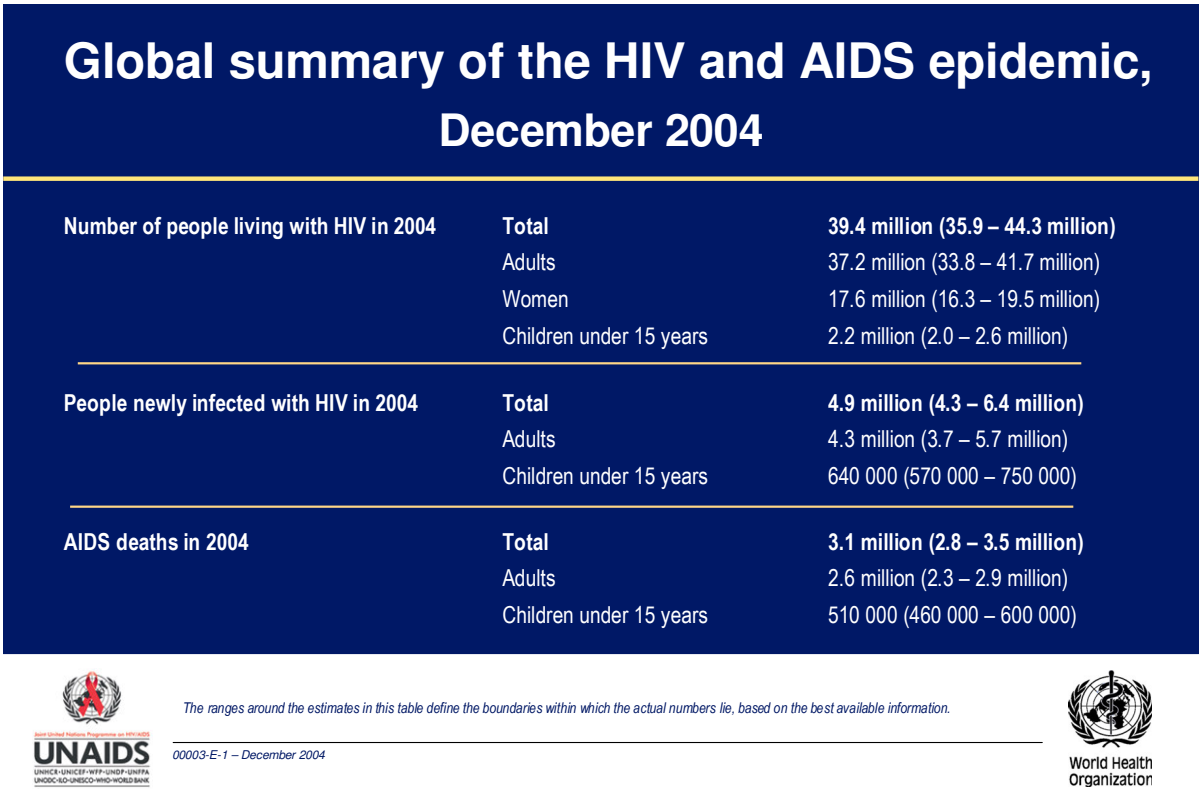

Figure 3. Global summary of the HIV/AIDS epidemic, December, 2004, WHO/UNAIDS:

HIV possesses unsurpassable features as directly destroying the immune system itself and evading treatment by uncontrollable mutation leading to drug multiresistance and in-target-cell DNA latency in various reservoir (sanctuary) organs of the human body. Due to the above characteristics of HIV no infection eradication is achieved until now. Up to date state-of-the-art therapy known as Highly Active Anti Retroviral Therapy (HAART) results only in relative improved quality of life and possible postponing of unavoidable death. The need of treatment of the infected patients for the rest of their lives is obviated by variously expressed drug toxicity and life threatening side effects limiting radical anti retroviral treatment as well as drug non compliance reaction on behalf of the patients.

Characteristics of the preparation.

Contents: The preparation consists of liposome (lipid vesicle) encapsulated combination of the antiretroviral agent Azydothymidine (AZT) and the Potent enzyme blocking agent (PEBA) of the HIV/host cell transmembrane signalling system (liposomized lithium ions).
Production: The preparation was produced at the Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Sofia, Bulgaria (IEPP, BAS) http://www.iepp.bas.bg/. State of the art high pressure Argon gas homogenization combined with in line extrusion technology was used for formation of the liposomes and encapsulation of the active substances.
Mechanism of action: AZT is a typical anti HIV substance destroying the virus by blocking of its vitally important enzyme reverse transcriptase (RT). PEBA is blocking the transmembrane signalling system between the virus host cells and the virus itself thus renders the cell refractive to virus attack and stops further replication of the virus. The role of the liposomes which are not an active drug is to enhance and focus the therapeutic power of the above active substances similar to the concave mirror does with the light beam not being an energy source by itself. Scientific details are given in our publication: E. E. Gabev et al. "Blocking of the polyphosphoinositide transmembrane signalling system is a novel and promising approach for AIDS therapy". European Journal of Parenteral Sciences (UK), 2002, 7 (1):3-11. The paper is also available at: http://www.geocities.com/hivaidsbg/.

Evaluation of the preparation.

In vitro.

Testing on HIV infected MT4 cell culture by quantitating of viral p24antigen (ELISA) gaves 67 fold increase anti HIV efficacy over the AZT free (non liposome encapsulated). No cell toxicity was demonstrated by MTT (Methyltetrazole) viability test. The obtained results undoubtedly illustrates the crucial role of the combination of AZT and PEBA and as well as the role of liposome encapsulation.

In vivo.

Toxicological studies by daily monitoring of experimental animal's condition, gross pathology, histological, electron microscopic and laboratory blood and biochemical tests testified that no short term (1 month, acute) and long term (7 months, chronic) toxic changes due the Lipohivir were observed. Ten and 100 fold doses over the expected human therapeutic one were used throughout the experiment.

Pilot clinical trial.

The pilot trial was carried out on 8 male HIV/AIDS suffering subjects 33 years of mean age (range 22-53), with median initial CD4+ T cells 256 per μl (range 19-518) and mean initial VL (viral load) of 93, 228 HIV-1 RNA copies/ml (range 2942-328, 125). Written Informed

consent from all of the patients and official permission from the National Drug Agency, Central Ethical Commission and Ministry of Health were obtained beforehand. The study was conducted in the State Infectious Diseases Hospital “Professor Ivan Kirov” in Sofia, Bulgaria according to the Good Clinical Practice (GCP). All files and clinical laboratory documentation are stored at the hospital.

At the beginning of the treatment 5 subjects were at A2, 1 at B3 and 2 at C3 stage of AIDS. We used PCR Amplicor HIV-1 Monitor test (Roche Diagnostic Systems, Nutley, New Jersey) for the quantitative measurement of HIV-1 RNA in plasma and Simultest IMK-Lymphocyte kit (Becton Dickinson, San Jose, California) for flow cytometric enumerating the percentage of CD4+ T cells. An absolute CD4+ T cell count was computed thereafter. The white blood cells and lymphocyte percentage from an independent differential white cell count were obtained using standard laboratory procedures. All patients were tested negative for Mycobacterium tuberculosis availability by PCR Amplicor MTB test (Roche Diagnostic Systems, Nutley, New Jersey).

Conclusion.

The preparation FTL/AZT/PEBA (Lipohivir) is not toxic. This is evident from Table 1. No pathological changes of the laboratory indices were obtained. Even improvement of about 80% of them was achieved especially during the second treatment period when the doses were increased. Our maximal dose relative to AZT is about 2,5 times less than the routinely used in the practice. Also no adverse drug reactions nor undesirable side effects were observed. Lack of progression of HIV infection together with considerable improvement of the clinical state of the patients also prove the efficacy of the Lipohivir. The therapeutic effect of the preparation is evident from Fig. 4. The baseline measurements (before treatment) of the viral load (VL) and CD4+ T cell count of our patients (Fig. 4A) showed upward VL and downward CD4+ T cells. This is typical for the progression of the untreated HIV/AIDS. Under the treatment these all-patients trends favorably inverted (Fig. 4B). indicating the positive therapeutic effect of our preparation.

Table 1

Measured clinical laboratory indices. Mean values for 2 treatment periods depending on the total dose received.

Laboratory Indices	Referent values	Units (SI)	Period 1 (1-23 weeks) (*n*)	Period 2 (24-46 weeks) (*n*)	*P*-value
Erythrocytes	4.6-6.2	10^{12}/l	*3.96 (111)	*4.15 (40)	$P < 0.0001$
Hemoglobin	140-180	g/l	133.35 (111)	*134.57 (40)	$P = 0.5811$
Hematocrit	0.40-0.54	l/l	0.39 (111)	0.39 (40)	$P = 0.3285$
Thrombocytes	140-440	10^9/l	179.29 (111)	183.63 (40)	$P = 0.0088$
ESR/Westergren	<11 (15)	mm/h	20.26 (111)	16.27 (40)	$P = 0.0295$
Leucocytes	4-10	10^9/l	5.00 (111)	*5.61 (40)	$P = 0.0012$
Protein	58-80	g/l	82.36 (104)	*79.10 (40)	$P = 0.0001$
SGOT	<22	U/l	19.39 (104)	*17.33 (40)	$P = 0.0613$
SGPT	<22	U/l	25.59 (104)	*25.73 (40)	$P = 0.5580$
Alkaline phosphatase	50-170	U/l	46.65 (104)	*53.70 (40)	$P < 0.00001$
Urea	1.67-8.2	mmol/l	*6.37 (104)	8.62 (40)	$P = 0.0032$
Creatinine	44.2-133.6	μmol/l	*77.46 (104)	*74.10 (39)	$P = 0.003$
Glucose	2.78-5.55	mmol/l	4.56 (104)	*4.80 (40)	$P = 0.0450$
Prothrombin time	80-100	%	87.51 (111)	88.80 (40)	$P = 0.0378$
P_i	0.77-1.36	mmol/l	*1.41 (104)	1.26 (40)	$P < 0.00001$
CPK	<80	U/l	46.12 (104)	*51.83 (40)	$P < 0.00001$
Creatinine clearance	1.3-3.0	ml/s	2.14 (60)	NP	
Bilirubin	3.4-21.00	μmol/l	*17.66 (104)	16.81 (40)	$P < 0.00001$
Cholesterol	3.36-7.76	mmol/l	3.88 (104)	*4.19 (40)	$P < 0.00001$

With the asterisk (*) are marked the mean values of the laboratory indices having Gaussian (normal) distribution in the respective group. In case of normal distribution in both groups, unpaired t test was applied. In case at least in one of the groups the distribution of the respective indices is not normal, then the nonparametric Mann-Whitney test was applied. The Kolmogorov-Smirnov test was used for testing whether the distribution is normal. NP means not performed.

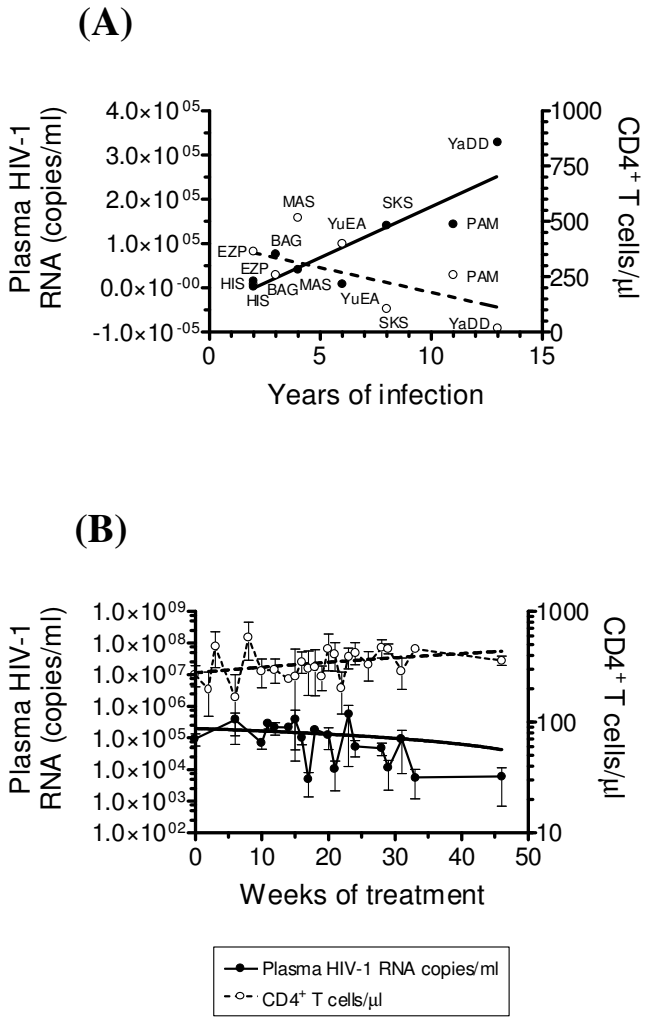

Fig. 4. Measured values of plasma HIV-1 RNA (VL) and CD4+ T cells. (A) Before treatment (baseline values). Y axes are both linear. (B) Mean values during the time course of treatment. Inverting of the trends illustrates the positive therapeutic effect of FTL/AZT/PEBA, i. e. the preparation decreases the VL and increases the CD4+ T cells. Y axes are both $\log_{10}$. From See E.E. Gabev et al. Blocking of the polyphosphoinositide transmembrane signalling system is a novel and promising approach for AIDS therapy. European Journal of Parenteral Sciences (UK), volume 7, No 1, p.3-11, 2002. The full text is given at http://www.geocities.com/hivaidsbg/.

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