HIV Universal Vaccine

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Original Research

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Abstract

Background

For many deadly viruses, there are no preventive and / or therapeutic vaccines approved by health authorities World-wide (e.g., HIV, Ebola, Dengue, and many others). Although, for some viruses, prophylactic vaccines are very effective (e.g., HBV, Polio, Rota, and many others). In this realm, we design, manufacture, test, and streamline into the clinics novel viral universal vaccines (VUV). VUV have such unique features, that medical vaccination or natural infection induced immunity against some viruses (e.g., HBV) in patients, who became infected with other viruses (e.g., HIV), upon the VUV's administration , is redirected against these other, newly infecting viruses (e.g., HIV).

Specific Aim

The specific aim of this work was biomolecular engineering of the HIV universal vaccine (HIVUV) comprising the two main functional domains: CD4 or anti-gp120 - as the HIV tagging domain and HBsAg - as the immune response eliciting domain, so that upon its administration the HBV medical immunization or natural infection induced immunity would be redirected, accelerated, and amplified to fight the HIV infection.

Healthy Donors and Patients

Per the Institutional Review Board approval and in compliance with the Declaration of Helsinki, all healthy donors and patients were presented with the Patients' Bill of Rights and provided Patient Informed Consent. All the procedures were pursued by the licensed medical doctors.

Methods & Results

We have biomolecularly engineered HIV universal vaccine (HIVUV) comprising human CD4 or anti-gp120 and HBsAg of HBV. By immunoblotting and magnetic activated molecular sorting, we have demonstrated high specificity of this vaccine in binding HIV. By flow cytometry and nuclear magnetic resonance, we have demonstrated high efficacy of these vaccines to engage HBV immunized patients' immune system to work against HIV. Administration of HIVUV to blood or lymph of the HIV+ patients resulted in rapid reduction of the HIV viremia down to undetectable. It also resulted in protection of populations of CD4+ cells against HIV caused decline.

Conclusions

We have demonstrated the proof of concept for high efficacy of VUV, specifically HIVUV, in annihilating HIV. Nevertheless, the same compositions, processes, and methods, for persons skilled in biotechnology, pharmacogenomics, and molecular medicine, are adaptable for other deadly viral infections, which we vigorously pursue.

Introduction

Viral infections cause debilitating diseases and deaths World-wide. [1-2]

Prophylactic vaccines approved by health authorities World-wide, including the ones approved by FDA and recommended by CDC in the USA, prevent some of them (e.g., Hepatitis B Virus Vaccine aka HBV Vaccine aka HBVV). [3-6] While there are very effective against conserved strains of viruses, the main problem with these prophylactic vaccines are that new strains emerge which are evading vaccination acquired immunity; thus making those prophylactic vaccines ineffective (e.g., Influenza

Virus Vaccine). Moreover, for many deadly viruses, there are no prophylactic vaccines approved by health authorities at all, that is resulting in very high incidence and mortality due to viral infections (e.g., HIV). For example, nearly 42.9 million people lived infected with HIV, 1.8 million became newly infected, and almost 1 million died due to AIDS in 2016. [6]

The main, currently approved by health authorities strategies for helping patients infected with viruses rely upon chemotherapeutics. The main classes of chemotherapeutics include: virus entry inhibitors (e.g., enfuvirtide or maraviroc), reverse transcriptase inhibitors (e.g., zidovudine or tenofovir), integrase inhibitors (e.g., elvitegravir), or maturation protease inhibitors (e.g., darunavir). They interfere with viruses' propagation mechanisms. The main problems with these methods of therapy include: mutations, mutagenesis, adverse effects, pharmacogenomic profiles, reservoirs. First, chemical small molecules repress the viruses' replication by docking into the domains with enzymes of host cells to lock them. Therefore, any spontaneous mutations in those docking sites make the chemotherapeutics ineffective. Second, nucleoside and nucleotide analogs prevent incorporation or do incorporate false nucleotides, which if erroneously repaired, become the sources of resistance. Third, all chemotherapeutics have very serious, systemic adverse effects. Fourth, the therapeutic efficacy is contingent upon personal response to specific therapeutic cocktails. Fifth, HIV may prevail chemotherapy hidden in the natural reservoirs. [2, 7-13]

The aforementioned problems stimulated ongoing works on immuno-therapeutics. For these the primary consideration is that the first step in the HIV invasion is docking of the HIV gp120 into cells' virus entry receptor (VER): CD4; thus only the viruses with the non-mutated gp120 are capable of docking into the non-mutated CD4 and infect. Mutations in gp120, gp41, as well as CD4, CXCR4, 5 make the infections impossible. [14-15] As such, CD4 recombinants were used for attempts of virus neutralization. [16-17] Anti-gp120 antibodies were tested in similar attempts. [18-19] They were often evaded due to HIV mutations in immunogenic domains, targeting non-docking domains, and poor immune response. Moreover, they might even promote enhanced infections. As those trials were failing, the hybrid molecules comprising of CD4 and IgG were engineered. [20-22] Additionally, the HIV-at-risk-of-infection or HIV-infected patients have compromised ability to develop a de novo immune response. None of those attempts led to an effective therapy to this date. [23-24]

The general objective of our work is to design, manufacture, and test molecules with such features, that their administration to the patients carrying medical-vaccination- or natural-infection-induced immunity against some viruses would immediately redirect, accelerate, and amplify that immunity against the other, different, newly infecting viruses.

While designing our new therapeutics, we were primarily concerned with two elements: securing selective, specific, and reliable docking of the virus, as well as eliciting amplified immune response against it. We did chose HIV as the lead therapeutic target.

Specific aim
The specific aim of this work was biomolecular engineering of the HIV universal vaccine (HIVUV) comprising the two main functional domains: CD4 or anti-gp120 HBsAg and HBV – HBsAg linked by an optional chemical or genomic linker aka junctional domain (JD). That was to be followed by testing of its efficacy in annihilation of HIV.

Patients and Healthy Donors
Per the Institutional Review Board approval and in compliance with the Declaration of Helsinki, all healthy donors and patients were presented with the Patient Bill of Rights and provided Patient Informed Consent with their identities entirely concealed, while all the procedures involving them were pursued by the licensed medical doctors (MDs).

Six healthy volunteers (three women, three men) had no past or present diseases in their medical records, so they were not taking any medications. Results of their routine laboratory tests of blood, with emphasis on erythrocyte sedimentation rates (ESR), immunoglobulin profiles, and of urine, with emphasis on potential presence of cells and proteins, were within laboratory norms.

Three oncology patients (all males) were diagnosed with Kaposi sarcomas. Their blood routine laboratory tests revealed low counts of CD4+ lymphocytes. These results prompted us to test for Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV), Cyto-megalo-virus (CMV), Human Papilloma Virus (HPV). The HIV test came out positive, but the HSV, HPV, and CMV tests came out negative. These patients had not received any therapy yet.

Five oncology patients (three women, two men) were diagnosed Multiple myelomas. Their blood routine laboratory tests revealed high ESR. These results prompted us to test for protein profiles, which revealed high immunoglobulin. Their urine routine laboratory tests revealed protein presence, identified as Bence-Jones protein. These patients were treated by aspiration of pleural and peritoneal effusion as the first therapeutic steps, but had not received prior chemotherapy.

Three oncology patients were diagnosed with Hepatocellular carcinoma and admitted for surgical treatment with intrahepatic artery infusion. Chronic Hepatitis B was in their medical histories. Their blood tests revealed high immunoglobulins. Four oncology patients were diagnosed with Acute or Chronic Hepatitis B, while admitted for other surgical therapy of non-liver cancers.

Materials & Methods
Hepatitis B Vaccine, anti-virus antibodies (AVA), anti-HBV antibodies
The details of generating HBV vaccines and anti-HBsAg,

anti-HBcAg, anti-HBeAg antibodies were published in peer-reviewed articles, that are freely available at PubMed. [25-28]. Briefly, HBsAg was isolated from the patients suffering from Acute Hepatitis B: either from the blood by PEG fractionations or from the liver biopsies by CsCl gradient centrifugation. To assure exact immunogenic compatibility with the immunity induced by vaccinations with the FDA approved HBV vaccine (HBsAg), which were produced in yeast, the HBsAg in this project were also generated in yeast as originally described. Biotechnology of the recombinant HBsAg, as the baits for in vitro selection and evolution of anti-HBV antibodies, was pursued based upon the published DNA coding sequences. [SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3][25-30]

Hepatitis B virus like particles (VLP)
Hepatitis B virus like particles (VLP) were designed based upon the DNA sequences of HBV from GenBank and expressed as described. [SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3] [25-30] The VLP were initially synthesized in yeast— Saccharomyces cerevisiae as originally described. In particular, the expression plasmid pHBS-16 included the HBsAg surface antigen (HBsAg) controlled by the yeast alcohol dehydrogenase (ADHI) promoter introduced through EcoRI restriction sites into the DNA construct of the pBR322 plasmid. That followed by yeast replication origin, yeast trp1 gene. This biotechnology was later modified to be pursued in Pichia pastoris. Briefly, yeast cultures of Pichia pastoris were grown at 30 °C in rich medium (YPD; 1% yeast extract, 2% bactopeptone, 2% glucose) initially and shifted either to synthetic media (YNM, 0.67% yeast nitrogen base supplemented with 0.5% (v/v) methanol) for immunoprecipitation and immunofluorescence experiments, or to mineral media (MMOT, 0.2% (v/v) oleate and 0.02% (v/v) Tween-40) for fractionation studies. Thereafter, we switched to entirely synthetic VLP, which were refolded from high pressure and low temperature. All the protocols' products—HBsAg VLP were referenced and validated to the FDA approved and the CDC recommended Engerix B and Recombivax and the anti-HBV antibody titer assays.

Blood, lymph, plasma, effusion, HIV
Processing, storage, and culture of blood, erythrocyte-free blood, peritoneal or pleural effusions, lymph, and plasma were pursued according to the original, detailed protocols published earlier and only briefly outlined here. [31-34]
　Blood was drawn according to the standard clinical procedure by venipuncture into citric acid / dextran receiving buffer. It was stored at 4 deg. C until further processing. For virus binding assays, the blood was depleted of fibrinogen and calcium (later re-adjusted to physiological levels). Erythrocyte-free (E-), CD3-, CD4- blood was prepared by magnetic apheresis aided by antibodies conjugated with magnetic beads. Plasma was prepared by simple sedimentation and collecting supernatant or alternatively sampled during plasmapheresis. T cell fractions were prepared by activated sheep erythrocyte reseting. B cells were

removed by complement receptor activated lysis. Desired cell fractions were enriched by FACS after labeling with fluorescent antibodies or by magnetic activated cell sorting (MACS) after labeling with superparamagnetic antibodies. Plasma and B cells were isolated from erythrocyte-free blood by MACS after labeling with anti-CD19 and anti-CD20 magnetic antibodies. [25, 29-30].
　Lymph was acquired according to the standard clinical procedure during surgeries on open abdomens. The viruses and cells from lymph were prepared according to the protocols for erythrocyte-free blood. [31-34]
　All the samples were processed on different ways depending upon the research plans: secured for long term storing or immediately upon being drawn.
　For storing, the samples were prepared by cryo-biobanking. The samples were equilibrated with 10% DMSO in the patients' serum at 4 deg C. They were frozen according to the gradual lowering temperature down to -35 deg C, -70 deg C, -196 deg C per 24 h on each step. (Cryo-immobilization were pursued on the cryo-immobilization apparatus, which was constructed and freezing protocols were developed in part thanks to the NSF and NIH Grants to MM). These samples were stored indefinitely without compromised quality. When needed, these samples were thawed according to the reverse-to-freezing protocol for processing. Alternatively, the total RNA was prepared and either stored as such or converted into cDNA for storing and / or shipping. For storing, alternatively, the samples were also retained on the sterile filter papers. [34-37]
　For immediate processing, the HIV+ patients' viremia in blood, lymph, or plasma were tested by reverse transcription and polymerase chain amplification of the sequences nested by the primers designed upon the published HIV-1 sequences in PubMed / GenBank from the samples generated by isolation of total RNA. [25-28, 38-41] [SEQ ID NO: 4; SEQ ID NO: 5; SEQ ID NO: 6]
　The healthy donors' blood, lymph, and plasma served as the controls and processed in parallel to those of the HIV+ patients.
　For research, the samples acquired from the HIV+ patients, whose HIV counts were adjusted to the experimental levels, were spiked with superparamagnetic genomically engineered molecules (GEM-SPM). The infected samples in vials were incubated at the desired time at 37 deg. C and 5 % CO2 while on the gyroscopic tables.
　For immunological test, lysis of the cells was pursued in two buffers: NP40 and RIPA, as outlined in the forthcoming section (IP and IL). In NP40, they were prepared for immunoprecipitation. In RIPA, they were prepared for immunoblotting.
　HIV was aspirated from the peritoneal or pleural effusions of the HIV+ oncology patients, who were diagnosed with Kaposi sarcoma. Of these sterile effusions, 100 microliters containing the HIV copy number determined by q-RT-PCR, was injected into in the H9 culture (ATCC) and propagated strictly according to the published protocols. [33-34]

Human Immunodeficiency Virus gp160, gp120, gp41, p24

HIV gp160, gp120, gp41, p24, were prepared from the effusions of the HIV+ oncology patients admitted primarily for treatment of Kaposi sarcomas either by in vitro expression or collected as soluble from cell cultures. Templates were generated by two ways. Total mRNA was isolated from HIV+ producing CD4+ lymphocytes of the HIV+ patients as described. [27-28] The gp160, gp120, and gp41 mRNA were converted from total mRNA into ds cDNA by reverse transcription and polymerase chain reaction aided by the primers for gp160, gp120, and gp41 having sequences imported from GenBank [SEQ ID NO: 4-6], primers designed on 5Prime (NIH), and synthesized on oligonucleotide synthesizers (Applied Biosystems). The yielded amplicons were inserted into plasmids comprising CMV promoters and metal binding coding sequences or bifunctional linker binding domains as in the details described elsewhere. [25] These plasmids were propagated in Escherichia coli grown in Luria-Bartani media on shakers at 37 deg. C. After isolation of plasmids on Maxipreps (Qiagen), the restriction sites were tested and plasmids cut opened to accept the inserts coding for gp160, gp120, gp41, and p24. The new plasmids (pCMV-MBS-gp160, pCMV-MBS-gp120, pCMV-MBS-gp41. pCMV-MBS-p24) were electroporated into the Myeloma cells in cultures established from effusions of the oncology patients diagnosed with Multiple myelomas. The culture media were based upon RPMI1640 supplemented with the patients' effusion fluids rather than bovine sera. Alternatively, the HIV gp120, gp41, p24 were isolated directly from the patients' effusions or culture media.

Anti-viral antibodies (anti-gp120, anti-gp160, anti-gp41, anti-p24) (AVA)

All the HIV+ patients manifested anti-HIV antibodies, which were detected in their blood and lymph with the FDA approved clinical diagnostic tests. One of these patients manifested also anti-CD4 antibodies validated with OKT4. The coding sequences of these human antibodies were extracted from this patients' plasma and B cells and expressed in human Multiple myeloma cells as described in our detailed protocols published in the peer-reviewed journals and freely available through PubMed. [25-30]

Taking advantage of these laboratory results and access to the human cells, we adopted coding sequences from the plasma and B cells of these patients, while using them for biomolecular engineering of genomically engineered antibodies (AVA), which in the process were also rendered magnetic and / or fluorescent. All the procedures of manufacturing of antibodies were pursued as previously described and briefly outlined here.

The plasma and B cells were selected from blood of HIV+ patients by MACS and FACS using anti-CD19 and anti-CD20 magnetic antibodies. Total mRNA was isolated and stored. After importing the human HC and LC sequences [Kabat], the primers were designed with the aid of 5Prime software [NIH] for heavy and light variable chains and synthesized. After reverse transcription, these primers primers served to create cDNA templates, which were cloned into the plasmid containing CMV promoter and metal binding domains. [25-26, 45-46] Plasmids were propagated in Escherichia coli grown in the Luria-Bartani media in cultures maintained on the shakers at 37 deg. C. After purification on MaxiPreps [Qiagen], the plasmids were electroporated into the cells in cultures of Multiple myelomas, which were established from the effusions of the oncology patients, who were diagnosed with Multiple myelomas. The cells were cultured in RPMI1640 media, but modified on such a way that they were supplemented with the supernatants of the patients' effusions, but not bovine sera. Later, the cells were conditioned to grow in serum-free media in roller bottles at 37 deg C and 5% CO_2. Therefore, supernatant could be easily used to test specificity of secreted antibodies. Metal binding domains (MBS) facilitated rendering them superparamagnetic and / or fluorescent, if MBS were saturated with Eu or Tb. The metal binding domains were chosen to provide strong direct binding to superparamagnetic nanoparticles used for MAMS. [50-51] The covalent bonds were aided through the classical chemistry. [50-51]

Human CD4 – Human Immunodeficiency Virus Entry Receptors (VERs)

Human CD4 was manufactured on two ways: cell lysis - immunoprecipitation - immunoblotting and genomic isolation - amplification - expression. [14-15, 25-28, 47] T lymphocytes from healthy volunteers were initially selected by sheep erythrocyte resetting precipitation followed by B cell complement lysis. Later, these fractions were enriched by CD4+ selection with our anti-CD4 superparamagnetic antibodies, raised from the plasma and B cells of the anti-CD4+ patient, on MACS or our anti-CD4 fluorescent antibodies by FACS.

For immunoprecipitation, the enriched fractions of the CD4+ cells were lysed with NP40. The soup was mixed with our anti-CD4, superparamagnetic, genomically engineered anti-CD4 antibodies, incubated for 1 h at 4 deg. C, and inserted into magnetic field at room temperature for 15 minutes. Diamagnetic content was rinsed off, while in the field. The fraction retained by anti-CD4 was released after the magnetic field ceased. Aliquots of this fraction were electrophoresed on PAGE and transferred onto the PVDF membranes (Amersham). The immunoblots were tested by the standard OKT4 antibody produced by the hybridoma cell line (ATCC), which was initially grown in the recommended cell culture conditions (ATCC), but which we modified so that the cells were grown in sera-free media as described. [27-28]

For immunoblotting, the enriched fractions of the CD4+ cells were lysed with RIPA. The lysates were electrophoresed by PAGE and validated as immunoprecipitated ones.

Alternatively, primarily for future expression of the full CD4 and its selected domains, total mRNA was isolated from these cells and stored. [27-28] The CD4 mRNA main transcripts' sequences were imported from GenBank

(NCBI) [SEQ ID NO: 9-13]. The primers flanking the CD4 coding sequence were designed with the aid of 5Prime software (NIH) and synthesized (Applied Biosystems). These primers guided creating the cDNA templates, which were cloned into the plasmids comprising CMV promoter and metal binding domains, and hetero-bifunctional linker domains linking chelates. [29] The choice of the coding cloning vector was contingent upon the future superparamagnetic molecule to be used with. Propagated plasmids were electroporated into the human Multiple myeloma cells, which were established from the oncology patients diagnose with Multiple myelomas, to express the recombinant CD4. The cells were conditioned to grow in the serum-free RPMI media in roller bottles at 37 deg. C and 5% CO2. Therefore, supernatant could be easily used to test secreted recombinant receptors expressed from all the transcript variant versions by NMR and FCM. [29-30, 45-46]

Separation of the HIV-infected cells from HIV

Added to the patients' blood or lymph, recombinant CD4 or anti-gp120 antibodies with superparamagnetic features not only tag HIV-infected cells through the HIV envelope molecules of budding viruses, but also the viruses themselves. These procedures facilitated apheresis of the all HIV+ fractions: viruses and virus budding cells. Further, separation of the HIV-infected and viral envelope displaying cells from the viruses was performed by spinning the apheresed HIV+ samples at 1000 rpm at room temperature. The supernatant contained the HIV tagged with antibodies, while the HIV-infected and HIV budding cells, but not healthy cells, were collected in the pellet. [52]

Immunoprecipitation

For immunoprecipitation, the enriched fractions of the CD4+ cells were lysed with NP40. The soup was mixed with our anti-CD4, superparamagnetic, genomically engineered antibodies (GEA-SPM), incubated for 1 h at 4 deg. C, and placed into magnetic field for 15 minutes. Diamagnetic content was rinsed off, while in the field. The fraction retained by anti-CD4 GEA-SPM was released after the magnetic field ceased. Aliquots of this fraction were electrophoresed on PAGE and transferred onto the PVDF membranes (Amersham). The immunoblots were tested by the standard OKT4 antibody produced by the cell line were initially grown in the recommended cell culture conditions, which we modified so the cells were grown in sera-free media (ATCC).

Immunoblotting

For immunoblotting, the enriched fractions of the CD4+ cells were lysed with RIPA. The lysates were electrophoresed by PAGE and validated the same way as immune-precipitated ones.

Alternatively, primarily for future expression of the CD4 selected domains, total mRNA was isolated from these cells and stored. [30-32] The CD4 mRNA main transcripts' The sequences were imported from GenBank. [SEQ ID

NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13] The primers flanking the CD4 coding sequence were designed with the aid of the 5Prime software (NIH) and synthesized (Applied Biosystems). These primers served to create cDNA templates, which were cloned into the plasmids comprising CMV promoter, metal binding domains, hetero-bifunctional linker domains linking chelates. The choice of the coding cloning vector was contingent upon the future superparamagnetic molecule to be used with: either solid superparamagnetic or core-shell. Propagated plasmids were electroporated into the human Multiple myeloma cells, which were established from the oncology patients diagnose with Multiple myelomas, to express the recombinant CD4. [25-26, 29-30] The cells were conditioned to grow in the sera-free RPMI media in roller bottles at 37 deg. C and 5% CO2. Therefore, supernatant could be easily used to test secreted recombinant receptors expressed from all the transcript variant versions by NMR and FCM. [25-26, 29-30]

VUV: HIV universal vaccine (HIVUV)

Viral universal vaccines (VUV) comprise the two main domains integrated into a single biomolecule: virus entry receptor (VER) and viral vaccine (VV). In this project, we were focused on manufacturing of the specific VUV: HIV Universal Vaccines. (HIVUV: CD4-HBsAg and anti-gp120-HBsAg were prepared on two ways: chemical conjugation and genetic engineering.

HIVUV by chemical conjugation

Chemical conjugation was initiated from purified components: CD4 and HBsAg. Terminal functional groups of CD4 molecules disclosed herein at 1 mg / ml of physiological buffer were reacted with the linkers disclosed herein particularly SMCC. That was followed by dialysis in PBS with 1000 Da cutoff at 4 deg C. To the buffer containing activated CD4, the buffer containing HBsAg at 1:1 - 1:10 ration was added. The conjugation reaction was completed by dialysis in a physiological buffer to retain HIVUV: CD4–HBsAg, but remove unconjugated reactants. The process resulted in HIVUV in the physiological buffer. HPLC served as the means of separating monomeric HIVUV from potential aggregates, as well as quality control.

Conjugation of anti-Virus Antibodies (AVA) - specifically anti-gp120, with VV - specifically HBV vaccine, was performed on the identical way to create an alternative HIVUV: anti-gp120-HBsAg.

HIVUV by genetic/genomic engineering

Genetic/genomic engineering was initiated with synthesis of the the CD4, polyGly, and HBsAg coding sequences and assembling them into the claimed complete CD4 – HBsAg or anti-gp120-HBsAg coding sequences. The resulting dsDNA was inserted into the plasmid comprising CMV promoter, signal peptide, and metal binding domain. After sealing into cc ds DNA, it was electroporated into Multiple myeloma patients' cells. The myeloma cells were

grown in the serum free media. The pure CD4–HBsAg was recovered through HPLC (Tosoh).

The coding sequences for variable domains of anti-gp120 were assembled into scFv or dcFv, which were then inserted and expressed as disclosed for CD4 above.

Superparamagnetic domains and molecules
Superparamagnetic molecules (SPM) were prepared to comprise solid homogenous or core-shell architecture. The choice of the supermagnets was driven by future applications. [25-26, 29-30, 45-47] The solid superparamagnetic particles were used for in vitro diagnosis and research. The core-shell particles were manufactured for in vivo, in patients therapy. Their inner core provided superparamagnetic properties. The outer layer - shells comprised biologically inert elements to protect the patients from potentially leaking, toxic magnetic material and to offer interfacing layer to link them with antibodies and recombinant receptors.

The solid, homogenous, magnetic nickel and iron metal binding domains / particles were synthesized according to classical protocols adopted in this project. Therefore, the magnetic properties of genomically engineered molecules were gained either by incorporation of superparamagnetic entities into the structure of genomically engineered molecules or by attaching of superparamagnetic particles to the genomically engineered molecules. Chemical reactions involved in linking biomolecules and metallic entities were described in the details. [46-47]

The core-shell particles comprise Fe_3O_4 or Ni cores and Au or SiO_2 shells. They are engineered according to classical protocols adopted in this project. [48-49] Briefly, the cores were synthesized by mixing aquous solutions of $FeCl_3 \times 6H_2O$ / $FeCl_2 \times 4H_2O$ in ½ molar ratio, followed by adding 1m NaOH and stirring initially at room temprerature, that was gradually increased to 90 deg. C for 1h. The process was completed by multiple cycles of rinsing with water. The superparamagnetic particles were then retained by magnets and dispersed in water as ferrofluid. The gradient centrifugation facilitated collecting particles of differenct sizes. The gold shells were prepared according to the modification of the classical Turkevich procedure. [48] The aliquots of the ferrofluid were mixed with 01.% $HAuCl_4$, that was followed by adding 100 mM NH_2OH. The thickness of the shells was determined empirically monitoring time and changes in absorption at 400 nm indicative of depletion of Au. The silica shells were prepared according to the modification of the classical Stober procedure. [49] The solution was added to the aliquots of the ferrofluid and multiple cycles of incubation for 1h at 90 deg. C, that were followed by multiple rinses with water. Covalent attachments were formed by the classical chemistry reactions. [50-51]

The cells were labeled with the superparamagnetic engineered antibodies, as described in details elsewhere. [25-26, 29-30, 45-46] Briefly, the AVA, VER, and VUV were dissolved and all washing steps carried in phenol-free, Ca+ / Mg+ - free, PIPES buffered saline solution, supplemented with 20 mM glucose, 10% human serum. The aliquots were dispensed into the magnetism-free NMR tubes (Shigemi). The relaxation times T1 and T2 were measured in resonance to the applied pulse sequences on the NMR spectrometers: DMX 400 WB or AVANCE II NMR (Bruker, Billerica, MA) or the Signa clinical scanners (General Electric, Milwaukee, WI).

The superparamagnetic AVA, VER, and VUV were used to isolate the labeled molecules and/or cells from the solution. The labeled cells rendered superparamagnetic properties, which facilitated their isolation on the magnetic, activated cell sorter (MACS) operated at 0.47 T – 9.4 T and / or clinical MRI instruments operating at 1.5 T – 3 T and / or NMR scanners operating at 0.47 T- 9.4 T (Bruker, Billerica, MA) (some results were validated thanks to the access to the NSF, NIH and Bruker instrumentation attained by MM).

Flow cytometry (FCM)
Fluorescent, activated cell sorting (FACS)
Multiphoton spectroscopy
The cells were labeled with the fluorescent antibodies as described in details elsewhere. [25-26, 29-30] They were sorted on the Calibur, Vantage SE, or Aria (Becton-Dickinson). The antibodies were dissolved and all washing steps carried in phenol-free, Ca+ / Mg+- free, PIPES buffered saline solution, supplemented with 20 mM glucose, 10% human serum. Sorting was performed on Aria, Calibur, Vantage SE (Becton-Dickinson) with the sheath pressure set at 20 pounds per square inch and low count rate. The sorted batches were analyzed on Calibur or Aria using FACS Diva software or on the FC500 (Beckman-Coulter). For the measurement of the fluorescently labeled cells, these settings were tuned at the maximum emission for the Eu chelated antibody at 500V with references to isotype antibodies and non-labeled cells. This assured the comparisons between populations of cells labeled with multiple antibodies without changing the settings on PMTs.

The fluorescently labeled cells or tissues were imaged with the Axiovert (Zeiss) equipped with the Enterprise argon ion (457 nm, 488 nm, 529 nm lines) and ultraviolet (UV) (364 nm line) lasers; Odyssey XL digital high-sensitivity with instant deconvolution confocal laser scanning imaging system operated up to 240 frames/s (Noran), and the Diaphot (Nikon) equipped with the diode-pumped Nd:YLF solid state laser (1048 nm line) (Microlase).

Nuclear magnetic resonance spectroscopy (NMR)
Magnetic, activated cell sorting (MACS)
Magnetic, activated, molecular sorting (MAMS)
Magnetic apheresis

Energy dispersive X-ray spectroscopy (EDXS)
Electron energy loss spectroscopy (EELS)
X-ray reflection fluorescence spectroscopy (XRFS)
Elemental analyses were pursued by EDXS, EELS, and

XRFS as described earlier. [25-26, 29-30, 46] The field emission, scanning transmission, electron microscope FESTEM HB501 (Vacuum Generators) was equipped with the energy dispersive x-ray spectrometer (EDXS) (Noran) and post-column electron energy loss spectrometer (EELS) (Gatan). The cryo-energy filtering transmission electron microscope 912 Omega was equipped with the omega, in-column, electron energy loss spectrometer (EELS) and the energy dispersive x-ray spectrometer (EDXS) (Zeiss). The cryo-energy filtering transmission electron microscopes 410 and 430 Phillips were equipped with the post-column, electron energy loss spectrometers (EELS) and the energy dispersive x-ray spectrometer (EDXS) (Noran). The field emission, scanning electron microscope SEM1530 (Zeiss) was equipped with the energy dispersive x-ray spectrometer (EDXS) (Noran). The field emission, scanning electron microscope H3400 (Hitachi) was equipped with the energy dispersive x-ray spectrometer (EDXS) (Hitachi). The S2 Picofox XRFS spectrometer was equipped with a molybdenum (Mo) X-ray target and the Peltier cooled Xflash Silicon Drift Detector (Bruker). Scan times ranged up to 1000 seconds. The ICP standard of 1000 mg/l of mono-element Gallium or Gadolinium (CPI International) was added to 500 microL of each sample to the final concentration of 10 mg/l. Instrument control, data collection, and analysis were under the SPECTRA 7 software (Bruker).

Effects of VUV on viral counts and infected-cell counts
To determine the efficacy of our HIVUV on the HIV particle and HIV-infected cell count, the blood or lymph acquired from the HIV+ patients with the HIV-infected cell count adjusted to 1000 cells per milliliter were supplemented with the erythrocyte-free blood of the healthy volunteers, who were previously vaccinated with HBV vaccine and had the anti-HBS titer adjusted above 100 IU, and with HIVUVs comprising: CD4 – HBsAg or anti-gp120-HBsAg. For comparison the blood or lymph of the HIV+ patients were spiked with the erythrocyte-free blood of healthy volunteers, who were vaccinated, but the mix was not spiked with our HIVUV.

To determine the efficacy of our VUV on the HIV count, the blood or lymph acquired from the HIV+ patients with the HIV count adjusted to 100 HIV particles per microliter were supplemented with the erythrocyte-free blood of the healthy volunteers, who were previously vaccinated with HBV vaccine and had the anti-HBS titer adjusted above 100 IU, and with HIVUVs comprising: CD4 – HBsAg or anti-gp120-HBsAg. For comparison the blood or lymph of the HIV+ patients were spiked with the erythrocyte-free blood of healthy volunteers who were vaccinated, but the mix was not spiked with our HIVUV.

Statistical analysis
All the measurements were run at least in triplicates for each sample from each of the patients and each of the healthy volunteers and. The numbers were analyzed and displayed using statistical and image quantification software GraphPad and ImageJ. Data were presented as mean of standard error of the mean (SEM). Statistical significance was calculated by t-test for two groups. To assure statistical significance multiple controls were performed. First, the measurements of the effects of the particular HIVUV were compared with the reading of the HIVUV engineered by introducing mutated CD4 or isotype anti-gp120. Second, the same HIVUV and isotype controls were administered to the HIV+ patient and healthy volunteer vaccinated or not with HBVV. Third, the measurement of every HIV+ patients were conducted in parallel with the HBV+ patient. Fourth, the long term studies were conducted in which HIV and CD4 counts varied with time. Fifth, the measurements were compared when obtained after administration of HIVUV according to different doses and regimens.

Result
Specificity and sensitivity of binding HIV by viral universal vaccine (VUV): Human Immunodeficiency Virus Universal Vaccine (HIVUV)
The first and foremost important feature of any targeted therapeutic is high specificity and sensitivity in marking all the targets and only the targets. We have determined these features for our viral universal vaccines' (VUV): HIVUV. Specifically, HIVUV's: CD4-HBsAg and gp120-HBsAg ability to bind HIV gp120 has been demonstrated in FIG. 1. For all targeting scenarios of testing, we wanted to demonstrate, if there is a formation of complexes between the viruses and virus universal vaccines (VUV). Specifically herein, that was to demonstrate formation of complexes between CD4 of HIVUV: CD4-HBsAg with gp120 of HIV. Similarly, we sought to see, if there is binding of anti-gp120 of HIVUV: anti-gp120-HBsAg with HIV gp120. In both cases, the tests conveyed positive answers. We were asserting absence of CD4+, CD3+ T,soluble CD4, gp120, gp160, gp41, as potential decoys. For these studies, blood of the HIV+ patients, HBV+ patients, healthy volunteers immunized with HBV vaccine, and healthy volunteers not immunized with HBV vaccine was acquired. Blood of these subjects was spiked with HIVUV, followed by non-denaturing lysis to retain potential molecular complexes intact. That was followed by labeling with superparamagnetic anti-gp120 or CD4 for magnetic activated molecule sorting (MAMS). The selection was loaded into acrylamide gels and electrophoresed. This highly selective procedures allowed us to determine that HIVUV, through its CD4 and / or anti-gp120 domain, was pulling out very efficiently HIV from the blood and lymph of the HIV+ patients. It also allowed us to demonstrate the HIVUV high specificity, as no other molecules were pulled out of the lysates, but only the HIV gp120. The lanes had only single bands. These features were critical to assure that no other molecules would be marked for annihilation by the immune system except the ones marked by HIVUV. The lanes running the samples from the HIV- patients, healthy volunteers immunized and non-immunized, as well as of mutated CD4 or isotype anti-gp120 guided HIVUV

appeared empty. These empty lanes were critical in demonstrating absence of risks of potential adverse effects resulting from binding other-than-targeted molecules resulting from non-specific irritation of the immune system of the patients.

Specificity and sensitivity in engaging vaccination induced immunity by Virus Universal Vaccine (VUV): Human Immunodeficiency Virus Universal Vaccine (HIVUV)

The first and foremost important feature of any immunotherapeutic, to be effective, is its ability to elicit new or to engage existing immune response system on a very focused, selective manner, i.e., with absent or at least minimized bystander effect or triggering autoimmune diseases. Antibodies directed against immunogens, which were used for vaccination or were inflicted by natural infection, are the primary responders, which guide effectors. They guide the two main modi operandi in annihilating all insulting microbial: ADCC and CDC. We tested efficacy of HIVUV to engage vaccination induced immunity by flow cytometry of FcR+ cells engaged through anti-HBV antibodies present in abundance in all HBV vaccinated people with sufficiently high titers. For all scenarios of testing efficacy of our viral universal vaccines (VUV), we wanted to demonstrate, if there is a formation of complexes comprising a virus, a redirecting vaccine, an antibody raised through vaccination or natural infection, and the effector cells. In our case, that was to demonstrate formation of complexes between: gp120 of HIV; CD4 of HIVUV: CD4-HBsAg or anti-gp120 of HIVUV: anti-gp120-HBsAg; anti-HBV; and FcR+ of the effector cells. However, these complexes should not be formed with HIVUV engineered with mutated CD4 or isotype anti-gp120 guided HIVUV. The affirmative results have been demonstrated in FIG. 2.

Blood of the HIV positive HIV+ (HIV+), HBV immunized (anti-HBV+), depleted CD4 T, CD3 T, erythrocyte (CD3-, CD4-, E-) patients had administered fluorescent HIVUV:

CD4-HBsAg or anti-gp120-HBsAg versus Human Papilloma Virus Universal Vaccine (HPVUV): anti-HPV16-HBsAg as the controls. That was followed by fluorescent labeling of CD16, CD64, and gp120 versus non-fluorescent antibodies and the isotypes as the controls. Alternatively, FcR+ fractions of cells were tagged with superparamagnetic HIVUV versus non-magnetic HIVUV or non-tagged cells. In this scenario, the changes in relaxivity time rates were determined by NMR. [40, 41] For all scenarios of testing viral vaccines targeting, they have to demonstrate formation of complexes comprising the HIV, HIVUV, anti-HBV, and CD16+ and / or CD64+ effectors.

Administration of VUV: HIVUV: CD4-HBsAg to blood of the HIV+, HBV vaccinated, CD3-, CD4-, E-, patients resulted in very high efficacy of engaging FcR+ effector cells against HIV, as shown (FIG. 2: 1-6). Omission of HIVUV: CD4-HBsAg resulted in lack of engagement of FcR+ by gp120 of HIV through HIVUV. Administration of VUV: HIVUV: anti-gp120-HBsAg to blood of the HIV+, HBV vaccinated, CD3-, CD4-, E-, patients resulted in good efficacy of engaging FcR+ effector cells against HBV as shown (FIG. 2: 7-12). However, quantitatively, this efficacy was not as high as with CD4-HBsAg. Any changes introduced to the design of the HIVUV resulted in a significant reduction down to disappearance of the FcR+ cells' engaging efficacy, as it was the case, when we incorporated mutated version of CD4, isotype of anti-gp120, isotype of anti-HBV, or anti-HPV. Moreover, the labeling was highly specific and efficient, if compared to the healthy donors controls (FIG. 2: 13-14) versus using isotype antibodies anti-CD16, anti-CD64, anti-gp120, anti-HBsAg.

Efficacy of HIVUV in reducing HIV viremia

Efficacy of HIVUV: CD4-HBsAg in reducing HIV viremia is demonstrated in FIG. 3. It was determined based upon quantitative, real time, reverse transcription - polymerase chain reaction (q-RT-RT-PCR) of HIV viremia – HIV counts - in blood of the HIV+ patients with administration

Figure 1. Left pane. Acrylamide electrophoretic gel of immune-pull-outs, which were generated after spiking with HIVUV: CD4-HBsAg (1-5) or anti-gp120-HBsAg (6-9) of the of the HIV+, HBV immunized, patients (2-7), healthy volunteers immunized with HBV vaccine (1) with mutated CD4 (3), mutated HBsAg (4), isotype anti-HBV (5), identical variations for anti-gp120-HBsAg (6-9), isotype anti-gp120 (10), isotype anti-HBV (12). Control ladder in the lane 11. This is the raw image, taken with orange filter, with no processing. The measurements were performed in triplicates on samples from each of the patients and healthy donors listed in the section: Patients and Healthy Donors. The data are statistically representative of all tests performed. Middle pane. Three-dimensional quantification of the bands' density reflecting the amount of material loaded in the lanes presented in the gel in the left pane. Right pane. Quantification of the top densities of the gel depicted in the left pane. In clinical practice, quantification of the bands' densities should facilitate assessment of the efficacy of HIVUV treatment upon the HIV viremia contingent upon the dose and regimen for each treated patient individually.
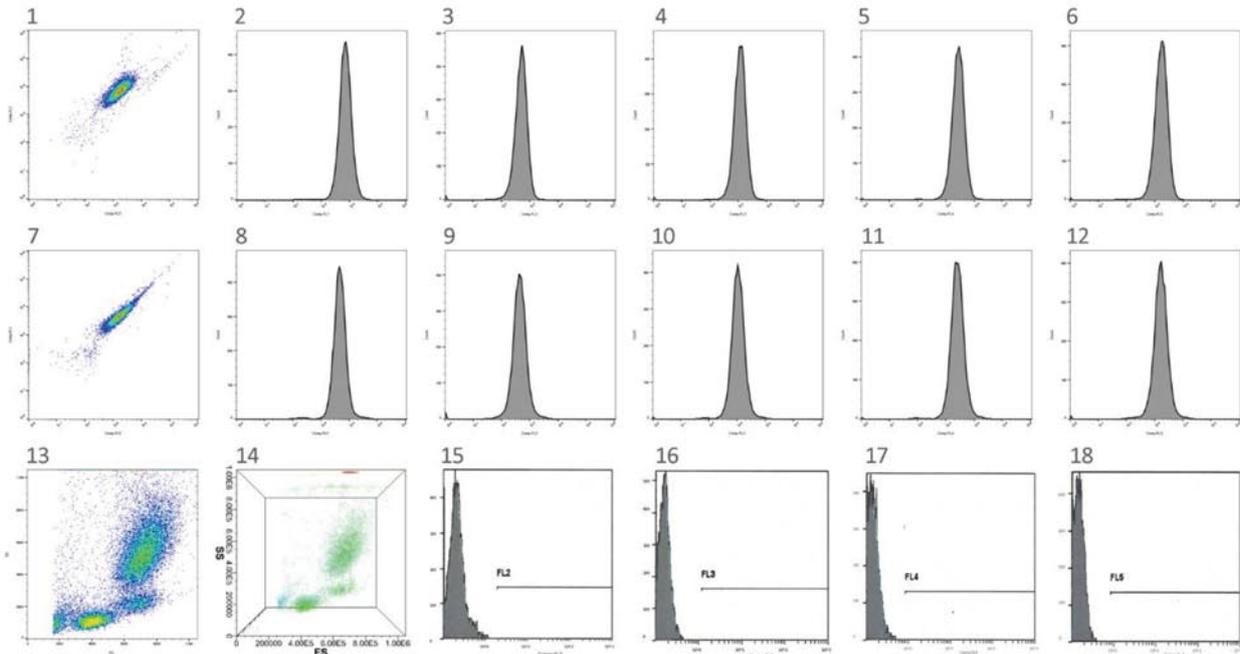
Figure 2. Flow cytometry (FCM) of blood of the HIV+, HBV immunized, CD4+ depleted, patient after having administered HIVUV: CD4-HBsAg (1-6) or HIVUV: anti-gp120-HBsAg (7-12) and the healthy, HBV vaccinated, donor (13-18). Channels: HIVUV: CD-HBsAg (2); HIVUV: anti-gp120-HBsAg (8); HIV (3, 9), anti-HBV (4, 10); anti-CD16 (5, 11); anti-CD64 (6, 12); HIVVUV, FcR+ cells (1, 8); FS / SS (13); FS / SS / CD16 (14); controls: mutated CD4 (15); isotype anti-gp120 (16); isotype anti-HBV (17); isotype anti-CD16. These are raw read-outs, with no gating, and no processing. The measurements were performed in triplicates on samples from each of the patients and healthy donors listed in the section: Patients and Healthy Donors. The data are statistically representative of all tests performed.
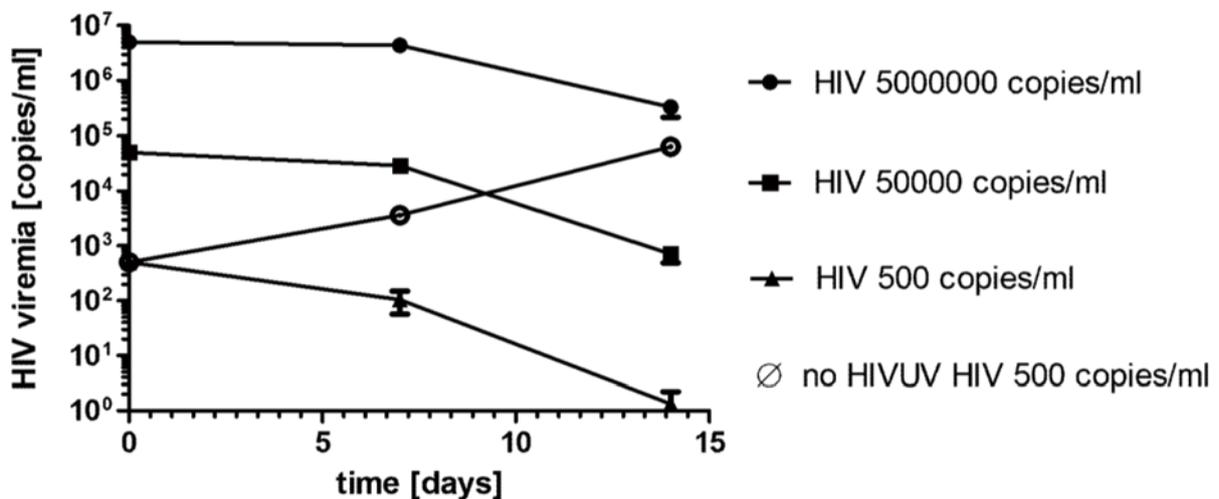

Figure 3. HIVUV aided repression of various levels of HIV viremia in blood of the HIV+ patients over time. Polymerase chain reaction (PCR) - based HIV counts in cultured blood from the HIV+ patient with no treatment versus treatment with our HIVUV: CD4-HBsAg. It demonstrates rapid eradication of HIV by HIVUV, which is contingent upon the initial HIV count; thus gravity of viremia. The measurements were performed in triplicates on samples from each of the patients and healthy donors listed in the section: Patients and Healthy Donors. The data are statistically representative of all tests performed.

of VUV: HIVUV: CD4-HBsAg versus no treatment. It was conducted as the function of time. Importantly, the HIV viremia could be brought down to non-detectable levels contingent upon the initial, low levels of viremia, the dose of administered VUV, and / or the number / frequency of the HIVUV administrations or maintaining constant levels of HIVUV, and assuring the stable levels of the FcR+ effector cells, over the entire tested period of time. Similar effects, but quantitatively lower, were observed upon administration of VUV: anti-gp120-HBsAg.

Efficacy of HIVUV in protecting CD4+ cells

Efficacy of HIVUV in protecting CD4+ cells was assessed by flow cytometry (FCM) and nuclear magnetic resonance (NMR) with the results summarized in FIG. 4. Flow cytometry (FCM) - based CD4+ cells' counts as the function of time in cultured blood from the HIV+ patient with administration of HIVUV: CD4-HBsAg versus no treatment determined at various time intervals. Similar effects, but quantitatively lower, were observed upon administration of VUV: anti-gp120-HBsAg. Infection of blood of the healthy volunteers with HIV, but not treated with HIVUV, resulted in rapid depletion of the CD4+ cells' population in a matter of weeks. Altogether, administration of HIVUV: CD4-HBsAg or anti-gp120-HBsAg very effectively reduced the speed of the CD4+ cell populations' depletion.

Discussion

Herein, we present the compositions, processes of making, and proof of concept for various methods of using for therapy of patients suffering from viral infections: viral universal vaccines (VUV). In principle, VUV comprise the two main functional domains: de novo infecting virus docking domain or antibody directed against viral targeting molecule and microbial, viral or bacterial or yeast, vaccine domain, which are conjugated by an optional joining domain (JD) a bifunctional chemical linker, a peptide, or integrated as fusion proteins by genetic engineering.

Specifically, we provide the composition, processes of making, and methods of utility of VUV for therapy of the HIV infected patients. Specifically, we focus on VUV for HIV, HIVUV: CD4HBsAg and anti-gp120-HBsAg, and methods of using them for therapy of the HIV infected patients, who have been immunized against HBV.

One of the main advantages of VUV is that they rely upon the patients' existing immunity, raised by vaccination or / and natural infection with one type of microbials. This existing immunity is redirected by VUV to instantly respond full force to a new infection. For patients, whose immune system, which was exhausted by a disease and / or rounds of chemotherapy, raising immune response de novo may be difficult. VUV overcome this problem. Moreover, raising immune response to a new vaccine takes time. In cases of VLP, it takes also booster shots. Only after a couple of months, the immune system of a healthy person is capable for switching from IgM to IgG class. Therafter, it may also take time to mount all components of the immune response to assure the effective response to the newly insulting hot virus. Unfortunately, in cases of HIV infections, a couple of months is enough to significantly deplete CD4+ of lymphocytes and practically disable the T helper cells' arm of immunity. It is a race for time between the immune system building a response and the virus rapidly propagating, while effectively dismantling that immune response. In a long run, this leads to Acquired Immunodeficiency Syndromes (AIDS) and deaths primarily due to opportunistic infections and cancers. In this realm, instant engagement by redirecting of the anti-HBV existing immune system remaining idle, on stand-by, addresses the problem, when time is of essence. Moreover, when the prophylactic immunity is on stand-by (e.g., after HBV vaccination with VLP), then upon the HIV infection, redirected response to HBV is rapidly amplified. The HIV viremia, contingent upon HIV counts, is corresponding to gravity of the HIV+ patients' symptoms. Therefore, the magnitude of amplified immune response, as the way to repress viremia, is another great advantage of instantly administered HIVUV, rather than introducing new therapeutic vaccines, while waiting for amplification of
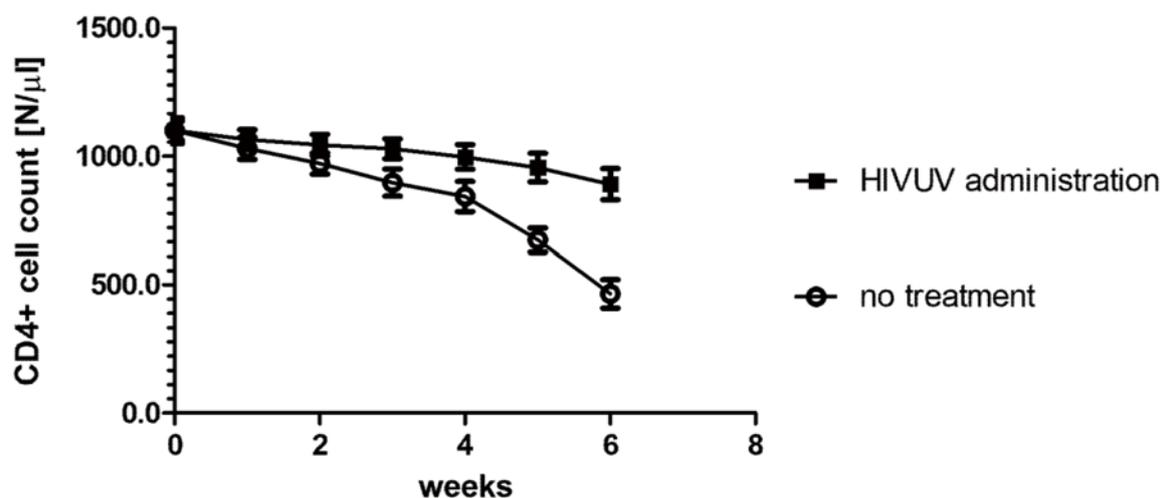

Figure 4. HIVUV aided protection of CD4+ cell populations. Flow cytometry (FCM) was based upon the CD4+ cell counts in cultured blood from the HIV+ patients with no treatment versus treatment with HIVUV: CD4-HBsAg as the function of time. It demonstrates solid protection of the CD4+ cells' population with HIVUV; thus preventing the CD4+ cell population decline due to the HIV induced cytopathic mechanisms. The measurements were performed in triplicates on samples from each of the patients and healthy donors listed in the section: Patients and Healthy Donors. The data are statistically representative of all tests performed.

efficacy of that new vaccine induced immunity.

The HIVUV efficacy relies upon components, which have outstanding record of safety. Moreover, HIVUV do not interrupt any of the elements of the immune response. Therefore, it is unlikely that they would trigger cytokine storms in patients, who tolerated HBV vaccination well. Furthermore, gp120, which is responsible for triggering cytokine storm, is directly neutralized by CD4 domain of HIVUV.

After the initial increase since the infection, the HIV viremia is temporarily reduced. It is also the case upon the administration of chemotherapies. However, the HIV is present in its natural reservoirs, while integrated into the genomic DNA of the infected cells. Nevertheless, progression of the disease is contingent upon cytopathic effects of HIVupon CD4+ cells, that eventually lead to AIDS. As soon as the HIV molecules progress through budding to appear on the surface, they become the targets for HIVUV, which promptly engage ADCC and CDC to eliminate these infected cells. These mechanisms may help in repressing progression of the initial infection into AIDS. Some of the therapeutic efficacy would be reduced by the presence of soluble gp120, becoming practically decoys for HIVUV. Therefore, in order to determine the most effective therapeutic dose of the HIVUV for a particular patient, the total gp120 concentration would have to be measured, while minding that it comprises HIV, HIV producing infected lymphocytes, and soluble components. Whereas, the primary target – HIV would absorb the amount of the administered HIVUV proportional to the HIV count, while each of HIV carries oligomers of gp120 per each of its ~ 72 spikes.

Since VUV rely upon existing immunity, there is no need to introduce new immunogens in the form of prophylactic vaccines, therapeutic vaccines, antibodies, and alike. Every new immunogens (including vaccines, antibodies, cytokines, etc) carries a risk of triggering adverse effects and / or autoimmune diseases. Pharmacogenomic testing may reduce such risks in the future. Meanwhile, relying upon existing vaccines, with well proven safety record, as it is the case with HBV vaccines (Engerix, Recombivax), while redirecting them, as it is the case with our VUV, against newly insulting viruses (e.g., HIV) is currently a far more efficient and safer option for the patients, than introducing new vaccines = immunogenes.

The main problem with biomolecular engineering of VUV against other viruses is identification of virus attachment molecules (VAM) docking them into to-be-infected cells' virus entry receptors (VER) or be neutralized by anti-virus attachment molecule antibodies (AVA). In our case of designing HIVUV, we relied upon gp120 as VAM and CD4 as VER, and anti-gp120 as AVA. Furthermore, we relied upon the safety record of HBV vaccine reported after years of administration VLP (Engerix, Recombivax). Following this novel paradigm, our vigorous effort is focused on identifying such molecules for other deadly viruses and biomolecular engineering VUV against them.

Our work was focused on very precise administration of the doses and regimens to the samples from individual patients, while adjusting very precisely HIV viral counts, CD4+ cell populations, concentrations of gp120 and CD4, and FcR+ effectors. This approach was meant to create the foundations for the large scale clinical trials, in which very specific doses and regimens would be administered to the patients having completed the required laboratory tests. This was focused on maximal personalization therapy. In those large scale studies, we work hard to have the personal pharmacogenomic assessment of individual patients' response performed. Only from those large scale studies, we plan to assess the patient-to-patient variability in response to the HIVUV aided therapy.

We envision streamlining HIVUV into clinics not only as a stand-alone therapy, but also complementing the currently approved chemotherapeutics, which rely upon repressing HIV replication. Administration of HIVUV supporting chemotherapy would not carry the risk of interference with various cocktails as it is the case with some currently approved therapeutic compositions.

We also envision a potential of HIVUV in elimination of HIV and HIV infected cells as enhancing efficacy of regenerative medicine applied to the HIV+ patients. Of particular interest would be attempts to introduce stem cells and CD4+ lineage precursor cells, as a way to replenish population of CD4+ cells, while repressing risks of them being newly infected; thus potentially annihilating therapeutic efficacy of stem cell therapy.

The principle of our approach for treatment of viral infections is that medical vaccination and / or natural infection induced immunity on stand-by against one virus in patients is redirected, accelerated, and amplified against a different, newly infecting viruses, to which the patients' immune system was not prepared. None of the published attempts and / or disclosures proposed and / or aimed at physically redirecting prophylactic immunity raised against one microbial to become a therapeutic immunity against another one. Nevertheless, the same compositions, processes, and methods are obviously and easily adaptable for a person skilled in molecular medicine, pharmacogenomic/pharmacogenetics, and pharmaceutical biotechnology, for all viral infections, which we vigorously pursue within the clinical trials.

Conclusions

We have demonstrated high efficacy of VUV: HIVUV: CD4 – HBsAg and anti-gp120-HBsAg in annihilating HIV through redirecting, accelerating, and amplifying immunity against HBV. Nevertheless, the same compositions, processes, and methods are obviously and easily adaptable for other deadly viral infections, which we vigorously pursue.

Abbreviations

HIV: Human Immunodeficiency Virus; VUV: Virus Universal Vaccine; HIVUV: Human Immunodeficiency Virus Universal Vaccine; HIVV: Human Immunodeficiency Virus Vaccine; HBV: Hepatitis B Virus; HBVV: Hepatitis B Virus Vaccine;

HPV: Human Papilloma Virus; HPVV: Human Papilloma Virus Vaccine; CD4: Cluster of Differentiation 4; gp120: glycoprotein 120; VER: virus entry receptor; AVA: anti-virus attaching molecule antibody; JD – joining domain; AIDS: Acquired Immunodeficiency Syndrome; MAMS: magnetic activated molecule sorting; FAMS: fluorescent activated molecule sorting; qRTPCR: quantitative real time polymerase chain reaction; NMR: nuclear magnetic resonance; FCM: fluorescent cytometry.

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Intellectual Property

All the designs, manufacturing processes, and utility methods are protected at USPTO and WIPO on behalf of Marek Malecki MD PhD and Bianka Saetre MD of PBMEF.

Conflict of interest

None.

References

1.   WHO. Infectious Diseases. http://www.who.int/topics/infectious_diseases/factsheets/en/ downloaded 01Jan2018

2.   Fauci AS; Lane HC. Chapter 226: Human Immunodeficiency Virus Disease: AIDS and Related Disorders. https://accessmedicine.mhmedical.com/content.aspx?bookid=1130&sectionid=79738808 In: Dennis Kasper, Anthony Fauci, Stephen Hauser, Dan Longo, J. Larry Jameson, Joseph Loscalzo eds. Harrison's Internal Medicine. 19th edition. https://accessmedicine.mhmedical.com/book.aspx?bookID=1130 downloaded 01Jan2018

3.   WHO Immunization, Vaccines and Biologicals http://www.who.int/immunization/diseases/en/ downloaded 01Jan2018

4.   CDC. List of Vaccines Used in United States https://www.cdc.gov/vaccines/vpd/vaccines-list.html downloaded 01Jan2018

5.   FDA. Vaccines. https://www.fda.gov/

BiologicsBloodVaccines/Vaccines/ downloaded 01Jan2018

6.   NIH. AIDS. Fact sheet – Preventive vaccine. https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/19/96/what-is-a-preventive-hiv-vaccine-

7.   NIH. AIDS. Fact sheet – Therapeutic vaccine. https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/19/91/what-is-a-therapeutic-hiv-vaccine-downloaded 01Jan2018

8.   Acosta EP, Flexner C. Chapter 58. Antiviral Agents. In: Brunton L. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 12th edition. https://accessmedicine.mhmedical.com/ downloaded 01Jan2018

9.   Flexner C. Chapter 59. Antiretroviral Agents and Treatment of HIV Infection. In: Brunton L. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 12th edition. https://accessmedicine.mhmedical.com/ downloaded 01Jan2018

10.   NIH. AIDS. Fact sheet - Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV https://aidsinfo.nih.gov/drugs downloaded 01Jan2018

11.   NIH. AIDS. Fact sheet - HIV medicines and side effects. https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/22/63/hiv-medicines-and-side-effects downloaded 01Jan2018

12.   Cummins NW, Sainski AM, Natesampillai S, Bren GD, Badley AD. Choice of antiretroviral therapy differentially impacts survival of HIV-infected CD4 T cells. Mol Cell Ther. 2014; 2:1.

13.   Cummins NW, Rizza S, Litzow MR, Hua S, Lee GQ, Einkauf K, Chun TW, Rhame F, Baker JV, Busch MP, Chomont N, Dean PG, Fromentin R, Haase AT, Hampton D, Keating SM, Lada SM, Lee TH, Natesampillai S, Richman DD, Schacker TW, Wietgrefe S, Yu XG, Yao JD, Zeuli J, Lichterfeld M, Badley AD. Extensive virologic and immunologic characterization in an HIV-infected individual following allogeneic stem cell transplant and analytic cessation of antiretroviral therapy: A case study. PLoS Med. 2017 Nov 28;14(11): e1002461.

14.   Dagleish AG, Beverly PCL, Clapham PR, Crawford DH, Greaves MF, Weiss RA. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature 1983; 312:763-765.

15.   Klatzmann D., Champagne E, Charamet S, Gruest J, Guetard D, Hercend T, Gluckman J-C, Montagnier L. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature 1983; 312, 766-767.

16.   Traunecker A1, Lüke W, Karjalainen K. Soluble CD4 molecules neutralize human immunodeficiency virus type 1. Nature 1988; 331(6151):84-6.

17.   Fisher RA1, Bertonis JM, Meier W, Johnson VA, Costopoulos DS, Liu T, Tizard R, Walker BD, Hirsch MS, Schooley RT, et al. HIV infection is blocked in vitro by recombinant soluble CD4. Nature. 1988; 331(6151):76-8.

18.   Bruel T, Guivel-Benhassine F, Amraoui S, Malbec M,

Richard L, Bourdic K, Donahue DA, Lorin V, Casartelli N, Noël N, Lambotte O, Mouquet H, Schwartz O. Elimination of HIV-infected cells by broadly neutralizing antibodies. Nature Communications 2016; 3; 7:10844.

19. Hodges TL, Kahn JO, Kaplan LD, et al. 1991. Phase 1 study of recombinant CD4-Immunoglobulin G therapy of patients with AIDS. Antimrobial Agents and Chemotherapy. 1991; 35 (12): 2580-2586.

20. Hodges TL, Kahn JO, Kaplan LD, et al. 1991. Phase 1 study of recombinant CD4-Immunoglobulin G therapy of patients with AIDS. Antimicrobial Agents and Chemotherapy. 1991; 35 (12): 2580-2586

21. Arthos J, Cicala C, Steenbeke TD, et al. Biochemical characterization of dodecameric CD4-Ig fusion protein. J. Biol. Chem. 2002; 277 (130: 11456-11464.

22. Bosch B, Blanco J, Pauls E., et al. Inhibition of co-receptor – independent cell-to-cell Human Immunodeficiency Virus type 1 transmission by CD4-Immunoglobulin G2 fusion protein. 2005; 49 (10): 4296-4305.

23. Kim JH, Excler JL, Michael NL. Lessons from the RV144 Thai phase III HIV-1 vaccine trial and the search for correlates of protection. Annu Rev Med. 2015; 66:423-37. doi: 10.1146/annurev-med-052912-123749. Epub 2014 Oct 17.

24. Excler JL, Robb ML, Kim JH. Prospects for a globally effective HIV-1 vaccine.Vaccine. 2015;33 Suppl 4: D4-12.

25. Malecki M, Hsu A, Truong L, Sanchez S. Molecular immunolabeling with recombinant single-chain variable fragment (scFv) antibodies designed with metal binding domains. Proc. Natl. Acad. Sci. U. S. A. 2001; 99, 213-219.

26. Malecki M, Szybalski W. Isolation of single, intact chromosomes from single, selected ovarian cancer cells for in situ hybridization and next generation sequencing. Gene. 2012; 493(1): 132-139.

27. Szybalska EH, Szybalski W. Genetics of human cell line. IV. DNA-mediated heritable transformation of a biochemical trait. Proc. Natl. Acad. Sci. U.S.A. 1962; 48:2026-2034.

28. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 1987; 162:156-159.

29. Malecki M, Putzer E, Caroline Quach C, DodivenakaC, Tombokan X. Novel paradigm for immunotherapy of breast cancer by engaging prophylactic immunity against hepatitis B. Clin Trans Med 2016; 5(32):1-12.

30. Malecki M, Sabo C, Foorohar A, Tombokan X. Novel paradigm for immunotherapy of ovary cancer by engaging prophylactic immunity against hepatitis B. Clin Trans Med 2016; 5(44):1-16.

31. Barre-Sinoussi F, Chermann JD, Rey F, et al: Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983, 220:868-871.

32. Gallo RC, Sarin PS, Gelmann EP, et al: Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science 1983, 220:865-868

33. Popovic M, Sarngadharan MG, Read E, et al: Detection, isolation and continuous production of cytopathic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. Science 1984, 224:497-500.

34. Gallo D, Kimpton JS, Dailey PJ. Comparative studies on use of fresh and frozen peripheral blood lymphocyte specimens for isolation of human immunodeficiency virus and effects of cell lysis on isolation efficiency. J Clin Microbiol. 1987; 25(7):1291-4.

35. Yourno J, Conroy J. A novel polymerase chain reaction method for detection of human immunodeficiency virus in dried blood spots on filter paper. J Clin Microbiol. 1992; 30(11):2887-92.

36. van Bueren J, Simpson RA, Jacobs P, Cookson BD. Survival of human immunodeficiency virus in suspension and dried onto surfaces. J Clin Microbiol. 1994 Feb;32(2):571-4.

37. Yourno J. Direct polymerase chain reaction for detection of human immunodeficiency virus in blood spot residues on filter paper after elution of antibodies: an adjunct to serological surveys for estimating vertical transmission rates among human immunodeficiency virus antibody-positive newborns. J Clin Microbiol. 1993; 31(5):1364-1367.

38. Wain-Hobson S, Sonigo P, Danos O, Cole S, Alizon M. Nucleotide sequence of the AIDS virus, LAV. Cell. 1985; 40(1):9-17.

39. Kwok S, Mach DH, Mullis KB, Poiesz B, Ehrlich G, Blair D, Friedman-Kien A, Sninsky JJ. Identification of human immunodeficiency virus sequences by using in vitro enzymatic amplification and oligomer cleavage detection. J. Virol. 1987; 61:1690-1694.

40. Wong-Staal F, Gallo RC, Chang NT, Ghrayeb J, Papas TS, Lautenberger JA, Pearson ML, Petteway SR Jr., Ivanoff L, Baumeister K, Whitehorn EA, Rafalski JA, Doran ER, Josephs SJ, Starcich B, Livak KJ, Patarca R, Haseltine WA, Ratner L. Complete nucleotide sequence of the AIDS virus, HTLV-III Nature 1985; 313 (6000): 277-284.

41. Muesing MA, Smith DH, Cabradilla CD, Benton CV, Lasky LA, Capon DJ. Nucleic acid structure and expression of the human AIDS/lymphadenopathy retrovirus. Nature 1985; 313 (6002): 450-458.

42. Maddon JP, Molineaux SE, Maddon DE, Zimmerman KA, Godfrey M, Alt FW, Chess L, Axel, R. Structure and expression of the human and mouse T4 genes. Proc. Natl. Acad. Sci. U.S.A. 1987; 84:9155-9159.

43. Molday RS, Yen SPS, Rembaum A. Application of magnetic microspheres in labelling and separation of cells. Nature 1977; 268: 437-438.

44. Glazer JK, Malecki M , Grist TM. Targeted MRI Contrast Agent Using Bioengineered scFv Fragments with Gadolinium Labelled Metal Binding Domain. INSRM 2004; 12: 001713

45. Malecki M. HER2/neu gene expression products evaluated with superparamagnetic single chain variable fragment (scFv) antibodies. Proc S Dak Acad

Sci. 2007; 86:297-308

46. Malecki M, L'Vanne K, Truong L, Hsu A, Hogan C, Albrecht R. Supramolecular Imaging of Immunolabeling with STEM EDX & EEL SI Microscopy and Microanalysis; Cambridge 2004; 10(S02): 1216-1217.

47. Söffge F, von Hörsten W. Ac-susceptibility measurements in small fields on fine superparamagnetic nickel particles. Zeitschrift für Physik B Condensed Matter. 1981; 42: 47–55

48. Turkevich J, Stevenson PC,   Hillier J. A study of the nucleation and growth processes in the synthesis of colloidal gold. Discussions of the Faraday Society. 1951; 11: 1-7.

49. Stöber W, Fink A, Bohn E. Controlled growth of monodisperse silica spheres in the micron size range. Journal of Colloid and Interface Science. 1968; 26 (1): 62–69.

50. Krejcarek GE, Tucker KL. Covalent attachment of chelating groups to macromolecules. Biochem Biophys Res Commun. 1977 Jul 25; 77(2):581-5.

51. Meares CF, Goodwin DA, Leung CS, Girgis AY, Silvester DJ, Nunn AD, Lavender PJ. Covalent attachment of metal chelates to proteins: the stability in vivo and in vitro of the conjugate of albumin with a chelate of 111indium. Proc Natl Acad Sci U S A. 1976; 73(11):3803-3806.

52. Malecki M., Saetre B. HIV Apheresis Tags (HIVAT) aided elimination of viremia. Molecular and Cellular Therapies, 2018, 6.

Sequences

SEQ ID NO 1: GenBank: V00866;
SEQ ID NO 2: GenBank: V00867.1;
SEQ ID NO 3: GenBank: X59795.1;
SEQ ID NO 4: GenBank: X01762.1;
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SEQ ID NO 6: GenBank: M27323.1;
SEQ ID NO 7: GenBank: NM_000616.4;
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SEQ ID NO 9: GenBank: NM_001195015.2;
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