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Original article

HIV-specific immunotherapy with DermaVir, the first pDNA/PEIm pathogen-like nanomedicine

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Abstract

Eradication of HIV requires the clearance of latently infected cells that remained in the reservoirs after highly active antiretroviral therapy (HAART). DermaVir is the first nanomedicine that induces long-lasting cytotoxic T cells (CTL) capable to kill these HIV-infected cells. DermaVir is a synthetic "pathogen-like" nanomedicine mimicking the size, shape, surface properties, cellular entry, endosomal escape, and antigen expression features of pathogens (e.g., viruses). We can optimize the biological activity of DermaVir during the manufacturing processes by controlling the physico-chemical properties of the nanoparticles that influence its structure and intracellular mode-of-action. In the clinic, targeted delivery of DermaVir to epidermal Langerhans cells is achieved with the DermaPrep medical device. Three clinical trials consistently demonstrated long-lasting CTL induced by DermaVir in HIV-infected people and killing of HIV-infected cells compared to Placebo. Since HAART and DermaVir are complementary, we envision that their combination might be suitable to achieve the cure: HAART to potent viral load suppression and DermaVir to kill latently infected cells that get activated to produce HIV.

Keywords: eradication; HIV cure; intracellular trafficking; Langerhans cell-targeted delivery; lymph node; plasmid DNA; polyethylenimine (PEI); reservoir; vaccine.

Nanomedicines in the space of HAART

HIV/AIDS is the leading infectious killer affecting more than 33 million people worldwide (1). HIV/AIDS is treated with the combination of three or more antiretroviral drugs (highly active antiretroviral therapy, HAART), because using any single drug has not been efficient in controlling infection due to the development of resistant strains of the virus. Currently, available HAART is potent in suppressing HIV replication and effective in decreasing HIV RNA level below the limit of detection (50 copies/mL) with only minimum side effects. Long-term HAART decreased morbidity and mortality associated with HIV infection (2). However, even optimal HAART, characterized by suppression of viral load to undetectable levels for years, has not provided a cure to the disease. Patients on optimal HAART have 12 years shorter life expectancy than HIV negative people (3, 4). In addition, increased AIDS-related and non-AIDS-related morbidity and mortality has been described in a significant proportion of individuals on optimal HAART due to the lack of normalization of their $CD4^+$ T cell counts (5). Optimal HAART also failed to decrease the viral reservoirs, especially in the gut mucosa, where the residual low-level viral replication may be the cause of persistent immune activation that facilitates the progression to AIDS and death (6). One barrier of cure is the stable latent reservoirs of HIV-infected resting memory T cells that are able to produce HIV after cellular activation. HIV producing cells in the reservoirs that are not eliminated by antiretroviral drugs (ARVs) would be susceptible to immune clearance, but long-term optimal HAART diminishes HIV-specific T cell responses (7). Therefore, the immune system of successfully treated HIV-infected people is unprepared to kill infected cells, decrease viral reservoirs and control the virus replication.

Immunotherapy improves the reactivity of the immune system to guard the body from internal and external intruders as infections (e.g., HIV), allergens and malignancies. The innate arm, composed of the phagocytic cells, circulating macrophages and the complement system, is responsible for the immediate defense. Later, the adaptive arm responds in a highly specific manner against molecular determinants of the intruders. Nanoparticles (NPs) made from different types of materials, having various sizes, shapes and surface charges activate the innate and adaptive immune system (8–10). Therefore, nanotechnology is suitable for improving efficacy and increasing specificity of products indicated as vaccine and immunotherapy. The immunotherapeutic efficacy of NPs is achieved by specific activation (e.g., cancer, infectious diseases) or suppression (e.g., allergy, autoimmune diseases) of the immune system. Efficacy of immune responses is improved by targeted delivery of antigens to professional immune cells specialized for either stimulation or suppression of immune reactivity. This is achieved by using NPs as antigen (virus-like particles; VLPs), NP formulation of soluble antigens (DNA, peptides, proteins) and new administration routes (transdermal, nasal, intratracheal). Increasing the specificity of immunotherapy is achieved by personalization of the antigens in the NP taking into account the diversity of the disease (e.g., tumor cells) and genomic background of the individual (e.g., HLA type).

More than 30 ARVs and drug combinations are currently available to achieve long-term suppression of HIV RNA

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to <50 copies/mL and control HIV disease. The failure to develop a vaccine for HIV has recently changed the treatment strategy from the long-term suppression of viral load to the cure of the disease. Approaches for HAART intensification with additional potent drugs also failed to provide a cure or any additional treatment benefits (4, 11–15). Consequently, it became evident that alternatives to vaccines and to HAART are required to eradicate HIV disease. The first HIV cure approach is eradication of the virus. Eradication was demonstrated in one HIV$^+$ patient after bone marrow transplantation with donor cells resistant to HIV infection (16). Alternative approach for HIV cure is the induction of a long-term remission, similarly to the cure of Hepatitis C infection. We have previously described the remission of an HIV$^+$ patient (Berlin patient, 1999) whose immune system was activated to kill infected cells by short interruptions of HAART (17). This work has led to the identification of "elite controllers" representing a model for remission. Elite controllers have large numbers of cells containing replication competent HIV and their viral load is suppressed by the cellular arm of the immune system (18, 19). Recently, Siliciano and his team have demonstrated that boosting of HIV-specific T cell responses (CTL) prior to reactivating latent HIV will be essential for eradication of the virus (20, 21). These results suggest that HIV-specific immunotherapy is essential for both remission and eradication of HIV, consequently for the cure of HIV.

Immunotherapeutic nanomedicines

Immunotherapeutic nanomedicines are new, complex, multimodular, targeted products that provide superior therapeutic effects compared to all previous vaccine approaches. Their physical size is usually over 50 nm, which is the approximate threshold of immune recognition (22). Soluble antigens, <50 nm in size, are generally not recognized by the immune system consequently poorly immunogenic. In fact, the size range of immunotherapeutic nanomedicines corresponds to the size range of viruses. Nature developed an effective and specific immune surveillance against viruses. Accordingly, triggering the immune system with nanomedicines provides exceptional immunogenicity since our body considers NPs as harmful viruses that need to be eliminated (23). The best examples for the superior immune recognition of nanomedicines are the human papilloma virus (HPV) vaccines, Gardasil and Cervarix. These vaccines are composed from one surface protein (L1) of the HPV that self-assemble to VLPs. These VLPs, morphologically similar to the wild type HPV, induce potent immune responses in the absence of adjuvants. In contrast, the L1 protein purified from bacteria remains a soluble protein, does not assemble to VLPs, and does not induce immune responses (24). These VLP vaccines are safe and protect young uninfected people from cancer. However, none of these vaccines prevent the development of cancer in HPV-infected people, since they unsuccessfully induce therapeutically beneficial T cell responses (25).

Creating "particulate vaccines" has recently been recognized in the HIV field to improve the immunogenicity of small soluble antigens. This approach involves an increase in the physical size of the antigen to the size of pathogens. There are so-called "natural" particulate vaccines, based on VLPs that induce both humoral and cellular immune responses against HIV (26, 27). HIV VLPs are essentially non-infective viruses consisting of self-assembled viral envelope proteins without the accompanying genetic material. A different approach is to use an adjuvant that increases the size of the antigen. One of the several proposed mechanism of aluminum salts, the adjuvant approved in the US and EU, is attributed to their particulate nature, however recently concerns have been raised regarding their safety (27).

A new approach in vaccine development is the use of a plasmid DNA (pDNA) that can express one or more protein antigens in the body. pDNA is attractive for immunotherapeutic nanomedicine development because (i) it has excellent safety profile, (ii) intracellularly expressed antigens are processed and presented on the host MHC molecules, and (iii) recently improved large-scale manufacturing capabilities provide cost effective production. Unfortunately, promising animal studies demonstrating the induction of immune responses with naked DNA injected intramuscularly or intradermally could not be reproduced in human subjects. Possible reasons of the weak immunogenicity is that the naked DNA poorly enters into the cells, does not reach the nucleus, and the expressed soluble protein antigens are not recognized by the immune system, similarly to the previously described soluble L1 protein of the HPV.

Various biodegradable and non-biodegradable polymeric and liposomal delivery systems have been explored for transforming HIV-antigens to synthetic NPs in order to increase their immunogenicity and to protect them against extra- and intracellular degradation (28, 29). Targeting dendritic cells (DCs) that are essential for initiating immune responses, can be achieved by different nanomedicine size; >100 nm nanomedicines target the peripheral immature DCs, and the smaller size ~50 nm nanomedicines drain to the lymph node resident DCs (30). Modification of the surface of the nanomedicine with DC-specific receptor ligands has been shown to increase the targeting specificity (31). However, several challenges including crossing physical barriers like the cell and nuclear membranes or adhesion to non-target tissues still need to be overcome during the development of a synthetic delivery system.

Pathogen-like features of DermaVir nanomedicine

DermaVir is the first synthetic pathogen-like nanomedicine that has been developed for HIV-specific immunotherapy. The active pharmaceutical ingredient (API) is a 12.5 kBp pDNA encoding 15 antigens of the HIV (32). The high number of antigens encoded in the DNAsupport the broadest epitope selection, and the assembly of replication-, transcription- and integration defective "complex virus-like particles" (VLP$^+$s).

DermaVir nanomedicine composed from the pDNA core that is covered by a synthetic polymer, a mannobyosilated polyethylenimine-mannobiose (PEIm) (33, 34). DermaVir NP not only looks like as a virus but also functions as a virus: it enters cells via receptor-mediated endocytosis, escapes from endosome and delivers the pDNA to the nucleus. The pDNA expresses fifteen HIV proteins that self-assemble to a complex VLP$^+$.

The pathogen-like character of DermaVir nanomedicine is based on the structure and function of the NP (Figure 1). It ensures the targeting of antigen presenting cells (APCs) of the immune system and effective expression of antigens. The PEIm polymer that contains covalently linked mannobiose residues outside of the NPscreatesa pathogen-like surface resembling viruses and other pathogens because they usually have sugar-like residues (e.g., glycoproteins) on their surface (35–39). Beside the pathogen-like surface elements, the cationic nature of the PEIm ensures the positive surface charge likewise specific for viruses. PEIm when mixed with the pDNA in solution spontaneously forms 70–300 nm spherical NPs, which resembles the size range of viruses (40, 41). The mannobiose residues not only responsible for the pathogen-like surface but also the pathogen-like entry via receptor-mediated endocytosis. Once entering the cell, the NPs translocate to the endosomes, where the cells degrade 'invaders' by lowering the pH with the use of proton pumps. In the edosomes the polymer envelope protects the pDNA core from the hydrolytic degradation since the PEIm is able to buffer the low pH with its proton sponge effect (34, 42). This way the loosened NPsare released into the cytoplasm and the free pDNA can reach the nucleus (43, 44). After the pDNA enters the nucleus, the encoded antigens can be transcribed and the expressed viral antigens can self-assemble to replication-, reverse transcription- and integration deficient VLP$^+$. These VLP$^+$s and also non-VLP$^+$-associated antigens then stimulate naïve T cells that will further proliferate to HIV-specific CTLs.

Controlling the biological activity of DermaVir during the manufacturing

During the development of DermaVir nanomedicine we explored the structure-activity relationship of the pathogen-like NPs and found that their fine structure determines the biological activity. As shown by atomic force microscopy the different DermaVir formulations have different fine structures; one has smooth, unified surface as envelop, the other has no coherent surface and the pDNA protrudes from the envelop (34). When we investigated the biological activity of these formulations we found that the coherent structured NPs have ~30% higher potency compared to the other one. The difference between the formulations was only the ionic strength and the pH of the nanomedicine solution thus these physico-chemical properties are responsible for the structure of the evolving NPs (34). At low pH and/or high ionic strength loose structure NPs form, because under these circumstances the pDNA and PEIm components have limited interactions. In this case, the biological activity will decrease since the loose NP cannot survive the endosome and the pDNA degrades in the lysosome (Figure 2A). At higher pH the pDNA and PEIm components can form compact NP with smooth surface, where the pDNA is protected from the extra- and intracellular degradation, the NP survives the endosome, and the pDNA is delivered to the nucleus (Figure 2B) (34).

Taking into account that both pDNA and PEIm are polyions explains why the ionic conditions influence their interactions; the dissociation and relative charge density of the components is determined by their ionic environment this way the compactness of the forming NPs also depends on these physico-chemical parameters. By fine-tuning the ionic properties of the formulation we can control the intracellular trafficking and biological activity of the NP: setting the optimal pH and ionic strength of the nanomedicine solution during the manufacturing processes makes us able to control the biological activity of pDNA/PEIm nanomedicines (33, 34).

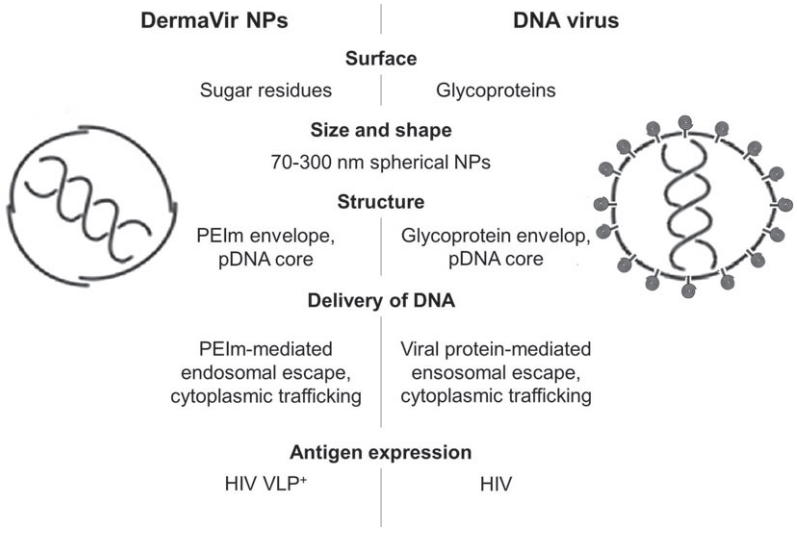

Figure 1 Pathogen-like features of DermaVir nanomedicine compared to a DNA virus.

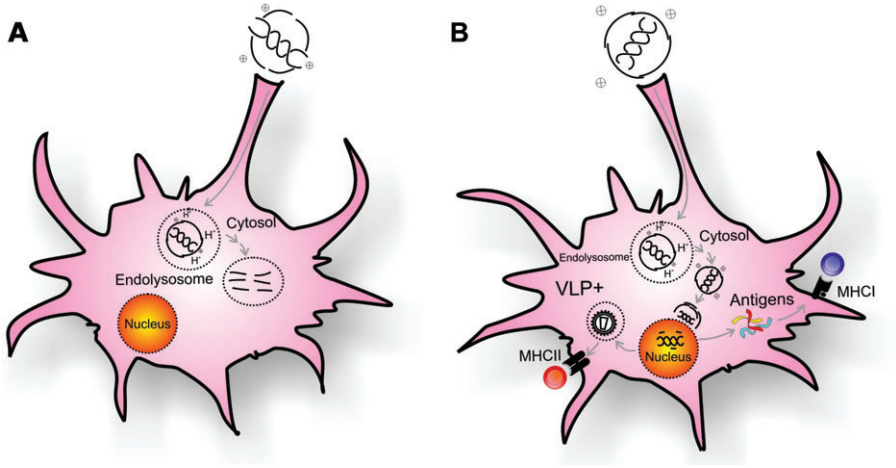

Figure 2 The fine structure of pDNA/PEIm NPs defines the intracellular trafficking and biological activity. (A) Loose structure NPs cannot survive the endosome. (B) Compact NPs survive the endosome and deliver the pDNA to the nucleus.

Langerhans cell-targeting delivery

Optimizing the biophysical properties of a nanomedicine is only one important issue when a product candidate is developed. As DermaVir nanomedicine was designed to target antigen-presenting cells (APCs) and mimic a natural "invasion of pathogens" it was obvious to target the first line defense organ of the body, the skin. Both intradermal injection and skin scarification would target the epidermal Langerhans cells (LCs) but we designed an alternative route of administration to enhance the number of targeted APCs. This CE marked medical device, DermaPrep combines the empty patch technology with a skin preparation method to activate and successfully target nanomedicines into the LCs of the epidermal layer of the skin. Using DermaPrep device various liquid nanomedicine formulation may be administered. The skin preparation method, applied before DermaPrep patch application, is essential (a) to create the "danger signal" for the LCs required to pick up the nanoparticles and migrate to the lymph nodes, and (b) to interrupt the stratum corneumenhancing nanoparticle penetration (45, 46). The area of a patch is 80 cm^2, this way the nanomedicine targets ~8 million LCs (47). Under each patch ~8×10^{12} DermaVir nanoparticles (correspond to 0.1 mg pDNA) are administered in liquid formulation, which means that about 1 million nanoparticles could be picked up by every LC. Activated LCs in the epidermis are looking for pathogens and pick up any "suspicious" nanoparticles such as pathogen-like nanoparticles like DermaVir (48). After taking them up they migrate to the local lymph nodes (49). Here, they express pDNA-encoded antigens and present most HIV epitopes to the passing naïve T cells (50, 51). HIV-specific CTLs primed in the lymph nodes seek to kill the virus-infected target cells.

The most unique property of DermaPrep device is the ability to target millions of LCs by modernizing the method of skin scarification, which is known be used for vaccination against smallpox, one of the very few diseases which were eradicated (52). The nowadays most studied vaccine administration systems such as electroporation and microneedles are able to target only a few mm^2 of the skin which allows them to deliver material to 3 orders of magnitude less APCs compared to DermaPrep (53–56).

DermaVir for the Cure of HIV/AIDS

DermaVir has successfully completed Phase II clinical development, therefore it is the most advanced nanomedicine developed for HIV-specific immunotherapy. A single DermaVir immunization in HIV-infected subjects on fully suppressive HAART demonstrated dose-dependent expansion of long lasting HIV-specific CTL with high proliferation capacity (57). However, frequency of DermaVir-boosted CTL decreased within a year suggesting that repeated DermaVir immunizations are required for durable CTL activity. Consequently, all subsequent clinical trials investigated repeated DermaVir doses. On HIV-infected adults receiving fully suppressive HAART DermaVir was as safe as placebo and potent CTL were induced in the 0.4 mg DNA dose group (58, 59). These results were confirmed in an independent placebo controlled randomized trial demonstrating the excellent safety and superior immunogenicity of the 0.4 mg DNA dose on HIV-infected, treatment naïve adults (60). In this dose group the medium HIV RNA significantly decreased by 70% compared to Placebo suggesting the killing of HIV infected cells. Viral load suppression occurred slowly, as predicted by DermaVir mechanism of action, similarly to cancer vaccines (61).

Based on the presently available preclinical and clinical results we hypothesize that DermaVir immunotherapy will overcome the following limitations of present HAART (Table 1): (i) reconstitution of HIV-specific immune responses that decreased during optimal HAART (ii) depletion of HIV-infected cells in contrast to current HAART that inhibit only one step in HIV life cycle. Compared to HAART the antiviral activity of DermaVir is delayed, slow and less potent, because recognizing and killing of millions of infected cells with CTL

Table 1 Comparison of the features of DermaVir and HAART.

Features	DermaVir immunotherapy	HAART
Therapeutic target	HIV-expressing cells	HIV life cycle
Time needed for effectiveness	Slow and durable	Rapid and transient
Adverse events	Transient, skin	Cumulative, systemic
Administration schedule	Yearly	Daily
Viral load	Slow decrease	Rapid decrease
HIV-specific cytotoxic T cells	Boosting	Decreasing
HIV-infected cells	Eliminated	No effect
Cure	Remission	No

in the presence of millions of new infections take more time to decrease viral load than blocking HIV replication with drugs. The effectiveness of killing infected cells is revealed by their capacity to manage the infection for ca. 15 years in the absence of any treatment. Therefore, 0.5 log reduction of HIV RNA in 24 weeks, demonstrated with DermaVir, should be sufficient to decrease the amount of HIV-infected cells that are not eliminated by HAART.

Cure of HIV from infected patient is defined by the absence of HIV rebound after HAART interruption. The different, complementary mechanism of action of HAART and DermaVir is suitable to achieve cure. The rapid and potent viral load reduction with HAART is essential to block HIV replication and reach undetectable viral load. After that, DermaVir immunotherapy could address what HAART intensification could not achieve; kill HIV-infected cells that remained in reservoirs and boost HIV-specific T cells to reconstitute immune responses. Since the immune system is slow to kill infected cells it will take time to substantially decrease the infected cells from the reservoirs and fully reconstitute HIV-specific immune responses. We envision that repeated DermaVir immune intensification could eliminate significant amount of infected cells from the reservoirs. Consequently, patients could decrease drug exposure and their immune system could maintain a low or undetectable HIV RNA level. Undetectable HIV RNA for 6 months after interruption of HAART could demonstrate cure of HIV similarly to the cure of HCV infection.

Potential advantages of DermaVir immunotherapy compared to HAART include its excellent safety, higher specificity, the longevity of an immune response and likely cost-savings as well as, at least theoretically, the chance to achieve a cure. However, despite decades of research, no therapeutic vaccine has reached the market. Challenges include (i) repeated failures of prophylactic vaccines, (ii) immune escape from T cell recognition based on the high genetic diversity of the virus and the HLA diversity of the host; (iii) the shortage of funding compared to vaccine and drug development. Any treatment that can eradicate HIV from infected patients or cure the disease by remission would have a huge commercial opportunity. Nanotechnology offers opportunities to develop new treatment approaches to the cure of HIV/AIDS. We envision that HIV-specific immunotherapy with synthetic pathogen-like nanomedicines might have the safety, efficacy and cost features to contribute to the cure of HIV/AIDS and significantly improve public health.

Disclosures and conflict of interest

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