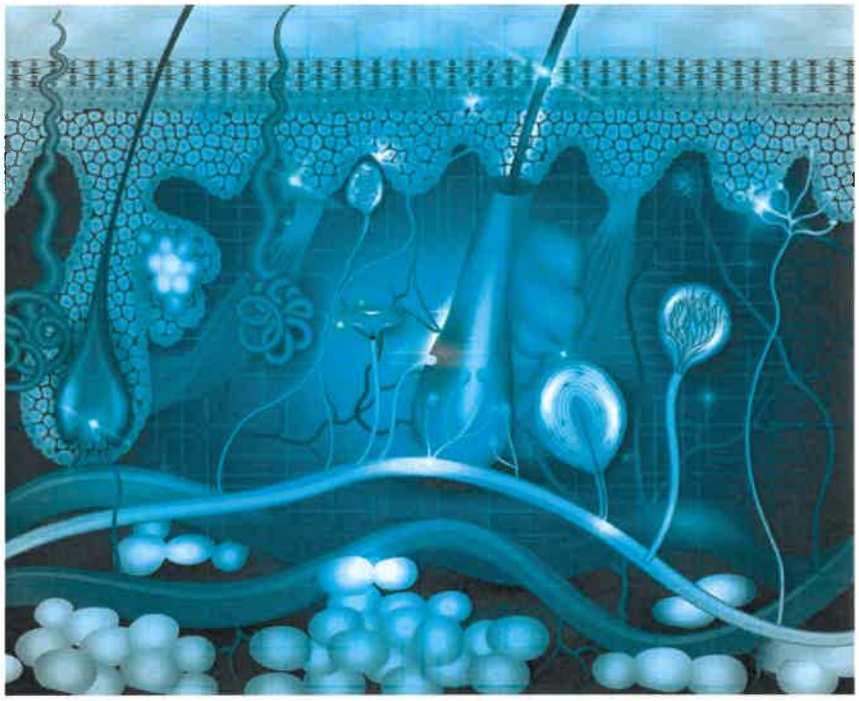

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Optimizing a Unique Cancer Vaccine for Intradermal Delivery

3M Drug Delivery Systems and Panacea Pharmaceuticals are collaborating to deliver an investigational cancer vaccine directly to the dermis using the 3M hollow microstructured transdermal system (hMTS). The hMTS offers high-volume, reproducible, direct delivery capability to the highly vascularized dermis.

STEVEN FULLER, MARK TOMAI, JOHN PRICE, MUCTARR SESAY, AND DAVE CUNNINGHAM

For decades, researchers have had limited success in developing effective vaccines to treat cancer because of the complexities of the immune system. It has become clear that cancer cells have different ways of eluding the immune system, which makes creating a vaccine very challenging. As a result, the greatest level of success to date has been with tumor vaccines that are autologous, meaning that the vaccine is made from killed tumor cells taken from the person in whom the vaccine will later be used.

A group of scientists at Panacea Pharmaceuticals, a clinical-stage biopharmaceutical company developing novel biologically targeted cancer therapies

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Figure 1. Immunohistochemical staining of human aspartyl (asparaginyl) β-hydroxylase (HAAH) in prostate tissue from normal (left panel), benign prostatic hyperplasia (center panel), and prostate cancer (right panel) subjects. Only the prostate cancer tissue shows dark staining using an anti-HAAH monoclonal antibody.

and diagnostics for unmet medical needs, is trying a different method. They noted that human aspartyl (asparaginyl) β-hydroxylase (HAAH) is an enzyme that is normally expressed in fetal development, where it plays a role in cell growth, movement, and cell-cell interaction in tissues during formation. At the time of birth, the gene is silenced and HAAH is internalized into the internal cellular compartments such as the endoplasmic reticulum. However, in cancer tissues of adults, HAAH is overexpressed and translocated to the tumor cell surface (see **Figure 1**). This surface localization is uniquely associated with cancer and is related to cancer cell growth, cell motility, and invasiveness. HAAH is prevalent in more than 20 different tumor types, but is not recognized by the immune system due to immune tolerance (1–5). However, a fully human monoclonal antibody to HAAH has been shown to be promising as an imaging agent and possible therapy for metastatic breast cancer (6). Therefore, it was determined that a vaccine incorporating an antibody to HAAH could be designed to specifically target the tumor cells.

PAN-301-1 is a *de novo*-engineered vaccine candidate that presents an N-terminal portion of the HAAH protein as a fusion protein with the gpD antigen on the surface of the bacteriophage lambda. This construct presents HAAH in a manner that is unfamiliar to the body, thereby overcoming self-tolerance. The bacteriophage component functions as an immune stimulator nanoparticle that contains DNA fragments that present the phage CpG motif to activate the major histocompatibility complex (MHC) class II pathway, along with hundreds of copies of an HAAH fragment on the surface of the nanoparticle. *In-vivo* testing has shown that this vaccine is highly immunogenic; it produces an HAAH-specific antibody response and significantly stimulates immune cells to target HAAH. The PAN-301-1 vaccine is easy to produce and has proven to be safe in animal toxicology testing.

DELIVERING THE CANCER VACCINE

The relative instability of biologic protein- and peptide-based molecules mean that biopharmaceuticals are nearly universally incompatible with oral, inhalation, or traditional transdermal delivery technologies. Therefore, biologics used to treat chronic diseases are mostly administered by intramuscular (IM)/subcutaneous injections, or via an infusion that takes up a considerable amount of time (7). Unfortunately, these delivery options can cause pain, deep tissue trauma, and anxiety, which consequently, has a negative impact on patient adherence to their medications. Patients dread monthly injections. Approximately 10% of patients are needle phobic, and an even higher percentage of individuals have noted that their dislike for needles was the reason for foregoing medical treatment and discontinuing therapy (8). Not only are there issues surrounding sharps disposal and the risk of accidental needle stick injuries, but conventional syringes also minimize the potential for patients to be able to self-medicate at home.

Furthermore, the production of biopharmaceuticals is resource-intensive, and as a result, biopharmaceuticals are more costly than traditional chemical pharmaceuticals. Efficiency from production through to delivery to the patient is, therefore, crucial (9).

Given that the PAN-301-1 vaccine is a biologic that cannot be administered orally, or via a transdermal patch, it was thought that intradermal delivery to the skin could potentially be the optimal route of administration. The skin is rich in dendritic cells, which are required for initiating an immune response.

Active transport strategies are being developed that use microneedle technology to transport drugs

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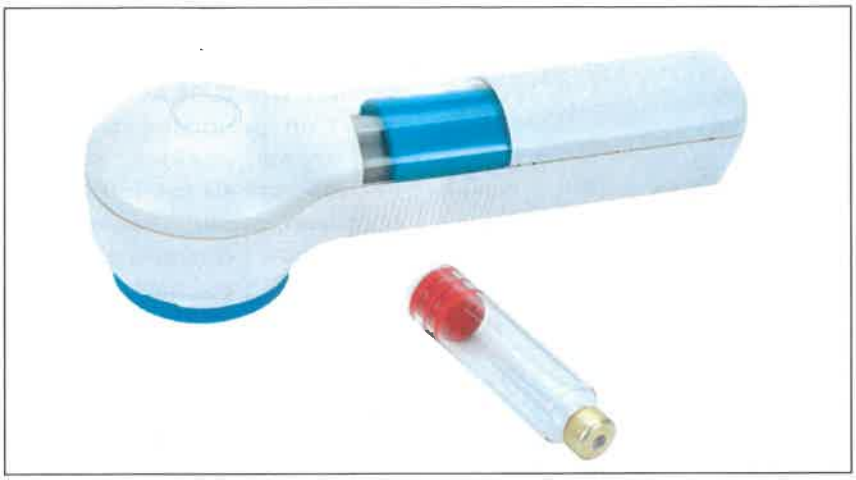
Figure 2. 3M hollow microstructured transdermal system (hMTS).



through the outer stratum corneum to the dermis. These smaller needles result in a more patient-friendly administration method. However, most commercially viable devices that provide intradermal delivery of liquid formulation remain confined to relatively small volumes, typically 200 µL or less.

TARGETING DELIVERY TO THE DERMIS

Intradermal delivery enables faster transport of the macromolecule to the systemic circulation through the highly vascularized intradermal tissue rather than the denser subcutaneous tissue. In addition, the lymphatic capillaries, abundant in the dermis, may be the primary source of uptake within the intradermal space. The lymphatic capillaries are larger than the adjacent blood vessels and are designed for rapid uptake and transport of large molecules. Importantly, for the efficacy of a nanoparticle vaccine, the dermis contains large numbers of dendritic cells (DCs) which are antigen-presenting cells (10). These DCs capture and process antigens for presentation to T cells so that the body can mount an effective immune response.

3M Drug Delivery Systems and Panacea Pharmaceuticals are collaborating to deliver Panacea's investigational cancer vaccine, PAN-301-1, directly to the dermis via the 3M hollow microstructured transdermal system (hMTS). The hMTS (see **Figure 2**) offers high-volume, reproducible, direct delivery capability to the highly vascularized and DC-containing dermis.

The drug candidate is delivered as a high-volume (up to 2 mL), minutes long injection via the hMTS, not requiring time-consuming and uncomfortable infusions, while greatly facilitating the ease of use and patient acceptance. The device applies the microstructures into the skin to a depth sufficient to penetrate the stratum corneum and epidermis and create direct access to the dermis. Delivery of the liquid formulation through the microstructures is powered by a spring. Further, the delivery surface area is spread across a 1 cm^2 array versus a single point of entry provided by a syringe.

With this cancer vaccine, one needs to deliver a 1-mL volume of the vaccine into the dermis. The hMTS has the ability to deliver formulations of up to 2 mL with viscosities up to 20 centipoise (cp) with specialized options for delivery of up to 80 cp for development purposes. Viscous formulations are more prone to plugging in the narrow channels of traditional single channel devices and require a long delivery time.

The 3M hMTS is the device constituent of a drug–device combination product. Because the drug constituent (i.e., the vaccine) is the primary mode of action, human studies using 3M hMTS are registered with FDA through Panacea's investigational new drug application with cross reference to 3M's device master file.

FILL/FINISH OF A BIOLOGIC

Aseptic fill, perhaps the most critical step in the manufacturing of biopharmaceuticals, has been associated with the highest possible risk because of the complex interaction between personnel, product, equipment, cleanrooms, and sterilized fill components during that phase of cGMP manufacturing. In fact, the greatest source of microbial contamination during the process comes from the personnel and his/her activities. Further, it is critical to maintain the integrity of the protein. Preserving the three-dimensional structural integrity of the protein, maintaining aseptic conditions, and being able to distinguish between inherent protein properties and extrinsic particulate defects upon visual inspection are essential for success. As a result, aseptic fill/finish is the subject of focused scrutiny by regulatory agencies.

Panacea Pharmaceuticals selected biopharmaceutical contract development and manufacturing organization (CDMO) Goodwin Biotechnology to perform aseptic filling of its cancer vaccine. For this project, two cGMP batches of 200 and one batch of 400 3M hMTS glass injector cartridges were filled under cGMP conditions for the clinical trials, which required two operators—one to perform the filling tasks and the other to

Figure 3. Custom aseptic filling and vial stoppering of a specialized product in Goodwin Biotechnology's ISO-5 cleanroom.

secure the closure of each container in a controlled International Organization for Standardization (ISO) 5 cleanroom environment (see **Figure 3**). Because this represented a new procedure, filling process qualification had to be performed prior to the actual cGMP filling operation. For this small-scale operation, the cartridges were filled by hand using a syringe and needle and a cap was hand crimped for the top closure. For future batches at a larger scale, the cartridges will be filled using a calibrated pumping system.

Once filled, the vaccine cartridges are kept refrigerated until they are ready to be used. They are inserted into the 3M hMTS device at the time of administration to the patient.

PRECLINICAL STUDIES HAVE SHOWN PROMISE

In passive immunotherapy studies, monoclonal antibodies to HAAH inhibited *in-vitro* tumor cell growth, motility, and invasiveness; they also inhibited *in-vivo* tumor growth in mouse xenograft models of cancer. Preclinical studies have shown a better immune response and good tolerability with the hMTS when compared to IM dosing of the vaccine.

The PAN-301-1 vaccine enables the immune system to generate polyclonal antibodies against HAAH and stimulates a cellular immune response that results in enhanced inhibition of tumor cell function and efficacy. It has been used in several animal models (mouse and rat) of liver, breast, and prostate cancers. In one study, the cancer vaccine inhibited tumor growth in liver, breast, and prostate cancers by more than 90%, reduced metastases by more than 80%, and increased survival from 12.5% to 100% (11).

The vaccine overcomes the self-tolerance to HAAH by the presentation of the altered HAAH antigen (the fusion proteins using HAAH fragments) on a solid phase and the immunomodulatory effect of the bacteriophage itself in recruiting dendritic cells to this antigen.

Following delivery using the 3M hMTS device, a red, array-sized blotch and a small wheal or dome was observed immediately after removal, but it faded to be almost indistinguishable within 10 minutes after delivery. The wheal was resolved to the touch after approximately 40 minutes post infusion. Further, the dome did not "leak" under gentle pressure.

MOVING INTO CLINICAL TRIALS

Based on promising data from the animal studies, Panacea Pharmaceuticals has initiated an open-label, parallel designed, multicenter Phase I clinical trial to evaluate the safety and immunogenicity of PAN-301-1 for the treatment of persistent prostate cancer. Initiation of the Phase I study for PAN-301-1 serves as a starting point for using HAAH as treatment to prevent the recurrence of cancer. PAN-301-1 will be administered at a microgram level through an intradermal injection in cohorts of patients with biochemically relapsed prostate cancer, using a fixed dose-escalation scheme at every 21 days to establish the recommended dose that will be used in Phase II studies. There is also an opportunity to extend the Phase I study at the same dose with approximately 18 patients being enrolled and studied.

As of this writing, three patients have been enrolled in the Phase I study and have had a total of 12 immunizations with zero adverse events and zero injection site issues.

FUTURE PLANS FOR A UNIQUE CANCER VACCINE AND DELIVERY SYSTEM

Future plans for the delivery device would allow the patient to self-administer a drug or cancer vaccine at home and avoid the trip to the doctor's office or an outpatient facility for an injection or infusion. This approach has the potential to enhance patient convenience, reduce patient anxiety, and reduce healthcare costs.

The future for this unique cancer vaccine is linked to the expression characteristics of HAAH. Given that HAAH is expressed on the cell

surface of more than 20 different tumor types, Panacea has interest in further study of this vaccine in Phase I/II clinical trials in breast, colon, ovarian, and bone cancers.

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Viral Vectors —*Contin. from page 30*

Typical affinity chromatography optimizations in terms of binding, washing, and elution conditions can be applied when utilizing AAV9 affinity resin. For example, in terms of binding considerations, for equilibration standard neutral buffers (pH 6–8) such as 10–50 mM sodium phosphate or Tris can be used. Elution conditions differ because the target molecules differ in their binding/elution behavior. When eluting most target molecules, however, reducing the pH to the range of pH 2–3 is generally successful. Other elution buffer components that can be used include phosphate, hydrochloric acid, glycine, acetate, or other components that buffer well at low pH. Other additives such as 2M magnesium chloride ($MgCl_2$) or 50% propylene glycol may be useful.

Lastly, the study results showed that when using the AAV9 affinity resin in the capture step during vector purification, a satisfactory vector recovery of ≥70% is obtained. **Figure 4b** shows that viral vector recovery is reproducible at different scales as process scale-up occurs 20-fold. Genethon also noted that viral vector purification processes are simplified when using affinity chromatography, increasing product yield from 20–60% and reducing cost by a factor of six over alternative methods. The utilization of an immunoaffinity column frequently requires only a single capture step and then a concentration step, significantly simplifying a purification process. Fewer unit operations means higher product yield obtained thereby enabling faster time to market, while helping reduce cost of goods. This is crucial because the industry is focusing on developing industrial capabilities to produce viral vectors in large amounts to meet clinical and future market demand.

CONCLUSION

Gene therapy shows great potential to treat a variety of diseases, and the industry is working to establish efficient commercial manufacturing capabilities for these unique therapies.

The methodology described above offers the following:

- One-step AAV purification from crude material with high purity and yield
- High specificity and capacity, reducing the process volume significantly for subsequent steps and maximizing yield
- Basis of platform purification (reproducible)
- Robust with less process optimization.

Affinity chromatography is set to have a significant impact on increasing process productivity and enabling the industry to meet market needs. The utilization of immunoaffinity columns will be an important improvement to downstream processing of viral vectors. A case study demonstrated that the columns can reduce the purification steps, maximize productivity, and offer the scalability and processing consistency needed for the production of clinical-grade gene therapy molecules.

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