Delivering on the Potential of DNA Vaccines

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Drew Hannaman is a co-inventor of the TriGrid™ electroporation technology and, during his seven years as the Vice President of Research & Development for Ichor Medical Systems, has been responsible for guiding development of the TriGrid Delivery System for intramuscular delivery (TDS-IM) from initial concept into clinical testing for multiple indications. He holds a degree in Cybernetics from the University of California, Los Angeles, and has over 12 years' experience in the development of novel medical technologies, specialising in device-based delivery systems for drugs and biologics.

Introduction

It was first established in animal models over 15 years ago that the *in vivo* administration of DNA sequences expressing immunogenic proteins had the capacity to elicit antigen-specific immune responses in the recipient. These observations provided the foundation for the investigation of a novel immunisation modality known as DNA vaccination. DNA vaccines are unique in that their biological activity is derived through the expression of antigen within the recipients' own cells without the use of a live vector. This approach offers several potential advantages when compared to conventional immunisation strategies using vaccines comprised of whole pathogens or isolated pathogen subunits. Of perhaps greatest significance, DNA vaccines can elicit potent cellular and humoral immune responses without the safety concerns associated with conventional vaccine technologies (including the use of live vector vaccines and/or immune modulating adjuvants). In addition, the sustained, endogenous antigen expression achievable with DNA vaccines offers the theoretical possibility of inducing potent, long-lasting immunity with a minimal number of immunisations. Finally, DNA vaccines are also relatively simple to manufacture and exhibit a good stability profile, facilitating transport and storage.

Taken together, these characteristics suggest that DNA immunisation has the capacity to provide a robust platform for the rapid, cost-effective development of vaccines that will be capable of preventing and/or treating a wide range of diseases. Compelling data with DNA vaccines in animal models have prompted the initiation of clinical trials in a wide range of therapeutic and prophylactic indications including cancer, infectious diseases, autoimmune disorders and allergy. The recent regulatory approval of three different DNA vaccines used in veterinary indications (two in 2005 and one in 2007) is an important step forward for the field. The impact of the technology on human health has been limited, however, which primarily reflects its inability to elicit target levels of immune response consistently when administered in humans.

DNA vaccines must be taken up into the cell, and thereafter the cell nucleus, in order to initiate production of the antigen of interest. Consequently, the inability of conventional administration methods to achieve efficient and consistent intracellular DNA delivery is recognised as an important factor contributing to suboptimal potency. Technologies capable of enhancing intracellular DNA uptake and antigen expression are therefore positioned to help realise the substantial, untapped promise of DNA vaccines.

Device-based approaches to DNA vaccine delivery have proved to be among the most promising methods of administration. Ballistic, particle-mediated delivery (the 'gene gun'), needle-free jet injection and electric field application ('electroporation') have each demonstrated the capacity to potentiate DNA vaccine delivery compared to conventional injection alone. These promising results suggest that device technology will play an important role in the clinical development of DNA vaccines.

Electroporation-mediated DNA Vaccine Delivery

Electroporation (EP) is a technique for intracellular delivery based on the brief application of electrical signals to target cells. Exposure of target cells to electrical fields of sufficient magnitude and duration can transiently destabilise their cell membranes. During this state, substances present in the extracellular environment that cannot efficiently cross the cell membrane on their own (e.g. DNA) can be taken up inside the cells at high levels. At the conclusion of EP application, cell membrane integrity is rapidly re-established, and the cells resume normal function (subject to the activity of the transferred material).

While EP was initially employed to enhance *in vitro* DNA transfection in cell culture, the discovery that it could also be effectively applied *in vivo* has led to its adaptation for DNA delivery in a wide variety of tissue including skeletal muscle, liver, brain, blood vessel, lung, skin and tumour.

EP has proved to be a particularly potent method for DNA delivery in tissues relevant to DNA immunisation (skeletal muscle and skin). EP-induced increases in DNA

expression of a hundredfold or more compared to conventional injection have been observed in a wide range of animal models with commensurate increases in immune response. Figures 1 and 2 provide examples of the protein expression levels (*Figure 1*) and antibody responses (*Figure 2*) that are observed following the intramuscular administration of DNA encoding expression of a reporter gene (human-secreted alkaline phosphatase) in rabbits. The dramatic increases in delivery efficiency achievable with EP, coupled with the findings from initial clinical studies which demonstrated that DNA immunisation with conventional administration methods exhibited suboptimal potency and consistency, have prompted widespread interest in the technology for DNA vaccine delivery.

The basic elements of the EP administration procedure are depicted in Figure 3 (A–C). They comprise: (A) applying the device and distributing DNA (blue) in the target tissue, and (B) propagating EP-inducing electrical fields (green) at the site of DNA distribution, which results in efficient uptake in the local tissue (C). Although DNA administration to muscle and skin prior to EP is most commonly accomplished using conventional needle injection, needle-free and topical administration methods are also feasible. The EP effect is induced by contacting the tissue with an array of two or more conductive electrodes, oriented such that the electrical fields are propagated at the site of DNA distribution. A wide range of EP parameters (including signal waveform, amplitude, number, duration and frequency) have been shown to induce effective delivery in muscle and skin. Since the application of EP has been

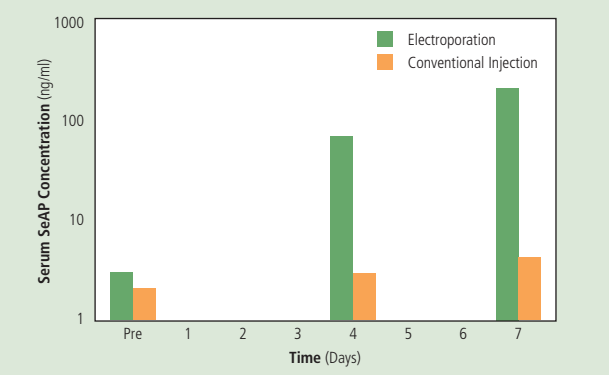

Figure 1 – Antigen expression levels following intramuscular administration of DNA in rabbits.

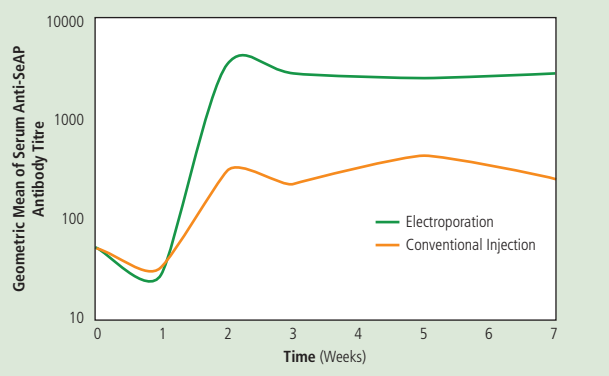

Figure 2 – Immune response following intramuscular administration of DNA in rabbits.

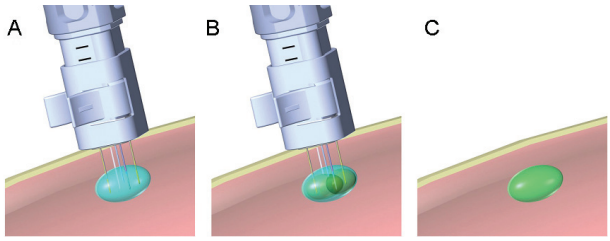

Figure 3 – Sequence of EP-mediated DNA delivery.

associated with acute discomfort during the EP delivery, administration conditions that can minimise the duration and intensity of this stimulation will be favoured for clinical development.

Used by research groups worldwide, these procedures have demonstrated dramatic enhancement in the magnitude of cellular and humoral immune responses across a broad spectrum of immunogens, including antigens associated with tumours as well as bacterial and viral infections. When compared to conventional administration of DNA vaccines, improvements in potency equivalent to a hundredfold increase in DNA dose have been routinely observed. EP has also been associated with increased breadth of immune response, a faster onset of immunity, and a reduction in the number of immunisations required to induce response. Because it provides a robust, adaptable method for increasing DNA vaccine delivery in multiple tissue types, EP is well positioned to address the suboptimal potency observed with DNA vaccines administered by conventional methods.

Device Technology for EP Administration in the Clinical Setting

The increased intracellular DNA delivery that is characteristic of EP occurs only in those tissues where the vaccine is present during propagation of the EP-inducing electrical fields. The key technical objective for clinical implementation of EP is therefore the development of devices and administration procedures that allow for the consistent induction of the EP effect at the site of DNA distribution, even when applied in heterogeneous subject populations. Widespread commercial adoption of the technology will also depend on the development of EP devices that are capable of applying the procedure in a manner evocative of conventional injection procedures (especially when contemplating prophylactic indications). Towards this end, devices should be simple to use and well tolerated by subject populations, permit rapid administration, and achieve consistent results with minimal operator training.

Although several early-phase clinical studies of EP-mediated DNA immunisation are under way with technology relying on manual control over DNA administration and electrode placement, this approach requires extensive operator training and is still likely to exhibit a high degree of variability from subject to subject. The device development efforts under way at leading EP technology firms – including **Inovio Biomedical**

Corporation (www.inovio.com), **Ichor Medical Systems, Inc.** (www.ichorms.com), **VGX Pharmaceuticals, Inc.** (www.vgxp.com) and **Cyto Pulse Sciences, Inc.** (www.cytopulse.com) – demonstrate an emerging consensus that clinical implementation of EP is best achieved using device technologies that integrate the means for agent administration and EP application into a single apparatus. Moreover, the implementation of automated control over key device functions is likely to reduce operator training requirements further and facilitate consistent delivery across subjects and operators. Finally, procedures capable of simple and rapid administration are likely to be essential for the widespread commercial acceptance of the technology.

The first integrated, fully automated EP device to enter clinical testing was the intramuscular TriGrid™ Delivery System (TDS-IM) developed by Ichor Medical Systems. Two clinical studies of the TDS-IM are currently under way. These include administration of a melanoma vaccine in patients at high risk for recurrent disease as well as a study in healthy subjects to compare EP delivery and conventional intramuscular injection of a HIV vaccine candidate.

The TDS-IM comprises three components (*Figure 4*). An electronic Pulse Stimulator controls the administration sequence and generates the EP-inducing electrical signals. The Integrated Applicator is a reusable hand-held device that provides the user with independent control over electrode placement as well as the site and rate of agent administration. This facilitates consistent application of EP in the tissues at the site of DNA distribution. The Application Cartridge is a sterile, single-use component that houses the DNA to be administered and a TriGrid electrode array for EP application. It is the only component of the TDS-IM that contacts the subject, which reduces the risk of cross-contamination. Visualisation and exposure to sharps is minimised by the use of deployable electrodes and injection needle coupled with an automated stick shield that engages at the conclusion of the procedure. A sliding depth gauge allows for consistent intramuscular

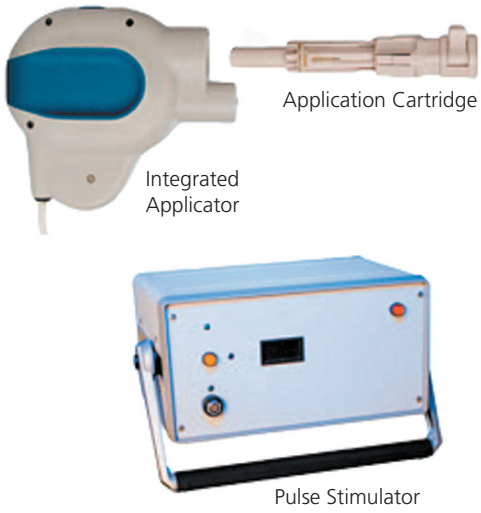

Figure 4 – The TDS-IM administration device.

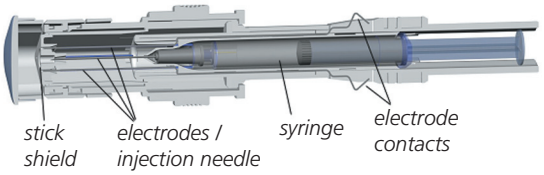

Figure 5 – Cutaway view of a TDS-IM administration cartridge.

injection in subjects with varying subcutaneous tissue thickness. Non-conductive insulation on the proximal portions of the TriGrid array minimises the propagation of electric fields in tissues outside of the administration site. The array comprises four electrodes arranged in two interlocking triangles (hence the name TriGrid) arranged around a central injection needle. By orienting the long axis of the electrode array in alignment with the myofibres in the target muscle, the electrical fields propagated within the array correspond to the ellipsoid distribution pattern that is characteristic of an intramuscular DNA injection. The efficient propagation of electrical fields enables the EP effect to be induced with stimulation conditions that last for less than half a second. In order to provide dosage flexibility during early-phase clinical studies, the Application Cartridges are currently configured to accommodate a standard syringe (*Figure 5*). To facilitate the eventual commercial implementation of the technology, development of device configurations accommodating pre-filled syringes and single-dose cartridges is now under way.

Overall, the integrated, automated design of the TDS-IM permits reproducible delivery to be achieved with a procedure that requires only a few seconds to complete. In light of the progress with the TDS-IM, the basic principles of the TDS technology are now being adapted for use in devices for other routes of administration, including intradermal delivery (the TDS-ID).

Conclusions

Despite their considerable promise, the suboptimal potency of DNA vaccines delivered using conventional administration techniques has, to date, minimised their clinical impact. EP has been demonstrated as a potent method of delivery in animal models that appears well suited for DNA vaccine applications. EP technology developers have made substantial progress towards the provision of devices that are capable of effective and reproducible DNA vaccine delivery in the clinical setting. With a number of these devices now in clinical testing and others expected to enter testing shortly, the field is well positioned for an initial assessment of the safety and effectiveness of the technology in humans. In parallel, additional refinements in device technology are expected to broaden the range of applications for EP and set the stage for the introduction of devices suitable to support product commercialisation.

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Taking on the challenge of disruptive lipid technology

LiPlasome Pharma is a Danish biotech company focusing on the development and commercialization of a targeted drug delivery platform using lipid technology.

LiPlasome Pharma has taken on the challenge of disruptive lipid technology and is currently entering into phase one with a chemotherapeutic platform. The company aims to have several other lipid formulated active pharmaceutical formulations as well as prodrugs in the pipeline within the next couple of years.

LiPlasome Pharma's drug delivery platform consists of:

- A lipid-based drug delivery system (LiPlasomes) for the intravenous transportation of anticancer drugs and prodrugs.
- An activating release mechanism ensuring that the prodrugs and drugs are released specifically at the target site. Examples include prostatic, pancreatic, colorectal, gastric and breast tumors.

LiPlasome Pharma is convinced that Drug Delivery Systems (DDS) will gain increasing importance over the coming years, bringing relief to severely ill patients in future.

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