EXPERT REVIEWS

Cutaneous delivery of prophylactic and therapeutic vaccines: historical perspective and future outlook

Expert Rev. Vaccines 7(9), 1329–1339 (2008)

John A Mikszta† and Philippe E Laurent
†*Author for correspondence*
BD Technologies, 21 Davis Drive, Research Triangle Park, NC 27709, USA
Tel.: +1 919 597 6158
Fax: +1 919 597 6402
john_mikszta@bd.com

The skin has long been recognized as an attractive target for vaccine administration. A number of clinical studies have tested the epidermal and dermal routes of delivery using a variety of vaccines over the years. In many cases, cutaneous administration has been associated with immunological benefits, such as the induction of greater immune responses compared with those elicited by conventional routes of delivery. Furthermore, there is a growing body of evidence to suggest that such benefits may be particularly important for certain higher-risk populations, such as the elderly, the immunocompromised and cancer patients. Despite the potential advantages of vaccination via the skin, results have sometimes been conflicting and the full benefits of this approach have not been fully realized, partly due to the lack of delivery devices that accurately and reproducibly administer vaccines to the skin. The 5-year outlook, however, appears quite promising as new cutaneous delivery systems advance through clinical trials and become available for more widespread clinical and commercial use.

Keywords: cutaneous • immune compromised • intradermal • microneedle • skin • vaccine

Targeting the skin to protect against infectious diseases has been practiced for many centuries. The first well-documented application dates from the 16th Century when variola virus, the causative agent of smallpox, was punctured into the skin to protect against later exposure to the virus [1]. The term 'vaccination' was coined as a result of Edward Jenner's demonstration in 1796 that cutaneous administration of the related, but much safer, vaccinia virus could also protect against smallpox [1]. Over the intervening centuries, a number of vaccines have been administered to the skin using a variety of instruments, ranging from crude to sophisticated [2]. Furthermore, advances in the field of immunology have led to an increased understanding of the basic mechanisms of innate and adaptive immunity and have identified the skin as an attractive site for vaccination, largely due to the presence of a dense network of immune-stimulatory antigen-presenting cells and lymphatic drainage networks [3–6]. This article reviews the results of published clinical vaccine trials in which the intradermal route has been investigated and provides a future outlook based on the coming availability of new delivery systems specifically designed for vaccine administration to the skin. Specific emphasis is placed on the potential of cutaneous delivery to overcome current limitations in vaccine potency in certain higher-risk subject populations, such as the elderly, the immunocompromised and cancer patients.

Classical intradermal delivery

The route of delivery for most vaccines is intramuscular. This is due more to the widespread availability of long needles and syringes that easily access the muscle tissue than to any compelling scientific or medical evidence to suggest that the muscle is an ideal tissue from an immunologic point of view. Although the skin is now known to be a potent immune-stimulatory tissue, it has not been exploited to its full extent for vaccination due to the lack of readily available delivery systems to easily and reproducibly target the tissue. The most widely used method of cutaneous delivery to date employs conventional needles and syringes according to a technique invented

 10.1586/14760584.7.9.1329 ISSN 1476-0584

by Mendel and Mantoux in the early 1900s [2]. This method, now referred to as the 'Mantoux technique,' is accomplished by inserting a 26- or 27-gauge (Ga) needle into the skin at a slight angle with the bevel pointed upwards. The needle is pushed into the dermis until the bevel is completely covered, after which the fluid, typically 100–200 µl, is slowly and carefully injected, resulting in a raised wheal on the skin surface (Figure 1). This technique requires extensive training and practice and must be performed by medical personnel. Furthermore, it is very difficult to control the injection depth using the Mantoux technique because it is determined by the angle of needle insertion, which varies with the user. Insertion of the needle too deeply may result in vaccine being administered into the less immunologically responsive subcutaneous tissue, while needles that are not inserted deeply enough can result in leakage of the fluid onto the skin surface either during or after the injection. Despite these difficulties, the Mantoux technique has been used in clinical trials investigating the intradermal route for at least 12 different vaccines. The most recently widely studied of these include hepatitis B (HepB), influenza and therapeutic cancer vaccines, which will be reviewed below.

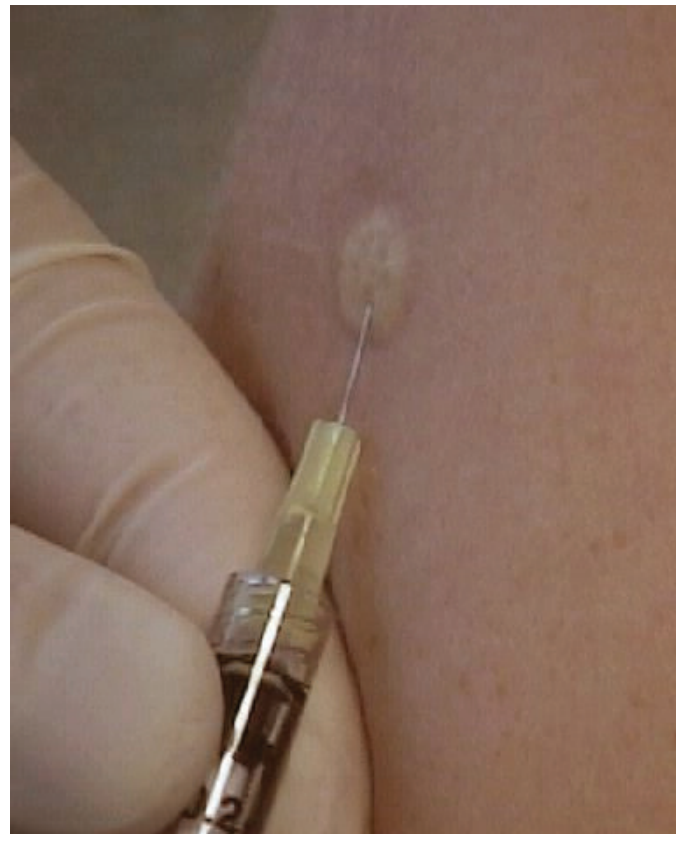

Figure 1. The classical Mantoux technique for intradermal administration.

Hepatitis B vaccine

Intradermal delivery of HepB vaccine has been investigated in numerous subject populations, including healthy infants, children and young adults [7–20], as well as in high-risk populations, such as patients undergoing dialysis due to chronic kidney disease or transplantation [21–33].

Healthy young adult healthcare workers who had previously failed to respond to the intramuscular dosing regimen have been shown, in several cases, to generate protective levels of antibody following immunization by the Mantoux method [15–17]. In other comparisons between intramuscular and intradermal routes in healthy volunteers, it has been suggested that Mantoux-style intradermal delivery can enable dose-sparing by inducing anti-HepB antibody responses using as little as a sixth of the dose typically used in conjunction with the conventional intramuscular route [9–11,19]. Conclusions from these studies must be made with caution, however, since low doses of vaccine administered by the intradermal route were not tested by conventional intramuscular injection. Nonetheless, dose-sparing benefits from intradermal delivery have been proposed as a means of reducing costs associated with HepB vaccination, particularly in the developing world where resources are limited, and in mass-vaccination settings [9,11,12,14,34].

The ability of intradermal delivery to induce more potent immune responses than intramuscular injection and the possibility of dose-sparing for HepB vaccine are particularly important for hemodialysis patients, of whom only approximately 50–60% respond to the standard intramuscular regimen [28,30]. In this regard, administration of HepB vaccine by the Mantoux method has been shown to be immunogenic in dialysis patients who were previously unresponsive by the intramuscular route [25,31,33]. Various dosing regimens have been examined over the years. In many cases, an accelerated intradermal dosing schedule consisting of weekly or biweekly administrations of a low dose (e.g., 2–10 µg) of vaccine over several months has been shown to induce similar or greater responses compared with a standard intramuscular dosing regimen consisting of three to four administrations of a high dose of vaccine (e.g., 20–40 µg) over a 6-month period [22,23,27–29,31,32]. Intradermal-induced serum antibody has been shown to persist in dialysis patients for as long as 3–5 years after dosing [26,30], while the level of long-term response in comparison with intramuscular may vary depending on the particular dosing regimen employed [23,26,30,32]. In a head-to-head comparison, Propst *et al.* showed that antibody responses following administration of 20 µg of vaccine by the intradermal route persisted at higher levels than those induced by the same dose of vaccine administered by subcutaneous or intramuscular routes for at least 3 years [30]. However, other studies using lower doses of HepB vaccine administered by the Mantoux method showed a waning response over time [20,23,26,32]. Additional dose-optimization studies will be required in order to further resolve the potential effects of antigen dose and dosing frequency on both primary and memory responses for intradermal compared with conventional routes of delivery. In addition, the field would benefit from more direct head-to-head comparisons between intradermal and intramuscular in which the same doses of vaccine and dosing regimens were employed between routes. Nonetheless, the prevailing evidence to date suggests that cutaneous delivery of HepB vaccine can provide benefits over conventional routes of administration. These benefits are likely to be better defined and exploited through the use of

new cutaneous delivery systems that are able to target the skin in a more reproducible manner than the current Mantoux method, as discussed later.

Influenza vaccine

Influenza infections are a major cause of morbidity and mortality worldwide; nevertheless, influenza vaccination remains greatly underutilized, even in developed countries, despite being a fully proven, cost-effective intervention against annual influenza infection [35]. Therefore, there is a need to establish more effective immunization protocols, including exploring new vaccine delivery systems that can contribute to improving prevention of influenza infection. Three major challenges remain to be solved with existing influenza vaccines:

- Improving postimmunization immune response in elderly and fragile subjects
- Increasing compliance of children and adults to the annual vaccination program
- Increasing vaccine dose availability to reduce the risk of influenza vaccine shortages

The currently recommended delivery route for inactivated trivalent influenza vaccine is intramuscular, but this delivery method has not adequately addressed the unresolved issues presented previously. Intranasal administration of live-attenuated vaccine has been approved recently, but the impact this new delivery route has in addressing the three concerns remains to be established [36].

Intradermal delivery of influenza vaccine has been investigated intermittently for over 30 years. In all of these studies, the Mantoux method was employed. In 1977, Brooks *et al.* reported that intradermal delivery of a reduced dose of vaccine compared with the standard regimen induced a fourfold or greater rise in antibody response, although the extent of response varied according to virus antigen [37]. In the same year, Brown *et al.* published the results of a clinical trial comparing 0.1 ml of vaccine administered by the Mantoux method with 0.5 ml delivered intramuscularly in naive subjects (without previous exposure to influenza antigens) and in subjects primed by a previous influenza infection. The results showed stronger antibody responses by the intramuscular route in naive subjects and equivalent responses for intramuscular versus intradermal routes in primed subjects [38]. In 1979, Herbert *et al.* and Halperin *et al.* conducted similar trials and concluded that intradermal injection promoted at least equivalent immune responses as compared with subcutaneous injection, with variations according to the antigen strain and the pre-existing immune status against influenza hemagglutinin antigens [39,40]. Among the confounding factors leading to mixed clinical study results with trivalent influenza vaccine is the effect of priming by previous natural infection.

In clinical studies published in 2004, it was shown that a reduced dose of influenza vaccine administered to healthy young adults by the intradermal route produced equivalent responses to a higher dose administered intramuscularly [41,42]. In a similar study conducted in 2006, delivery of a low dose of vaccine by the Mantoux method was shown to induce weaker responses than the full dose administered intramuscular, although the response levels were still greater than the minimum levels required to achieve licensure in Europe [43]. Chiu *et al.* conducted a similar study in young children and reached similar conclusions as the above studies conducted in healthy young adults [44]. All of these studies, however, lacked the appropriate low-dose intramuscular controls required to make clear head-to-head comparisons between the two routes of administration. Recently, Belshe *et al.* conducted such a comparison in healthy young adults and showed that a low dose of vaccine administered intramuscular was just as effective as a low dose delivered by the Mantoux method [45]. Thus, the potential for the Mantoux-style intradermal injections to enable dose sparing and improved immune responses for influenza vaccine remains a subject of debate that perhaps can be better resolved through the use of new delivery systems that more reproducibly and effectively deliver to the skin. Recent studies using one such delivery system (described in greater detail below) suggest that controlled intradermal delivery can induce humoral immune responses in elderly subjects that are superior to responses achieved by conventional intramuscular injection when the same dose is administered by both routes [46,47]. Additional studies with this new delivery system and others are eagerly anticipated and will contribute to a greater understanding of the role of delivery route on influenza vaccine potency.

Therapeutic cancer vaccines

Intradermal delivery by the Mantoux method has been widely studied in the field of therapeutic cancer vaccines. Clinical trials of the intradermal route have been conducted for a wealth of indications, to varying degrees of success. These include melanoma [48–57], non-small cell lung cancer [58–60], hepatocellular carcinoma [61,62], pancreatic cancer [63,64], brain cancer [65], renal cell carcinoma [66–71], breast cancer [72–75], ovarian cancer [72,75,76], prostate cancer [69,77–83], non-Hodgkins lymphoma [84] and B-cell lymphoma [85]. However, most of these studies did not compare intradermal delivery with alternate routes of administration, thus making it difficult to ascertain the potential benefits associated with delivery to the skin. Nonetheless, the overwhelming majority of studies demonstrated an acceptable safety profile regardless of the class of vaccine administered. These include anti-idiotype antibodies [48,76,86], peptides [51,58,62–64,72,75,82,87], proteins [84], autologous tumor cells [49,50,60,67,70,80], DNA [81] and dendritic cells [54,57,61,66,68,69,71,78,83,88]. In some cases, Mantoux-based injection was combined with other routes, such as intravenous [65], subcutaneous [52,55,56,59,74,86,89] or intranodal [55].

A small number of clinical studies have directly compared the Mantoux method with other routes of administration for cancer vaccines [53,77,79,90]. Fong *et al.* reported that IFN-γ producing T-cell responses were evident in patients with metastatic prostate cancer immunized by the intradermal and intralymphatic routes, but not following intravenous administration [77]. By contrast, more patients generated antibody responses when immunized by the intravenous route compared with intradermal and intralymphatic routes. These results suggested that the type

of immune response can vary depending on the route of administration. In most cases, cell-mediated immunity will be critical to the success of therapeutic cancer vaccines, so delivery methods that induce strong cell-mediated immunity will probably be preferred. Using autologous dendritic cells pulsed with tumor RNA, Kyte *et al.* showed an increased frequency of patients generating tumor specific T-cell responses following Mantoux-style intradermal injections compared with nodal delivery in both melanoma and prostate cancer patients [53,79]. Using radiolabeled dendritic cells, Morse *et al.* showed evidence of cells migrating to the draining lymph nodes (where the adaptive immune response is initiated) in subjects treated by the intradermal route, but not following administration by intravenous or subcutaneous routes [90]. Evidence of dendritic cell migration to lymph nodes following intradermal administration has also been shown in other imaging studies [57]. Some investigators have sought to exploit the benefits of accessing the lymphatic drainage networks in the skin for cancer vaccines. Vieweg and colleagues showed that immature dendritic cells migrate to draining lymph nodes with efficiency similar to, or greater than, that of mature cells when administered to skin sites that had been pretreated with adjuvants, such as imiquimod or polyarginine [88]. Based on the large number of clinical trials evaluating the Mantoux method for therapeutic cancer vaccines, it is likely that this area of investigation will benefit greatly from the introduction of new skin delivery systems that are easier to use and more effective.

Expert commentary & five-year view

The vaccine delivery technology landscape is highly competitive and has been evolving rapidly over the past decade. The technology research environment involves numerous startup companies, major players in the medical device industry and the major vaccine manufacturers. Recent progress in skin immunology and a better understanding of the physiology of the adaptive immune response is fueling creativity and rapid evolution in vaccine delivery technologies. However, this innovation, is hampered by two challenging bottlenecks. The first bottleneck for start-up companies and medical device manufacturers is the need to form a research and development alliance with vaccine manufacturers to evaluate efficiency and safety of new delivery systems in full compliance with good manufacturing practice (GMP), good laboratory practice (GLP) and good clinical practice (GCP) without losing freedom to operate with any potential partners. The second bottleneck is the regulatory path for a combined vaccine-delivery system and formulated antigens, which can make it difficult for startup companies and even established medical-device manufacturers to proceed alone at an acceptable project risk level. In addition to these practical matters, the field has been hampered to date by a limited understanding of the cellular and molecular immunological mechanisms associated with vaccine administration using the various delivery systems under development. A background in business and product development strategy as well as a solid scientific basis will be critical to addressing the technology challenges in vaccine delivery in the future. Table 1 summarizes many of the cutaneous vaccine delivery technologies being researched and developed by commercial entities.

Table 1. Selected cutaneous vaccine delivery technologies under commercial development.

Technology	Vaccine loading	Companies	Products
Hollow microneedles			
Microneedle affixed to specialized syringe	Prefilled	Becton Dickinson	BD Soluvia™
Detachable microneedle for syringe	Nonprefilled	Becton Dickinson	BD micro injection needle
Detachable microneedle arrays for syringe	Nonprefilled	Debiotech	Nanoject
	Nonprefilled	Nanopass	MicroPyramid MicronJet needle
	Nonprefilled	Valeritas	Micro-Trans™
Solid microneedles			
Coated microneedle array	Prefilled	Zosano	Transdermal microprojection delivery system
Coated microneedle array or pretreatment for patch	Prefilled or nonprefilled	3M	Microstructured transdermal system
		Valeritas	Micro-Trans
Dissolvable microneedle vaccine array	Prefilled	TheraJect	VaxMAT
Transcutaneous			
Needle-free patch on abraded skin	Prefilled	Iomai/ Intercell	Transcutaneous immunization patch
Jet injection			
Needle-free delivery of liquid jet	Nonprefilled	Bioject	Biojector® 2000
	Nonprefilled	PharmaJet	PharmaJet system
	Prefilled	Valeritas	Mini-Ject™
Powder injection			
Needle-free ballistic delivery	Prefilled	Pfizer/ Powder Med	PMED™ device

To achieve public-health goals, vaccine-delivery systems must allow efficient and consistent delivery to the targeted body site without compromising the stability of vaccine antigens and adjuvants during storage and shipment and without negatively influencing subject acceptance. The market launch of the nasal, live-inactivated influenza vaccine in the USA for children and adults (FluMist™, MedImmune) with clinically proven efficacy and safety on a large population size gives hope that nasal delivery might be a viable alternative delivery method for influenza vaccination [91]. The unexpected adverse events (Bell's palsy syndrome) with another nasal influenza vaccine in Europe after market launch in 2001 (NasaFlu™, Berna Biotech), however, pointed out that the safety of the nasal route needs to be rigorously investigated, especially when adjuvants are included in the formulation [92].

Transdermal and transcutaneous delivery are other options for needle-free vaccine administration via the dermis and epidermis, respectively. Although the skin in its natural baseline condition is not permeable to large proteins and viral particles, it has been shown that if the outermost layer of the epidermis (the stratum corneum) is disrupted, large molecules and particles can reach the deeper epidermis where Langerhans' cells are present [93,94]. Needle-free technologies for disrupting the epidermal skin barrier are numerous and can be classified broadly as biological/chemical approaches that employ nanoparticles, liposomes or other carriers to transport large molecules across the stratum corneum [6,95–97] or physical/mechanical approaches such as abrasion [98–102], skin pretreatment with 'solid microneedles' [103,104], sonoporation [105] and laser ablation [106–109]. Many of these technologies are at a relatively early stage of development for vaccine delivery, but have shown progress in early preclinical models. One technology that has shown considerable progress in the clinic is transcutaneous immunization (TCI). This approach combines vaccine antigens with heat-labile enterotoxins from *Escherichia coli* as an adjuvant that is applied in a patch to the epidermis of skin pretreated by mechanical disruption of the stratum corneum [93,98,110]. TCI has recently been used to immunize travelers against diarrhea caused by enterotoxigenic *E. coli* in a Phase II clinical trial [110]. Advantages of this technology are that it is needle free and simple to administer. However, some uncertainty remains regarding how broadly applicable TCI may be, as well as how the approach compares with standard injection-based delivery, especially with regard to dosing efficiency. Others have shown that live-attenuated measles vaccine applied topically to disrupted stratum corneum without adjuvant induced T-cell responses to a similar extent as subcutaneous injection, but failed to induce measles-neutralizing antibodies [111].

Other needle-free alternatives include liquid jet injection [112] and epidermal powder immunization [113,114]. Both of these approaches use high pressure to blast the liquid or powder through the stratum corneum and into the body. Epidermal powder immunization was specifically designed to deliver vaccines to the skin, while jet injection has classically been used for subcutaneous or intramuscular delivery, although some jet injectors are also intended for intradermal delivery [2,112]. One challenge associated with the powder delivery approach is the need to reformulate the vaccine into dry powder form and to fill the powder into the device. Liquid jet injectors, on the other hand, are compatible with conventional vaccine formulations intended for injection by needle and syringe. By concept, liquid jet injectors are combination products associating a vial or cartridge with the vaccine and the jet injector as a medical device. One advantage of multiuse jet injectors is the ability to immunize many people very rapidly. Devices designed to inject several subjects using the same jet nozzle, however, were removed from the market due to inter-individual bloodborne contamination [115]. These concerns led to the development of safer jet injectors that use disposable cartridges. Although not strictly intended for cutaneous delivery, jet injection has been demonstrated to be applicable to a wide range of vaccine types, such as inactivated viruses, polysaccharide–protein conjugates, toxoids, naked DNA and whole-cell vaccines [2,112]. In contrast to needle-based injection, however, jet injection often results in wide distribution of the injected fluid that, in some cases, can extend from the skin epidermis to as deep as the muscle, depending on the kinetics of the fluid jet injection and the penetration force. One concern related to jet-injector systems that are not prefilled is the need to manipulate the vaccine solution by transferring vaccine dose from a single or multidose vial to the jet injector cartridge at the time of use, which can potentially lead to vaccine dose wastage, contamination and dosing error. Nonetheless, new prefilled jet injectors designed specifically for cutaneous delivery represent a promising future technology platform for vaccination via the skin.

Another approach for cutaneous delivery has been to continue to use needles, but dramatically reduce the size of such needles so that they are barely perceptible to the subject and are capable of delivering vaccines accurately and reproducibly to the epidermal and dermal tissue. Three approaches have been investigated; the first involves 'solid microneedles' that are coated with the vaccine in the form of a powder or film [116,117]. The coated microneedles are inserted into the skin and then removed, thus depositing their payload into the skin at a depth determined by the length of the microneedle. This approach has shown some promise in preclinical studies; however, the dosing efficiency is typically low relative to conventional needle-based injection. As such, it is often necessary to coat the microneedles with a large excess of antigen, which could be problematic in situations in which vaccine supply is limited. In addition, this approach requires reformulation of the vaccine and the development of new manufacturing processes to coat the vaccine powders or films onto the surface of the microneedles or tines. In order to overcome some of these issues, a second approach involves the use of solid microneedles that consist of a dissolvable material into which the vaccine antigen has been incorporated [116,118–120]. This approach is at a much earlier stage of development than the approaches described previously and has many challenges remaining before it can be implemented at a commercial scale. Nonetheless, continued development of these types of delivery systems could one day offer a promising alternative to needle-based injections for cutaneous immunization. The third approach in this category involves microneedles through which fluids are injected [100,102,104,121–123]. These devices

Figure 2. Microneedle intradermal injection device. (A) Scanning electron micrograph of the stainless steel microneedle extending 1.5 mm from the specially designed hub. **(B)** BD Soluvia™ device for intradermal delivery. **(C)** The intradermal injection technique using the BD Soluvia device. The needle is inserted perpendicularly to the skin surface so that the injection depth is controlled by the length of the microneedle rather than the user technique.

are intended to overcome the deficiencies of the Mantoux method by more accurately and reproducibly targeting the dermis. This is accomplished by inserting the microneedles perpendicularly to the skin surface, such that the injection depth is controlled precisely by the length of the microneedle. One potential disadvantage of this approach is that it still involves the use of sharp needles, albeit the needles are very small and, in many cases, barely perceptible to the subject. Nonetheless, the delivery devices associated with the microneedles will ideally be engineered with safety features to avoid accidental needle stick after use and to prevent reuse. Given the small size of the microneedle, it will also be challenging to develop delivery systems that enable fluid injection through the microneedle without leakage and that can be paired with existing vaccine manufacturing and filling processes.

A hollow microneedle-based injection system in advanced clinical development is shown in Figure 2. This device (BD Soluvia™, Becton Dickinson) employs a microneedle that penetrates 1.5 mm into the skin to deliver vaccine to the dermis. Although the microneedle on this system is longer than that found on other devices at earlier stages of development, clinical studies have shown that the injection depth is appropriate for intradermal delivery across multiple body sites and in subjects of varying age, gender, body characteristics and ethnicities [124]. The system is prefilled with a preadjusted vaccine dose and is engineered with safety features to reduce the risk of accidental needle stick after use and prevent reuse. Clinical studies show that the device is intuitive to use, even without any previous training of the healthcare worker [121]. In elderly volunteers, administration of trivalent inactivated influenza vaccine (Sanofi Pasteur) using this device resulted in superior immune responses for all three strains compared with responses elicited by the conventional intramuscular delivery route [46,47]. The incorporation of microneedle technology into prefilled syringes overcomes many of the logistical burdens of immunization because the vaccine–device combination does not need to be filled at time of use. In addition, vaccine wastage is greatly reduced by switching from vials to prefilled syringes because syringes require much less overfill volumes and are not prone to dosing errors associated with filling the syringe by end users. In addition, the device employs a glass syringe on the interior of the device that can be filled on existing filling lines by vaccine manufacturers and contract fillers. Devices incorporating stainless steel microneedles of this sort have been used in numerous preclinical studies for influenza, anthrax and Japanese encephalitis vaccines [100,102,122,123]. The continued clinical and commercial development of this device and others similar to it are likely to have a major positive impact on cutaneous vaccination in the future.

In summary, we believe that the 5-year outlook for new skin-targeting vaccine delivery systems is bright. Innovation in alternative vaccine delivery is being driven by emerging vaccine delivery systems combined with new and existing vaccines to address the specific needs of reducing logistical burdens of vaccination campaigns, ensuring high quality and stability of vaccines with at least equivalent efficacy relative to standard methods in a manner that does not increase health risk exposure and that corresponds to the industrial manufacturing constraints required for GMP products. Furthermore, vaccine delivery to the skin has been shown, in many cases, to be a particularly effective method of inducing immune responses in high-risk populations such as the elderly, the immunocompromised and cancer patients. Increasingly, development of the delivery device is being integrated early in the vaccine clinical development pathway to optimize the efficacy and cost–effectiveness of the new combination product. Innovation in the vaccine delivery field requires strategic alliances to mitigate project risks and to ensure complex and competing technology investment returns, making the new technology affordable in the context of the varying health economic factors and infectious diseases in different parts of the world.

Financial & competing interests disclosure

Both authors are employed by BD (Becton Dickinson & Co.) and own shares of stock in BD. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- The skin is an attractive site for vaccination.
- Clinical trials with numerous vaccines have shown that vaccines administered by intradermal delivery are safe and effective.
- In several cases, benefits such as increased immune responses have been demonstrated for intradermal delivery compared with traditional routes, such as subcutaneous or intramuscular.
- Increased immune responses are particularly desirable in high-risk subject populations, such as the elderly, the immunocompromised and cancer patients.
- Intradermal delivery using standard needles and syringes is difficult to accomplish and requires extensive training.
- New delivery systems for more accurate, reproducible and easier vaccine administration to the skin are currently being developed.
- The future of vaccine delivery to the skin looks promising as new skin-delivery systems become available.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Henderson DA, Borio LL, Grabenstein JD. Smallpox and vaccinia. In: *Vaccines (5th Edition).* Plotkin SA, Orenstein WA, Offit PA (Eds). Elsevier, Amsterdam, The Netherlands 773–803 (2008).

2 Weniger BG, Papania MJ. Alternative vaccine delivery methods. In: *Vaccines (5th Edition)*. Plotkin SA, Orenstein WA, Offit PA (Eds). Elsevier, Amsterdam, The Netherlands, 1357–1392 (2008).

•• **Extraordinarily comprehensive review on vaccine delivery methods of the past, present and future.**

3 Babiuk S, Baca-Estrada M, Babiuk LA, Ewen C, Foldvari M. Cutaneous vaccination: the skin as an immunologically active tissue and the challenge of antigen delivery. *J. Control Release* 66, 199–214 (2000).

4 Larregina AT, Falo LD. Changing paradigms in cutaneous immunology: adapting with dendritic cells. *J. Invest. Dermatol.* 124, 1–12 (2005).

5 Randolph GJ, Angeli V, Swartz MA. Dendritic-cell trafficking to lymph nodes through lymphatic vessels. *Nature Rev. Immunol.* 5, 617–628 (2005).

6 Reddy ST, van der Vlies AJ, Simeoni E *et al.* Exploiting lymphatic transport and complement activation in nanoparticle vaccines. *Nature Biotechnol.* 25, 1159–1164 (2007).

7 Cardell K, Fryden A, Normann B. Intradermal hepatitis B vaccination in health care workers. Response rate and experiences from vaccination in clinical practise. *Scand. J. Infect. Dis.* 31, 197–200 (1999).

8 Carlsson T, Struve J, Sonnerborg A, Weiland O. The anti-HBs response after 2 different accelerated intradermal and intramuscular schemes for hepatitis B vaccination. *Scand. J. Infect. Dis.* 31, 93–95 (1999).

9 Egemen A, Aksit S, Kurugol Z, Erensoy S, Bilgic A, Akilli M. Low-dose intradermal versus intramuscular administration of recombinant hepatitis B vaccine: a comparison of immunogenicity in infants and preschool children. *Vaccine* 16, 1511–1515 (1998).

10 Ghabouli MJ, Sabouri AH, Shoeibi N, Bajestan SN, Baradaran H. High seroprotection rate induced by intradermal administration of a recombinant hepatitis B vaccine in young healthy adults: comparison with standard intramuscular vaccination. *Eur. J. Epidemiol.* 19, 871–875

11 Henderson EA, Louie TJ, Ramotar K, Ledgerwood D, Hope KM, Kennedy A. Comparison of higher-dose intradermal hepatitis B vaccination to standard intramuscular vaccination of healthcare workers. *Infect. Control Hosp. Epidemiol.* 21, 264–269 (2000).

12 Jaiswal SP, Asolkar MV, Vijayvargiya R, Chitnis DS. Immunogenicity of low-dose hepatitis-B vaccine by the intradermal route and persistence of anti-HBs after 3 years. *Indian J. Med. Res.* 102, 129–133 (1995).

13 Kurugol Z, Erensoy S, Aksit S, Egemen A, Bilgic A. Low-dose intradermal administration of recombinant hepatitis B vaccine in children: 5-year follow-up study. *Vaccine* 19, 3936–3939 (2001).

14 Kyi KP, Oo KM, Htun MM *et al.* Clinical trial of the intradermal administration of hepatitis B vaccine produced at the Department of Medical Research, Myanmar. *Vaccine* 20, 1649–1652 (2002).

15 Levitz RE, Cooper BW, Regan HC. Immunization with high-dose intradermal recombinant hepatitis-B vaccine in health-care workers who failed to respond to intramuscular vaccination. *Infect. Control Hosp. Epidemiol.* 16, 88–91 (1995).

16 Playford EG, Hogan PG, Bansal AS *et al.* Intradermal recombinant hepatitis B vaccine for healthcare workers who fail to respond to intramuscular vaccine. *Infect. Control Hosp. Epidemiol.* 23, 87–90 (2002).

17 Rahman F, Dahmen A, Herzog-Hauff S, Bocher WO, Galle PR, Lohr HF. Cellular and humoral immune responses induced by intradermal or intramuscular vaccination with the major hepatitis B surface antigen. *Hepatology* 31, 521–527 (2000).

18 Struve J, Aronsson B, Frenning B, Forsgren M, Weiland O. Response to a booster dose 18 months after a low anti-HBs response (10–99 IU/l) to 3 doses of intradermally or intramuscularly administered recombinant hepatitis-B vaccine. *Infection* 23, 42–45 (1995).

19 Yamashiki M, Kosaka Y, Nishimura A. An effective intradermal hepatitis B vaccination. *Vaccine* 15, 1618–1623 (1997).

20 Zhuang GH, Yan H, Wang XL *et al.* Hepatitis B revaccination in healthy non-responder Chinese children: five-year follow-up of immune response and immunologic memory. *Vaccine* 24, 2186–2192 (2006).

21 Angelico M, Di Paolo D, Trinito MO *et al.* Failure of a reinforced triple course of hepatitis B vaccination in patients transplanted for HBV-related cirrhosis. *Hepatology* 35, 176–181 (2002).

22 Charest AF, McDougall J, Goldstein MB. A randomized comparison of intradermal and intramuscular vaccination against hepatitis B virus in incident chronic hemodialysis patients. *Am. J. Kidney Dis.* 36, 976–982 (2000).

23 Chau KF, Cheng YL, Tsang DNC *et al.* Efficacy and side effects of intradermal hepatitis B vaccination in CAPD patients: a comparison with the intramuscular vaccination. *Am. J. Kidney Dis.* 43, 910–917 (2004).

24 Choy BY, Peiris JSM, Chan TM, Lo SKF, Lui SL, Lai KN. Immunogenicity of intradermal hepatitis B vaccination in renal transplant recipients. *Am. J. Transplant.* 2, 965–969 (2002).

25 Fabrizi F, Andrulli S, Bacchini G, Corti M, Locatelli F. Intradermal versus intramuscular hepatitis B re-vaccination in

non-responsive chronic dialysis patients: a prospective randomized study with cost–effectiveness evaluation. *Nephrol. Dialysis Transplant.* 12, 1204–1211 (1997).

26 Fabrizi F, Dixit V, Magnini M, Elli A, Martin P. Meta-analysis: intradermal vs. intramuscular vaccination against hepatitis B virus in patients with chronic kidney disease. *Aliment. Pharmacol. Ther.* 24, 497–506 (2006).

27 Karahocagil MK, Buzgan T, Irmak H, Karsen H, Akdeniz H, Akman N. Comparison of intramuscular and intradermal applications of hepatitis B vaccine in hemodialysis patients. *Renal Failure* 28, 561–565 (2006).

28 Mettang T, Schenk U, Thomas S *et al.* Low-dose intradermal versus intramuscular hepatitis B vaccination in patients with end-stage renal failure – a preliminary study. *Nephron* 72, 192–196 (1996).

29 Morais EO, Resende MR, Oliveira AM *et al.* Intradermal hepatitis B vaccination in patients with advanced chronic renal failure: immunogenicity and follow-up. *Aliment. Pharmacol. Ther.* 25, 849–855 (2007).

30 Propst T, Propst A, Lhotta K, Vogel W, Konig P. Reinforced intradermal hepatitis B vaccination in hemodialysis patients is superior in antibody response to intramuscular or subcutaneous vaccination. *Am. J. Kidney Dis.* 32, 1041–1045 (1998).

- **One of the first investigators to propose a high-dose intradermal hepatitis B immunization regimen for dialysis patients and to show benefits over the same dose administered by intramuscular injection.**

31 Vlassopoulos D, Arvanitis D, Lilis D, Hatjiyannakos D, Louizou K, Hadjiconstantinou V. Complete success of intradermal vaccination against hepatitis B in advanced chronic renal failure and hemodialysis patients. *Renal Failure* 19, 455–460 (1997).

32 Vlassopoulos DA, Arvanitis DK, Lilis DS, Louizou KI, Hadjiconstantinou VE. Lower long-term efficiency of intradermal hepatitis B vaccine compared to the intramuscular route in hemodialysis patients. *Int. J. Art. Organs* 22, 739–743 (1999).

33 Waite NM, Thomson LG, Goldstein MB. Successful vaccination with intradermal hepatitis-B vaccine in hemodialysis-patients previously nonresponsive to intramuscular hepatitis-B vaccine. *J. Amer. Soc. Nephrol.* 5, 1930–1934 (1995).

34 Nagafuchi S, Kashiwagi S, Imayama S, Hayashi J, Niho Y. Intradermal administration of viral vaccines. *Rev. Med. Virol.* 8, 97–111 (1998).

35 Scuffham PA, West PA. Economic evaluation of strategies for the control and management of influenza in Europe. *Vaccine* 20, 2562–2578 (2002).

36 Vajdy M, Baudner B, Del Giudice G, O'Hagan D. A vaccination strategy to enhance mucosal and systemic antibody and T cell responses against influenza. *Clin. Immunol.* 123, 166–175 (2007).

37 Brooks JH, Criep LH, Ruben FL. Intradermal administration of bivalent and monovalent influenza vaccines. *Ann. Allergy* 39, 110–112 (1977).

38 Brown H, Kasel JA, Freeman DM, Moise LD, Grose NP, Couch RB. The immunizing effect of influenza A/New Jersey/76 (Hsw1N1) virus vaccine administered intradermally and intramuscularly to adults. *J. Infect. Dis.* 136 Suppl. S466–S471 (1977).

39 Halperin W, Weiss WI, Altman R *et al.* A comparison of the intradermal and subcutaneous routes of influenza vaccination with A/New Jersey/76 (swine flu) and A/Victoria/75: report of a study and review of the literature. *Am. J. Pub. Health* 69, 1247–1250 (1979).

40 Herbert FA, Larke RP, Markstad EL. Comparison of responses to influenza A/New Jersey/76-A/Victoria/75 virus vaccine administered intradermally or subcutaneously to adults with chronic respiratory disease. *J. Infect. Dis.* 140, 234–238 (1979).

41 Belshe RB, Newman FK, Cannon J *et al.* Serum antibody responses after intradermal vaccination against influenza. *N. Engl. J. Med.* 351, 2286–2294 (2004).

- **Reinvigorated the field and has led to a greatly increased interest in re-exploring the intradermal route of delivery for influenza vaccine.**

42 Kenney RT, Frech SA, Muenz LR, Villar CP, Glenn GM. Dose sparing with intradermal injection of influenza vaccine. *N. Engl. J. Med.* 351, 2295–2301 (2004).

- **Reinvigorated the field and has led to a greatly increased interest in re-exploring the intradermal route of delivery for influenza vaccine.**

43 Auewarakul P, Kositanont U, Sornsathapornkul P, Tothong P, Kanyok R, Thongcharoen P. Antibody responses after dose-sparing intradermal influenza vaccination. *Vaccine* 25, 659–663 (2006).

44 Chiu SS, Peiris JSM, Chan KH, Wong WHS, Lau YL. Immunogenicity and safety of intradermal influenza immunization at a reduced dose in healthy children. *Pediatrics* 119, 1076–1082 (2007).

45 Belshe RB, Newman FK, Wilkins K *et al.* Comparative immunogenicity of trivalent influenza vaccine administered by intradermal or intramuscular route in healthy adults. *Vaccine* 25, 6755–6763 (2007).

- **Much-anticipated follow-up to the 2004 publication, this time including the critical low-dose intramuscular control group.**

46 Booy R, Weber F, Saville M. Immunogenicity of a novel influenza vaccine delivered by intradermal microinjection in over 60-year-olds. Presented at: *Options for the Control of Influenza VI.* Toronto, Canada, 17–23 June 2007.

- **New injection technique and device for intradermal delivery of influenza vaccine.**

47 Holland D, Booy R, De Looze F *et al.* Intradermal influenza vaccine administered using a new microinjection system produces superior immunogenicity in elderly adults: a randomized controlled trial. *J. Infect. Dis.* 198(5), 650–658 (2008).

- **New injection technique and device for intradermal delivery of influenza vaccine.**

48 Alfonso M, Diaz A, Hernandez AM *et al.* An anti-idiotype vaccine elicits a specific response to *N*-glycolyl sialic acid residues of glycoconjugates in melanoma patients. *J. Immunol.* 168, 2523–2529 (2002).

49 Berd D, Sato T, Maguire HC, Kairys J, Mastrangelo MJ. Immunopharmacologic analysis of an autologous, hapten-modified human melanoma vaccine. *J. Clin. Oncol.* 22, 403–415 (2004).

50 Dessureault S, Noyes D, Lee D *et al.* A Phase-I trial using a universal GM-CSF-producing and CD40L-expressing bystander cell line (GM.CD40L) in the formulation of autologous tumor cell-based vaccines for cancer patients with stage IV disease. *Ann. Surg. Oncol.* 14, 869–884 (2007).

51 Hunger RE, Brand CU, Streit M *et al.* Successful induction of immune responses against mutant ras in melanoma patients using intradermal injection of peptides and GM-CSF as adjuvant. *Exp. Dermatol.* 10, 161–167 (2001).

52 Kruit WHJ, van Ojik HH, Brichard VG *et al.* Phase 1/2 study of subcutaneous and intradermal immunization with a

recombinant MAGE-3 protein in patients with detectable metastatic melanoma. *Int. J. Cancer* 117, 596–604 (2005).

53 Kyte JA, Mu L, Aamdal S *et al.* Phase I/II trial of melanoma therapy with dendritic cells transfected with autologous tumor-mRNA. *Cancer Gene Ther.* 13, 905–918 (2006).

- **One of very few studies comparing T-cell responses in cancer patients treated by intradermal or intranodel routes of administration.**

54 O'Rourke MGE, Johnson M, Lanagan C *et al.* Durable complete clinical responses in a Phase I/II trial using an autologous melanoma cell/dendritic cell vaccine. *Cancer Immunol. Immunother.* 52, 387–395 (2003).

55 Salcedo M, Bercovici N, Taylor R *et al.* Vaccination of melanoma patients using dendritic cells loaded with an allogeneic tumor cell lysate. *Cancer Immunol. Immunother.* 55, 819–829 (2006).

56 Schreiber S, Kampgen E, Wagner E *et al.* Immunotherapy of metastatic malignant melanoma by a vaccine consisting of autologous interleukin 2 transfected cancer cells: outcome of a Phase I study. *Hum. Gene Ther.* 10, 983–993 (1999).

57 Trakatelli M, Toungouz M, Blocklet D *et al.* A new dendritic cell vaccine generated with interleukin-3 and interferon-β induces $CD8^+$ T-cell responses against NA17-A2 tumor peptide in melanoma patients. *Cancer Immunol. Immunother.* 55, 469–474 (2006).

58 Brunsvig PF, Aamdal S, Gjertsen MK *et al.* Telomerase peptide vaccination: a Phase I/II study in patients with non-small cell lung cancer. *Cancer Immunol. Immunother.* 55, 1553–1564 (206).

59 Morse MA, Clay TM, Hobeika AC *et al.* Phase I study of immunization with dendritic cells modified with fowlpox encoding carcinoembryonic antigen and costimulatory molecules. *Clin. Cancer Res.* 11, 3017–3024 (2005).

60 Nemunaitis J, Sterman D, Jablons D *et al.* Granulocyte–macrophage colony-stimulating factor gene-modified autologous tumor vaccines in non-small-cell lung cancer. *J. Natl Cancer Inst.* 96, 326–331 (2004).

61 Butterfield LH, Ribas A, Dissette VB *et al.* A Phase I/II trial testing immunization of hepatocellular carcinoma patients with dendritic cells pulsed with four α-fetoprotein peptides. *Clin. Cancer Res.* 12, 2817–2825 (2006).

62 Butterfield LH, Ribas A, Meng WS *et al.* T-cell responses to HLA-A*0201 immunodominant peptides derived from α-fetoprotein in patients with hepatocellular cancer. *Clin. Cancer Res.* 9, 5902–5908 (2003).

63 Bernhardt SL, Gjertsen MK, Trachsel S *et al.* Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: a dose escalating Phase I/II study. *Br. J. Cancer* 95, 1474–1482 (2006).

64 Gjertsen MK, Buanes T, Rosseland AR *et al.* Intradermal ras peptide vaccination with granulocyte–macrophage colony-stimulating factor as adjuvant: clinical and immunological responses in patients with pancreatic adenocarcinoma. *Int. J. Cancer* 92, 441–450 (2001).

65 Caruso DA, Orme LM, Neale AM *et al.* Results of a Phase 1 study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children and young adults with brain cancer. *Neurooncology* 6, 236–246 (2004).

66 Dannull J, Su Z, Rizzieri D *et al.* Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. *J. Clin. Invest.* 115, 3623–3633 (2005).

67 Jocham D, Richter A, Hoffmann L *et al.* Adjuvant autologous renal tumour cell vaccine and risk of tumour progression in patients with renal-cell carcinoma after radical nephrectomy: Phase III, randomised controlled trial. *Lancet* 363, 594–599 (2004).

68 Oosterwijk-Wakka JC, Tiemessen DM, Bleumer I *et al.* Vaccination of patients with metastatic renal cell carcinoma with autologous dendritic cells pulsed with autologous tumor antigens in combination with interleukin-2: a Phase 1 study. *J. Immunother.* 25, 500–508 (2002).

69 Pandha HS, John RJ, Hutchinson J *et al.* Dendritic cell immunotherapy for urological cancers using cryopreserved allogeneic tumour lysate-pulsed cells: a Phase I/II study. *Br. J. Urol. Int.* 94, 412–418 (2004).

70 Simons JW, Jaffee EM, Weber CE *et al.* Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by *ex vivo* granulocyte–macrophage colony-stimulating factor gene transfer. *Cancer Res.* 57, 1537–1546 (1997).

71 Su Z, Dannull J, Heiser A *et al.* Immunological and clinical responses in metastatic renal cancer patients vaccinated with tumor RNA-transfected dendritic cells. *Cancer Res.* 63, 2127–2133 (2003).

72 Disis ML, Grabstein KH, Sleath PR, Cheever MA. Generation of immunity to the HER-2/neu oncogenic protein in patients with breast and ovarian cancer using a peptide-based vaccine. *Clin. Cancer Res.* 5, 1289–1297 (1999).

73 Guthmann MD, Castro MA, Cinat G *et al.* Cellular and humoral immune response to *N*-glycolyl-GM3 elicited by prolonged immunotherapy with an anti-idiotypic vaccine in high-risk and metastatic breast cancer patients. *J. Immunother.* 29, 215–223 (2006).

74 Morse MA, Hobeika A, Osada T *et al.* Long term disease-free survival and T cell and antibody responses in women with high-risk Her^{2+} breast cancer following vaccination against Her2. *J. Transl. Med.* 5, 42 (2007).

75 Murray JL, Gillogly ME, Przepiorka D *et al.* Toxicity, immunogenicity, and induction of E75-specific tumor-lytic CTLs by HER-2 peptide E75 (369–377) combined with granulocyte macrophage colony-stimulating factor in $HLA\text{-}A2^+$ patients with metastatic breast and ovarian cancer. *Clin. Cancer Res.*8, 3407–3418 (2002).

76 Nicholson S, Bomphray CC, Thomas H *et al.* A Phase I trial of idiotypic vaccination with HMFG1 in ovarian cancer. *Cancer Immunol. Immunother.* 53, 809–816 (2004).

77 Fong L, Brockstedt D, Benike C, Wu L, Engleman EG. Dendritic cells injected via different routes induce immunity in cancer patients. *J. Immunol.* 166, 4254–4259 (2001).

- **Both cell-mediated and humoral immune responses were compared for a cancer vaccine administered by various routes of delivery.**

78 Heiser A, Coleman D, Dannull J *et al.* Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL responses against metastatic prostate tumors. *J. Clin. Invest.* 109, 409–417 (2002).

79 Kyte JA, Gaudernack G. Immuno-gene therapy of cancer with tumour-mRNA transfected dendritic cells. *Cancer Immunol. Immunother.* 55, 1432–1442 (2006).

80 Michael A, Ball G, Quatan N *et al.* Delayed disease progression after allogeneic cell vaccination in hormone-resistant prostate cancer and correlation with immunologic variables. *Clin. Cancer Res.* 11, 4469–4478 (2005).

81 Mincheff M, Tchakarov S, Zoubak S *et al.* Naked DNA and adenoviral immunizations for immunotherapy of prostate cancer: a Phase I/II clinical trial. *Eur. Urol.* 38, 208–217 (2000).

82 Perambakam S, Hallmeyer S, Reddy S *et al.* Induction of specific T cell immunity in patients with prostate cancer by vaccination with PSA146-154 peptide. *Cancer Immunol. Immunother.* 55, 1033–1042 (2006).

83 Su Z, Dannull J, Yang BK *et al.* Telomerase mRNA-transfected dendritic cells stimulate antigen-specific $CD8^+$ and $CD4^+$ T cell responses in patients with metastatic prostate cancer. *J. Immunol.* 174, 3798–3807 (2005).

84 Oki Y, McLaughlin P, Fayad LE *et al.* Experience with heat shock protein–peptide complex 96 vaccine therapy in patients with indolent non-Hodgkin lymphoma. *Cancer* 109, 77–83 (2007).

85 Bertinetti C, Zirlik K, Heining-Mikesch K *et al.* Phase I trial of a novel intradermal idiotype vaccine in patients with advanced B-cell lymphoma: specific immune responses despite profound immunosuppression. *Cancer Res.* 66, 4496–4502 (2006).

86 Mosolits S, Markovic K, Frodin JE *et al.* Vaccination with Ep-CAM protein or anti-idiotypic antibody induces Th1-biased response against MHC class I- and II-restricted Ep-CAM epitopes in colorectal carcinoma patients. *Clin. Cancer Res.* 10, 5391–5402 (2004).

87 Lomas M, Liauw W, Packham D *et al.* Phase I clinical trial of a human idiotypic p53 vaccine in patients with advanced malignancy. *Ann. Oncol.* 15, 324–329 (2004).

88 Nair S, McLaughlin C, Weizer A *et al.* Injection of immature dendritic cells into adjuvant-treated skin obviates the need for *ex vivo* maturation. *J. Immunol.* 171, 6275–6282 (2003).

• **Study showed the impact of skin-administered adjuvants on dendritic cell migration and immune response.**

89 Morse MA, Deng YP, Coleman D *et al.* A Phase I study of active immunotherapy with carcinoembryonic antigen peptide (CAP-1)-pulsed, autologous human cultured dendritic cells in patients with metastatic malignancies expressing carcinoembryonic antigen. *Clin. Cancer Res.* 5, 1331–1338 (1999).

90 Morse MA, Coleman RE, Akabani G, Niehaus N, Coleman D, Lyerly HK. Migration of human dendritic cells after injection in patients with metastatic malignancies. *Cancer Res.* 59, 56–58 (1999).

91 Treanor JJ, Kotloff K, Betts RF *et al.* Evaluation of trivalent, live, cold-adapted (CAIV-T) and inactivated (TIV) influenza vaccines in prevention of virus infection and illness following challenge of adults with wild-type influenza A (H1N1), A (H3N2), and B viruses. *Vaccine* 18, 899–906 (1999).

92 Mutsch M, Zhou WG, Rhodes P *et al.* Use of the inactivated intranasal influenza vaccine and the risk of Bell's palsy in Switzerland. *N. Engl. J. Med.* 350, 896–903 (2004).

93 Glenn GM, Flyer DC, Ellingsworth LR *et al.* Transcutaneous immunization with heat-labile enterotoxin: development of a needle-free vaccine patch. *Expert Rev. Vaccines* 6, 809–819 (2007).

94 Godefroy S, Peyre A, Garcia N, Muller S, Sesardic D, Partidos CD. Effect of skin barrier disruption on immune responses to topically applied cross-reacting material, CRM197 of diphtheria toxin. *Infect. Immun.* 73, 4803–4809 (2005).

95 Cui Z, Mumper RJ. Topical immunization using nanoengineered genetic vaccines. *J. Control. Release* 81, 173–184 (2002).

96 Shi ZK, Curiel DT, Tang DC. DNA-based non-invasive vaccination onto the skin. *Vaccine* 17, 2136–2141 (1999).

97 Vyas SP, Khatri K, Mishra V. Vesicular carrier constructs for topical immunisation. *Exp. Opin. Drug Del.* 4, 341–348 (2007).

98 Glenn GM, Villar CP, Flyer DC *et al.* Safety and immunogenicity of an enterotoxigenic *Escherichia coli* vaccine patch containing heat-labile toxin: use of skin pretreatment to disrupt the stratum corneum. *Infect. Immun.* 75, 2163–2170 (2007).

99 Frerichs DM, Ellingsworth LR, Frech SA *et al.* Controlled, single-step, stratum corneum disruption as a pretreatment for immunization via a patch. *Vaccine* 26, 2782–2787 (2008).

100 Dean CH, Alarcon JB, Waterston AM *et al.* Cutaneous delivery of a live, attenuated chimeric flavivirus vaccine against Japanese encephalitis (ChimeriVax-JE) in non-human primates. *Hum. Vacc.* 1, 106–111 (2005).

101 Mikszta JA, Alarcon JB, Brittingham JM, Sutter DE, Pettis RJ, Harvey NG. Improved genetic immunization via micromechanical disruption of skin-barrier function and targeted epidermal delivery. *Nature Med.* 8, 415–419 (2002).

102 Mikszta JA, Sullivan VJ, Dean C *et al.* Protective immunization against inhalational anthrax: a comparison of minimally invasive delivery platforms. *J. Infect. Dis.* 191, 278–288 (2005).

103 Wermeling DP, Banks SL, Huclson DA *et al.* Microneedles permit transdermal delivery of a skin-impermeant medication to humans. *Proc. Natl Acad. Sci. USA* 105, 2058–2063 (2008).

104 Prausnitz MR. Microneedles for transdermal drug delivery. *Adv. Drug Del. Rev.* 56, 581–587 (2004).

105 Tezel A, Paliwal S, Shen ZC, Mitragotri S. Low-frequency ultrasound as a transcutaneous immunization adjuvant. *Vaccine* 23, 3800–3807 (2005).

106 Fang JY, Lee WR, Shen SC, Wang HY, Fang CL, Hu CH. Transdermal delivery of macromolecules by erbium:YAG laser. *J. Cont. Rel.* 100, 75–85 (2004).

107 Lee S, McAuliffe DJ, Flotte TJ, Kollias N, Doukas AG. Photomechanical transdermal delivery: the effect of laser confinement. *Lasers Surg. Med.* 28, 344–347 (2001).

108 Lee WR, Shen SC, Liu CR, Fang CL, Hu CH, Fang JY. Erbium: YAG laser-mediated oligonucleotide and DNA delivery via the skin: an animal study. *J. Cont. Rel.* 115, 344–353 (2006).

109 Jacques SL, McAuliffe DJ, Blank IH, Parrish JA. Controlled removal of human stratum corneum by pulsed laser. *J. Invest. Dermatol.* 88, 88–93 (1987).

110 Frech SA, DuPont HL, Bourgeois AL *et al.* Use of a patch containing heat-labile toxin from *Escherichia coli* against travellers' diarrhoea: a Phase II, randomised, double-blind, placebo-controlled field trial. *Lancet* 371, 2019–2025 (2008).

• **Phase II clinical trial showing immunization via skin abrasion followed by application of a patch containing heat-labile toxin from *Escherichia coli*.**

111 Etchart N, Hennino A, Friede M *et al.* Safety and efficacy of transcutaneous vaccination using a patch with the live-attenuated measles vaccine in humans. *Vaccine* 25, 6891–6899 (2007).

112 Baxter J, Mitragotri S. Needle-free liquid jet injections: mechanisms and applications. *Exp. Rev. Med. Dev.* 3, 565–574 (2006).

113 Dean HJ, Chen DX. Epidermal powder immunization against influenza. *Vaccine* 23, 681–686 (2004).

114 Chen DX, Endres R, Maa YF *et al.* Epidermal powder immunization of mice and monkeys with an influenza vaccine. *Vaccine* 21, 2830–2836 (2003).

115 Hoffman PN, Abuknesha RA, Andrews NJ, Samuel D, Lloyd JS. A model to assess the infection potential of jet injectors used in mass immunisation. *Vaccine* 19, 4020–4027 (2001).

116 Gill HS, Prausnitz MR. Coated microneedles for transdermal delivery. *J. Control. Release* 117, 227–237 (2007).

117 Widera G, Johnson J, Kim L *et al.* Effect of delivery parameters on immunization to ovalbumin following intracutaneous administration by a coated microneedle array patch system. *Vaccine* 24, 1653–1664 (2006).

118 Gill HS, Prausnitz MR. Coating formulations for microneedles. *Pharma. Res.* 24, 1369–1380 (2007).

119 Miyano T, Tobinaga Y, Kanno T *et al.* Sugar micro needles as transdermic drug delivery system. *Biomed. Microdev.* 7, 185–188 (2005).

120 Sullivan SP, Murthy N, Prausnitz MR. Minimally invasive protein delivery with rapidly dissolving polymer microneedles. *Adv. Mater.* 20, 933–938 (2008).

121 Laurent PE, Bonnet S, Alchas P *et al.* Evaluation of the clinical performance of a new intradermal vaccine administration technique and associated delivery system. *Vaccine* 25, 8833–8842 (2007).

122 Alarcon JB, Hartley AW, Harvey NG, Mikszta JA. Preclinical evaluation of microneedle technology for intradermal delivery of influenza vaccines. *Clin. Vacc. Immunol.* 14, 375–381 (2007).

123 Mikszta JA, Dekker JP, Harvey NG *et al.* Microneedle-based intradermal delivery of the anthrax recombinant protective antigen vaccine. *Infect. Immun.* 74, 6806–6810 (2006).

124 Laurent A, Mistretta F, Bottigioli D *et al.* Echographic measurement of skin thickness in adults by high frequency ultrasound to assess the appropriate microneedle length for intradermal delivery of vaccines. *Vaccine* 25, 6423–6430 (2007).

Affiliations

- John A Mikszta
BD Technologies, 21 Davis Drive, Research Triangle Park, NC 27709, USA
Tel.: +1 919 597 6158
Fax: +1 919 597 6402
john_mikszta@bd.com
- Philippe E Laurent
BD Medical Pharmaceutical Systems, 11 rue Aristide Berges, Le Pont de Claix, 38800 France
philippe_laurent@europe.bd.com